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Thomas J. Hope
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Editors-in-Chief

Encyclopedia of AIDS

 Springer

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With 341 Figures and 123 Tables

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Preface

The HIV/AIDS epidemic has spanned the past three-and-a-half decades. During this time, the epidemic has caused suffering on a global scale. In the fight to end this epidemic, researchers, healthcare agencies, and governments have mobilized and, as a result of these efforts, have fundamentally altered the course of the epidemic and transformed the prognosis for millions of individuals living with HIV/AIDS. Research has been central to the effort to control the HIV/AIDS epidemic. Early studies aimed at defining key processes in the viral replication cycle helped promote the identification of antiviral agents that have dramatically altered the outlook for infected individuals. This has created a *status quo* where HIV-1 infection is no longer a death sentence but a manageable, chronic infection. Now the frontier in the fight against HIV/AIDS has shifted to preventing infection, either through use of antivirals or through development of a vaccine. The field has also embarked on seeking ways to eliminate the virus from infected individuals. The hope is that these endeavors will bring an end to the “Age of AIDS.”

The human endeavor around three-and-a-half decades of research on HIV/AIDS has produced a large body of scientific information. There are many excellent treatises that have focused on condensing and highlighting different areas of the HIV/AIDS field. Therefore, why an AIDS Encyclopedia? Do we really need another work to summarize what we know about HIV/AIDS?

The AIDS Encyclopedia represents an AIDS “Wikipedia.” It is a dynamic, online reference work that can be updated by researchers and by editors that will be able to keep pace with developments in the field. The ability to continuously update the encyclopedia essentially means that it will retain its relevance as an easily accessible and important source of information for students, researchers, and clinicians whether they be working in academia, industry, or the hospital sectors.

Work on the encyclopedia began in 2010 and has seen fruition, thanks to the efforts of many individuals. The editors would like to thank Ms. Tina Shelton, Ms. Rita Beck, and Ms. Sunali Mull at Springer for much of the groundwork in structuring the project and maintaining timelines. Thank you for your persistence and commitment to bringing the project to completion. We would also like to thank the sub-editors who took on responsibility for the 282 individual chapters. We hope the research community will further help in keeping the AIDS Encyclopedia an important and current reference work for the

HIV/AIDS field, and we welcome contributions in this regard. Finally, the editors would like to acknowledge individuals living with HIV/AIDS who, by participating in research studies, have been central in the effort to end this epidemic.

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La Jolla, CA, USA
Miami, FL, USA
August 2017

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Section Editor: *Babafemi O. Taiwo*

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Circumcision and AIDS

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Section Editor: *Hansjakob Furrer*

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Section Editor: *Robert Yarchoan*

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Section Editor: *Sten H. Vermund*

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Section Editor: *Frank Kirchhoff*

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Section Editor: *William A. Paxton*

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Thomas J. Hope is currently a Professor at Northwestern University in the Departments of Cell and Molecular Biology and Obstetrics and Gynecology in the Feinberg School of Medicine and Professor in Biomedical Engineering in the Robert R. McCormick School of Engineering in Chicago, IL. He received his Ph.D. from the University of California, Berkeley, in Immunology. Tom began to study HIV in 1988, during his postdoctoral training at UC, San Francisco. He was one of the pioneers to utilizing the methods of cell biology to study HIV. In 2004, Tom was selected as an Elizabeth Glaser Scientist. For more than two decades, Tom has combined this cell biology approach, supplemented with molecular biology, biochemistry, and genetics, to study various aspects of HIV from virus entry, uncoating, and assembly to defining the earliest steps of sexual transmission and developing interventions to decrease transmission. Tom is currently the Editor-in-Chief of *AIDS Research and Human Retroviruses*, a Section Editor of *PLoS Pathogens*, serves on editorial boards and NIH/NIAID review panels, is a past co-organizer of HIVR4P, Keystone HIV, and Cold Spring Harbor retroviruses, and currently presents and teaches worldwide.



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Dr. Richman joined the University of California, San Diego (UCSD), in 1976 and is currently Distinguished Professor of Pathology and Medicine and holds the Florence Seeley Riford Chair in AIDS Research. He is Director of the Center for AIDS Research at UCSD, and he attends in infectious diseases at the San Diego VA Healthcare System. He is a Fellow of the American Association for the Advancement of Science, the American Association of Physicians, and the Infectious Disease Society of America.



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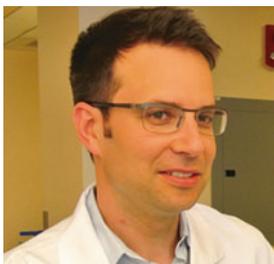
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A

Acetylation

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Definition

Acetylation is a protein post translational modification (PTM) catalyzed by a family of cellular enzymes named histone acetyltransferases (HATs). HATs catalyze the transfer of an acetyl group from the acetyl coenzyme A (acetyl-CoA) to the ϵ -amine group of specific lysine residues of protein substrates. The addition of an acetyl group alters the positive charge of specific lysines, thus modifying the electrostatic properties of the proteins targeted by HATs. The protein chemical modification occurring following acetylation results in modified protein functions. Proteins modified by acetylation might be both of cellular or viral origins.

The Diversity of HATs

Acetylation together with other types of PTMs (e.g., sumoylation, phosphorylation) extends the range of possible molecular structures beyond the limits imposed by the twenty encoded amino acids

and, if reversible, gives a means of control and signaling.

In the past decades, protein acetylation has been widely explored starting from histones up to a wide variety of targets either of cellular or viral origins. In fact, although histone acetylation is traditionally linked to transcription activation through histone modification, numerous studies demonstrate that HAT-mediated acetylation is involved in broad cellular and virological functions (Sterner and Berger 2000; Glozak et al. 2005).

HATs are evolutionary conserved from yeast to mammals and are grouped into two general classes: A-type and B-type HATs, depending on their subcellular localizations and functions (Roth et al. 2001). A-type HATs are located in the nucleus, and many are involved in the regulation of gene expression by functioning as transcriptional coactivators. B-type HATs are located in the cytoplasm and are intimately involved in chromatin synthesis and assembly of nascent histones into chromosomes by acetylating de novo synthesized free histones and promoting their nuclear localization and deposition onto newly synthesized DNA. To date, only one B-type HAT has been discovered: the yeast *HAT1* and its homologues in other species. On the contrary most HATs are nuclear, A-type. These HATs acetylate nucleosomal histones within the chromatin and are potentially linked to transcriptional activation through acetylation of transcription factors or by acting as coactivators at the level of gene

promoters. Although most HATs are able to acetylate free histones *in vitro* when assayed as a single polypeptide, in cells they are usually found in high-molecular-weight complexes, containing also several transcription regulators and chromatin-binding proteins (Sterner and Berger 2000).

The A-type HATs are grouped into five families based on their sequence similarities: the GNAT (GCN5-related *N*-acetyltransferase), MYST (MOZ, Ybf2, Sas2, and Tip60), p300/CBP, the general transcription factor HATs (including the TFIID subunit TAF250), and the nuclear-hormone-related HATs SRC1 and ACTR (SRC3).

The GNAT family members include the highly homologous HATs GCN5 (general control nonderepressible-5) and PCAF (p300/CBP-associated factor), as well as other more distantly related enzymes. GCN5 and PCAF appear to primarily function as histone-acetylating transcriptional coactivators but also catalyze the acetylation of nonhistone substrates, leading to changes in their activities.

p300 and CBP were isolated independently as factors interacting with the adenovirus E1A protein or the transcription factor CREB, respectively. These two factors are highly interchangeable in functions and are thus commonly referred as p300/CBP (Chan and La Thangue 2001). Both factors contain a bromodomain and are often found within the same macromolecular complexes. The four core histones (H2A, H2B, H3, and H4) and also a wide variety of nonhistone proteins can be acetylated by p300/CBP; in fact, these HATs seem to have the broadest substrate acceptance for histones and nonhistone proteins as compared to other HATs. p300/CBP is a potent transcriptional coactivator, which is recruited at specific promoters through interaction with a large number of transcription factors, like E1A, c-Jun, c-Myc, c-Fos, TFIID, MyoD, nuclear-hormone receptor, and E2F-1. In addition, lysine-specific acetylation of histones by p300/CBP together with chromatin remodeling and other covalent modifications establishes the active state of chromatin in a gene-specific manner. More in general, p300/CBP has been

implicated in a number of diverse biological functions such as proliferation, cell cycle regulation, apoptosis, differentiation, and DNA damage response (Yang and Seto 2007).

The MYST family of proteins constitutes a third major group of HATs. The acronym MYST derives from its four founding members: human MOZ (monocytic leukemia zinc finger protein), yeast Ybf2/Sas3, yeast Sas2, and mammalian Tip60 (HIV-1 Tat interacting 60 kDa protein). As compared with the GCN5/PCAF and p300/CBP groups, the MYST family is larger and more heterogeneous in domain organization and biological functions (Roth et al. 2001).

Histone Acetylation in the Regulation of Gene Expression

Histones are heavily modified through several pathways including acetylation, methylation, ubiquitination, sumoylation, phosphorylation, and ADPribosylation. These modifications alter chromatin dynamics by influencing histone-DNA interactions, as well as the recruitment and binding of protein factors to chromatin. Histone acetylation affects chromatin mainly in two ways. First, acetylation neutralizes the positive charge of lysine residues within the histone tails, thus reducing their ability to form electrostatic interactions with the negatively charged phosphodiester backbone of associated DNA. The acetylation of histones is therefore associated with chromatin decondensation, which results in an increased accessibility of transcription factors to their target sequences on DNA. Second, histone acetylation provides a platform for the recruitment of transcriptional regulatory proteins through acetyl-lysine interacting motifs, known as bromodomains. The bromodomain, found in HATs and chromatin-associated proteins, functions as the sole protein module known to bind acetyl-lysine motifs. These domains associate with acetylated lysines of both cellular and viral proteins allowing the proper transduction of the molecular information conveyed by lysine acetylation. For instance, transcriptional activation of HIV-1 proviral DNA depends on a molecular

interaction between acetylated Tat and the bromodomain of the cellular coactivator PCAF (see below).

Acetylation of Nonhistone Proteins

In addition to histones, HATs target nonhistone substrates, such as transcription factors and other nuclear proteins. Cytoplasmic structural components (e.g., tubulin), metabolic enzymes, and signaling regulators have also been reported to be modified by acetylation. Emerging evidence demonstrates that acetylation is an important regulatory mechanism for virally encoded proteins. Acetylation of nonhistone factors is important to modulate the affinity of the proteins with DNA, protein-protein interactions, and subcellular localization or stability.

Acetylation of Viral Proteins

Similarly to cellular factors, proteins encoded by viruses can be modified by acetylation. The oncoprotein E1A of the adenovirus was found to be acetylated by p300/CBP and PCAF at a single lysine residue located at position 239 in the C-terminal domain. Even though Lys 239 has a clear role in the interaction between E1A and the transcriptional corepressor C-terminal-binding protein (CtBP), the functional outcome of E1A acetylation has been debated. One report suggests that acetylation of K239 inhibits the interaction between E1A and CtBP, leading to the loss of CtBP-mediated transcriptional repression and consequent increase in the transforming potential of E1A. Conversely, other studies indicate that, rather than interfering with CtBP recruitment, acetylation of K239 prevents the nuclear import of E1A by abrogating its interaction with importin- α . In this case, acetylation would result in attenuation of E1A nuclear functions and redirection of part of the protein to the cytoplasm. Also viral proteins encoded by the Hepatitis delta virus (HDV) are modified by acetylation. In fact, the small hepatitis delta antigen (S-HDAg), a viral nucleocapsid protein

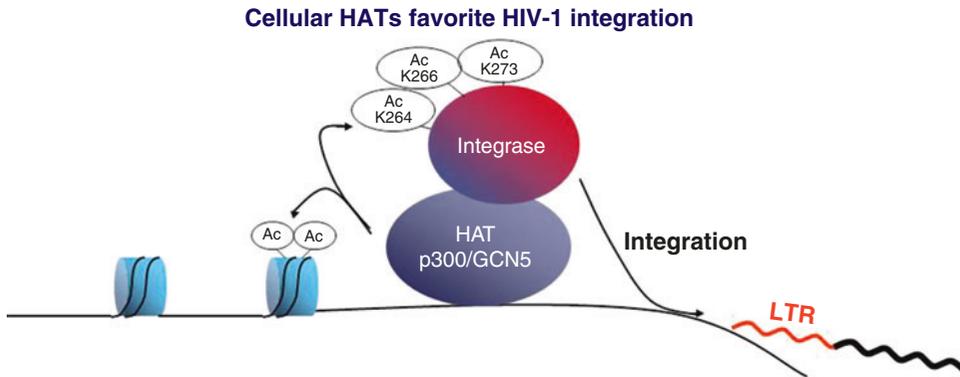
essential for HDV RNA replication, can be acetylated *in vitro* at K72 by the HAT domain of p300/CBP. Most importantly, substitution of K72 with Arg determines a relocalization of the mutated protein from the nucleus, where HDV replicates, to the cytoplasm preventing the accumulation of HDV genomic RNA in the infected cell. Another viral factor functionally modulated by acetylation is the simian virus 40 large T antigen (SV40 T-Ag), a multifunctional protein possessing cellular transforming activity. In this case, acetylation seems to be involved in the regulation of the protein stability, likely enhancing T-Ag degradation through a pathway independent of the ubiquitin-proteasome system.

Cells infected with the human T-cell leukemia virus type 1 (HTLV-1) or overexpressing the HTLV-1 transcriptional activator Tax have been reported to contain a pool of Tax molecules acetylated at a single lysine residue by p300/CBP. Overexpression of p300 markedly increases Tax acetylation and the ability of a wild type HTLV-1 provirus, but not of a mutant provirus encoding an acetylation deficient Tax protein, to activate gene expression from an integrated NF- κ B-controlled promoter. Acetylation together with other PTMs (phosphorylation and sumoylation) controls Tax intracellular localization and its transcriptional activity.

HIV-1 has been described to use the cellular acetylation pathways to regulate viral functions at the nuclear level. Two viral proteins, Tat and integrase, have been reported to be functionally regulated by acetylation through multiple HATs.

Acetylation of HIV-1 Tat

Tat ([► Tat Expression and Function](#)) can be acetylated by three different HATs at three specific lysine residues: K28 is targeted by PCAF, while K50 and K51 are substrates for p300/CBP and GCN5. Most interestingly, the acetylation of each individual lysine differently affects Tat at the molecular level by either increasing Tat affinity to macromolecular complexes (K28 acetylation) or decreasing Tat affinity for its target RNA (K50 acetylation) (Kiernan et al. 1999).



Acetylation, Fig. 1 Model of action of HATs during HIV-1 integration. HATs located at the level of active transcription units acetylated chromatin and integrase

from HIV-1 pre-integration complexes. Acetylation of integrase in turn enhances viral integration

The current model (Nakatani 2002) proposes that acetylation of Tat K28 enhances P-TEFb (► [Cellular Cofactors for HIV-1 Transcription](#)) recruitment on TAR through its interaction with cyclin T1 leading to enhanced RNA Pol II processivity. Subsequently acetylation of Tat K50 leads to Tat recycling by inducing its dissociation from TAR. Tat release from TAR seems to be promoted by the bromodomain of PCAF, through its competition with TAR in binding the acetylated K50. In agreement with the proposed model, the three HATs capable of acetylating Tat efficiently cooperate to stimulate transcription from the 5' LTR of HIV-1 proviral DNA.

Finally, additional functions have been attributed to Tat acetylation. One report shows that acetylation at K50 and K51 increases the affinity of Tat for p32, a cellular splicing factor, thus regulating the splicing efficiency of the HIV-1 transcripts (Berro et al. 2006). Finally, Tat acetylation at K28 increases the affinity for microtubules leading to increased apoptosis in T lymphocytes (Huo et al. 2011).

Acetylation of HIV-1 Integrase

HIV-1 integrase (► [Integration](#)) is acetylated by at least two different cellular HATs, p300/CBP and GCN5 (Cereseto et al. 2005; Terreni et al. 2010). In particular three lysines at the carboxy-terminal domain of the viral enzyme (K264, K266, K273)

are acetylated by both p300 and GCN5, while a fourth lysine, K258, is acetylated exclusively by GCN5.

Similarly to transcription factors, such as p53, E2F, MyoD, c-Myb, and GATA1, acetylated integrase has a higher affinity for DNA which determines increased in vitro catalytic activity (strand transfer activity). Consistently, substitution of acetylatable lysines with arginines (an amino acid with chemical properties similar to lysines) in integrase negatively affects the infectivity of the mutated viruses by specifically reducing their integration efficiencies. A comparative analysis, aimed at establishing the roles of p300/CBP and GCN5 during the HIV-1 replication cycle, revealed that mutated viruses expressing either integrase K264,266,273R or K258,264,266,273R exhibited the same replication deficiency, specifically affecting the step of integration. These results indicate that acetylation of integrase C-terminal lysines 264, 266, and 273 is required for HIV-1 maximal integration efficiency, while acetylation of K258, although observed in vitro, does not appear to play any significant role during infection (Fig. 1).

Similarly integrase of the Moloney murine leukemia virus (M-MuLV), a gammaretrovirus, was demonstrated to be acetylated at Lys 376 by p300 (Schneider et al. 2012). Interestingly, K376 is homologous to the acetylatable K266 in HIV-1. Mutations at K376 decrease retroviral integration; nevertheless, the role of integrase

acetylation has not been clarified yet in this type of retrovirus.

Thus, the same HATs, p300/CBP and GCN5, affecting viral transcription through Tat modification also regulate integration through integrase acetylation.

At the cellular level, acetylation regulates protein functions by modulating protein-protein interactions. Since HIV-1, as all viruses, requires to interact with numerous cellular factors during its replication cycle, acetylation was evaluated as a factor determining differential affinity of integrase with cellular proteins. A yeast two-hybrid screening aimed at identifying factors binding specifically to acetylated integrase led to the discovery of thirteen new integrase-binding partners (Allouch and Cereseto 2011). The identified binding factors are either nuclear or cytoplasmic and are involved in different pathways including chromatin remodeling, nuclear transport, RNA binding, protein synthesis regulation, and microtubule organization. The majority of the two-hybrid hits showed preferential binding for acetylated integrase, while few of them bind with the same affinity the acetylated and the unmodified integrase. One of the factors identified with this screening is KAP1 (also known as TRIM28 or TIF-1 beta), a protein belonging to the TRIM family of antiviral proteins. KAP1 binds acetylated IN and induces its deacetylation through the formation of a protein complex including the deacetylase HDAC1. KAP1-mediated deacetylation results in integrase inactivation and inhibition of viral integration. In fact, modulation of intracellular KAP1 levels through either over expression or knockdown technique (shRNA) revealed that KAP1 curtails viral infectivity by selectively affecting HIV-1 integration (Allouch et al. 2011).

KAP1 has also been described to restrict M-MLV in embryonic carcinoma and embryonic stem cells. However, in the last case viral inhibition occurs at the level of transcription and not during integration (Wolf and Goff 2009).

Conclusions

Acetylation is a PTM that was originally demonstrated at specific lysine residues within the

histone basic N-tail region. Subsequent studies demonstrated that acetylation substrates include also nonhistone proteins of both cellular and viral origins. Two HIV-1 viral proteins are modified by acetylation: Tat and integrase. Both proteins function mainly at the nuclear level. Tat acetylation increases viral transcription efficiency. Acetylation of integrase increases the efficiency of viral integration. Therefore, acetylation of so far identified HIV-1 target proteins positively regulates different stages of viral replication.

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Actin

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Definition

Actin is a ubiquitous highly conserved 42-KDa globular protein in eukaryotic cells. There are three actin isoforms: alpha, beta, and gamma. The alpha actins are mainly found in muscle cells where they form thin filaments, part of the muscle contractile apparatus. The beta and gamma actins are found in most cell types and exist either as a monomeric form (G-actin) or as assembled, double helical, filamentous polymers called filamentous actin (F-actin or microfilaments). In non-muscle cells, the actin cytoskeleton provides mechanical support to cells and is a major driving force for cell motility. Actin also participates in many cellular processes such as cell division, vesicle and organelle movement, and signal transduction.

Introduction

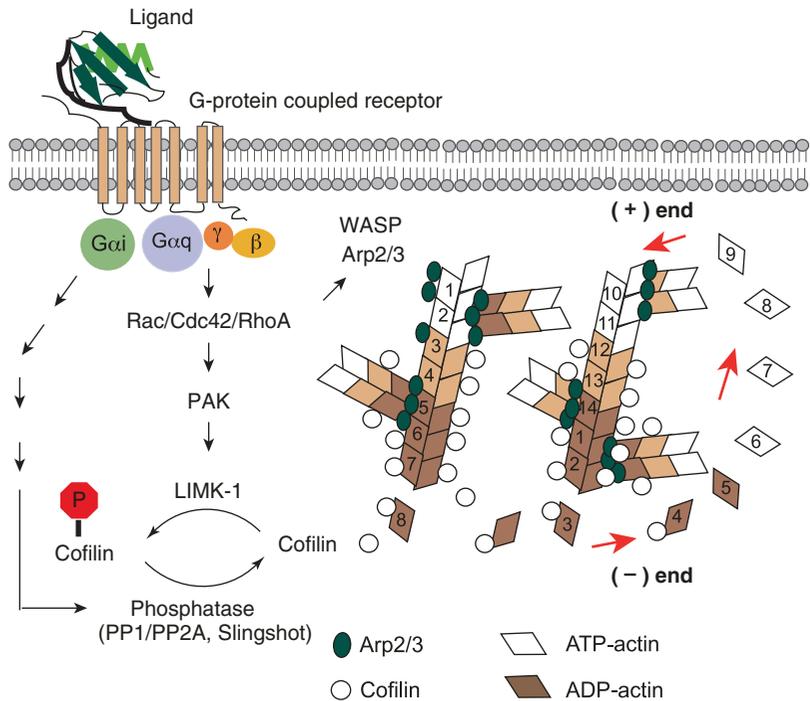
Actin dynamics are regulated through actin polymerization and depolymerization. In vitro, the monomeric G-actin can automatically polymerize in salt solution at concentrations above the critical concentration (C_c) at which actin polymerization

and subunit dissociation reach the equilibrium. Structurally, polymerized actin filaments have polarity, with (+) and (–) ends (or barbed and pointed ends, respectively), as revealed by binding of myosin S1 head domains, which generates directional arrowhead-like decorations pointing towards the (–) end in electron micrographs. In test tubes, the rates of actin polymerization at the (+) and the (–) ends are not the same because the C_c value is lower at the (+) end (100 nM) than at the (–) end (700–800 nM). This results in an elongation rate 5–10 times faster at the (+) end than at the (–) end. When actin polymerization reaches a steady-state phase, the G-actin concentration is between the C_c values of the (+) and (–) ends. This permits selective addition of actin monomers at the (+) end and dissociation of them at the (–) end, and the length of the actin filaments remains constant. This process, called actin treadmilling, resembles a “walking” process of actin subunits moving from the (+) end towards the (–) end along actin filaments (Pollard and Borisy 2003; Fig. 1).

Monomeric G-actin also binds to ATP, and once incorporated into actin filaments, ATP can hydrolyze into ADP-actin through the release of the γ phosphate. Although ATP binding to G-actin is not required for polymerization, the dissociation equilibrium constant (K) for ATP-actin is different, much lower at the (+) end than that at the (–) end. This lower K value promotes ATP-actin addition and actin growth at the (+) end. In contrast, the K value for ADP-actin is similar at both the (+) and (–) ends; this ADP-actin K value is much higher than that of ATP-actin at the (+) end but slightly lower than that of ATP-actin at the (–) end. The difference selectively promotes ADP-actin disassociation from the (–) end (Pollard and Borisy 2003).

In cells, the cytosolic G-actin concentration (0.5 mM) is much higher than the C_c of both (+) and (–) ends. This concentration would trigger the polymerization of almost all G-actin in test tubes, depleting the G-actin pool. However, the intracellular actin pool is stably maintained and actin dynamics are tightly regulated by multiple cellular factors to ensure proper and speedy response to stimulation. G-actin is normally

Actin, Fig. 1 Model of actin treadmilling in cell migration. Extracellular ligand binding to cognate surface receptors such as the G-protein coupled receptors transduces signals to activate actin modulators such as the Arp2/3 complex and cofilin. The Arp2/3 complex binds to the side of an existing filament and serves as an F-actin nucleus, promoting rapid ATP-actin polymerization at the (+) end. With filament growth, the γ phosphate is dissociated from ATP-actin by hydrolysis. Cofilin also promotes phosphate dissociation and servers ADP-actin filaments from the (-) end (Modified from (Wu and Yoder 2009))



associated with factors such as profilin and thymosin β 4. Profilin acts as a nucleotide-exchange factor, allowing the switch from ADP-actin to ATP-actin for actin polymerization. Thymosin β 4 is an actin-sequestering protein and is considered a buffering protein for the maintenance of the monomeric actin pool. Actin filaments (F-actin) are also associated with multiple cellular factors such as the Arp2/3 complex and ADF/cofilin. The Arp2/3 complex is a seven-subunit protein that promotes the growth of new actin filaments. Two of the subunits of the Arp2/3 complex, Arp2 and Arp3, resemble G-actin and serve as nucleation sites for actin growth. ADF/cofilin is a family of actin-severing proteins that depolymerize actin filaments mainly at the (-) end. The Arp2/3 complex and cofilin work together to mediate cytoskeletal actin reorganization and actin dynamics. In addition, actin-capping proteins such as CapZ and gelsolin can cap the ends of actin filaments, preventing actin polymerization or depolymerization. Gelsolin also has a calcium-dependent actin-severing activity that can break actin filaments into shorter fragments (Pollard and Borisov 2003).

In non-muscle cells, the actin cytoskeleton provides mechanical support and drives cell motility. Actin also participates in diverse cellular processes such as cell division, vesicle movement, and signal transduction. In immune cells, actin is involved in multiple processes such as cell adhesion, cell migration, chemotaxis, and T-cell activation. In chemotaxis, directed cell movement towards chemoattractants is controlled by localized cortical actin polymerization. In antigen-specific T-cell activation, the reorganization of the cortical actin plays a critical role in the formation of the immunological synapse. The actin cytoskeleton is the driving force for receptor clustering and the formation of the supramolecular activation cluster (SMAC). This process involves the actin-dependent activation of LFA-1, which is required for sustained signaling to reach full T-cell activation (Wulfing and Davis 1998).

Role of Actin in HIV Entry

HIV entry into cells is mediated through the binding of viral glycoprotein gp120 to the specific cell

receptor and coreceptor, CD4 and CXCR4 or CCR5. This interaction triggers membrane fusion between the virus and the cell and delivers the viral core into the cytoplasm. During entry, gp120 binding to blood resting CD4 T cells also initiates a transient course of actin polymerization and depolymerization, which is important for viral entry and the early postentry process (Vorster et al. 2011; Yoder et al. 2008). For viral entry, actin may be involved in the initial receptor clustering and stabilization. Actin polymerization may also transiently block CXCR4 internalization upon gp120 binding and may provide a support to stabilize the fusion complex (Vorster et al. 2011). In addition, actin may facilitate signal transduction from viral binding to CXCR4 in blood CD4 T cells (Vorster et al. 2011).

Mechanistically, the sequential binding of gp120 to CD4 and the coreceptor is suggested to enhance CD4-CXCR4 or CD4-CCR5 colocalization, which may be dependent on actin activity. However, several studies have demonstrated that CD4 is constitutively associated with or closely (within 100 nm) juxtaposed to CXCR4 or CCR5 in the absence of HIV or gp120. In addition, it has been suggested that gp120 binding to CCR5 requires juxtaposition of CD4 and CCR5, because the engagement has to occur very fast when gp120 is still attached to CD4. These studies suggest that actin may not be involved in the initial gp120-receptor engagement; instead, actin is likely involved in the stabilization of the fusion complex following gp120 binding to CD4 and the coreceptor (Harmon et al. 2010; Vorster et al. 2011). It has been found that the actin inhibitor cytochalasin B inhibits fusion mediated by the CD4-independent gp120, suggesting that steps sensitive to the actin inhibitor may not involve CD4-CXCR4 clustering. In addition, blocking actin activity inhibits gp120-mediated cell-cell fusion at the step of fusion pore formation (Harmon et al. 2010).

Binding of HIV to blood resting CD4 T cells triggers a transient course of actin polymerization and depolymerization mediated through the activation of the signaling pathway Rac1-PAK1/2-LIMK1 (Vorster et al. 2011). The LIM domain kinase 1, or LIMK1, is a cellular serine/threonine

kinase responsible for the phosphorylation of cofilin. Stable shRNA knockdown of LIMK1 decreases cortical actin density and increases the rate of CXCR4 internalization (Vorster et al. 2011). In addition, the entry of HIV is also slightly inhibited at high viral dosages. This LIMK1 knockdown study suggests that HIV-mediated LIMK1 activation and early actin dynamics may be required to block the rapid internalization of CXCR4 following gp120 binding to permit fusion to occur. This LIMK-mediated actin polymerization may also prolong coreceptor signaling to prime postentry events.

Several other actin-binding proteins such as filamin-A and moesin have also been identified as possible factors involved in HIV entry. Filamin-A is an adaptor protein that may anchor both CD4 and CXCR4/CCR5 to F-actin following receptor clustering. siRNA knockdown of filamin-A resulted in a reduction of HIV-1 infection (Jimenez-Baranda et al. 2007). Moesin belongs to the ezrin-radaxin-moesin (ERM) family of proteins that act as cross-linkers between the plasma membrane and actin filaments. HIV gp120 binding to CD4 alone can increase ezrin and moesin phosphorylation, which has been suggested to promote receptor clustering (Barrero-Villar et al. 2009; Naghavi et al. 2007). The siRNA knockdown of moesin has been shown to enhance or inhibit viral infection (Barrero-Villar et al. 2009; Naghavi et al. 2007).

HIV gp120-mediated cell-cell fusion has also been shown to extensively rely on gp120-induced signaling, which is often linked to actin dynamics (Harmon et al. 2010). siRNAs or inhibitors against molecules such as Pyk2, Rac1, GTPase Ras, phospholipase C, protein kinase C, Tiam-1, Abl, IRSp53, and Wave2 have been shown to inhibit gp120-mediated cell-cell fusion (Harmon et al. 2010). siRNA knockdown of the Arp2/3 complex has been shown to inhibit gp120-mediated cell-cell fusion or to have no effect on the cell-cell fusion (Harmon et al. 2010; Komano et al. 2004). However, inhibiting Arp2/3 with an inhibitor, CK548, or through stable shRNA knockdown of Arp3 has been shown to mainly inhibit viral DNA nuclear migration in human CD4 T cells (Spear et al. 2014).

Role of Actin in HIV DNA Synthesis

The involvement of actin cytoskeleton in HIV reverse transcription has been suggested based on multiple actin inhibitor studies and siRNA knockdown of actin modulators. cytochalasins A, D, and E inhibit HIV infection of a HeLa-CD4 indicator cell line at 5 μM (Bukrinskaya et al. 1998). Cytochalasin D reduces viral early DNA synthesis four to fivefold in MT-4 cells (Bukrinskaya et al. 1998). In addition, another actin inhibitor, jasplakinolide, effectively inhibits HIV latent infection of resting CD4 T cells at dosages of 120 nM and above (Cameron et al. 2010; Yoder et al. 2008). The inhibition by jasplakinolide is at the step of viral DNA synthesis and nuclear migration in resting CD4 T cells (Yoder et al. 2008), and this inhibition can be partially overcome by increasing actin dynamics through spinoculation (Guo et al. 2011). The actin inhibitor studies are supported by several siRNA knockdown studies in which the activity of actin modulators such as cofilin and LIMK1 are directly targeted. Inhibition of cofilin activity in blood CD4 T cells through siRNA triggers a drastic increase in the cortical actin density, and this increase correlates with an increase in HIV DNA synthesis (Yoder et al. 2008). In addition, siRNA knockdown of LIMK1 decreases cortical actin density, and this decrease is also associated with the inhibition of viral DNA synthesis (Vorster et al. 2011).

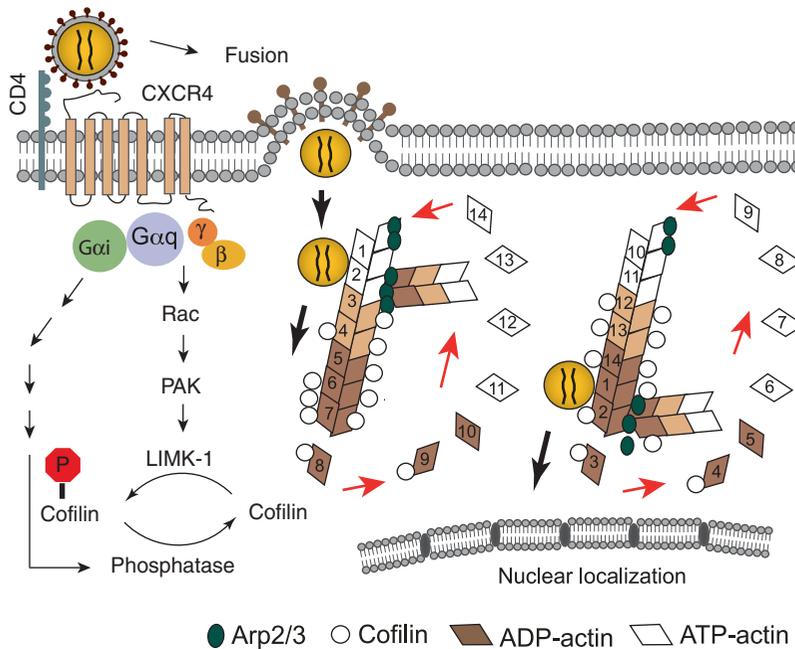
The mechanism of how actin is involved in HIV DNA synthesis remains unclear. It has been proposed that the process of actin polymerization may simply act as a driving force for viral uncoating, and thus, decreasing actin dynamics may impair proper uncoating (Vorster et al. 2011). Alternatively or additionally, the cortical actin may function as an anchorage for the viral reverse transcription complex, and a decrease in the actin cortex density may result in less contact time and suboptimal reverse transcription (Vorster et al. 2011). The actin cytoskeleton has been proposed to be a major site for viral reverse transcription in infected cells (Bukrinskaya et al. 1998; Vorster et al. 2011; Yoder et al. 2008). Multiple viral proteins in the preintegration complex are found to interact with

actin. These proteins include the Gag nucleocapsid protein (NC), the large subunit of viral reverse transcriptase, the viral integrase, and Nef. Among these actin interacting proteins, Nef, in particular, has been shown to enhance viral DNA synthesis. This positive effect of Nef is diminished by actin inhibitors, indicating that the Nef-mediated enhancement of viral DNA synthesis may be related to the cortical actin (Wu and Yoder 2009).

In HIV infection of blood resting CD4 T cells, viral DNA synthesis is a slow process that takes about two days to maximize (Yoder et al. 2008). This slower viral DNA synthesis may be attributed a lower deoxynucleoside level in resting T cells. It has also been suggested that the slower synthesis of viral DNA correlates with lower cortical actin dynamics in resting T cells (Yoder et al. 2008). Pre-stimulation of resting CD4 T cells with anti-CD4/CXCR4 antibody-coated magnetic beads reorganizes the cortical actin and increases actin dynamics, and this stimulation enhances viral DNA synthesis and nuclear migration (Yoder et al. 2008). In addition, spinoculation triggers dynamic actin and cofilin activity, and this process dramatically increases viral DNA synthesis and nuclear migration in resting CD4 T cells (Guo et al. 2011). It has also been shown that stimulation of resting CD4 T cells with chemokine CCL2 augments gp120-induced F-actin polymerization, which enhances viral DNA synthesis about fivefold (Campbell and Spector 2008).

Role of Actin in HIV Intracellular Migration

It has been observed, using live cell imaging, that HIV may use the cytoskeleton, particularly microtubules, to move inside epithelial cells. However, treatment of CD4 T cells with multiple microtubule modulators such as taxol (1 μM), vinblastine (1 and 10 μM), colchicine (10 and 100 μM), and nocodazole (10 and 100 μM) is not able to block HIV infection of CD4 T cells (Yoder et al. 2011). In contrast, actin inhibitors such as cytochalasins and jasplakinolide inhibit HIV infection of CD4 T cells (Yoder et al. 2008, 2011). It has been suggested that HIV may mainly use actin-based mobility for



Actin, Fig. 2 Model of HIV-mediated actin dynamics in the initiation of viral infection. HIV binding to CD4 and the chemokine coreceptor, CXCR4 or CCR5, mediates entry and signal transduction that activates actin modulators such as LIMK and cofilin. HIV-mediated actin dynamics may facilitate viral entry, and postentry DNA synthesis. The viral preintegration complex (*PIC*) may also be

anchored onto F-actin for reverse transcription. HIV-mediated cofilin activity increases actin treadmilling which may promote the migration of viral PIC across the cortical actin layer, facilitating PIC localization to the perinuclear or nuclear region (Modified from (Wu and Yoder 2009))

intracellular migration in T cells (Yoder et al. 2008, 2011). It has also been proposed that in blood resting CD4 T cells, the cortical actin is relatively static in the absence of T-cell activation or chemotactic stimulation. This lack of actin activity may represent a realistic limitation for viral early processes such as intracellular migration; HIV uses the chemokine receptor CXCR4 to trigger the activation of actin modulators such as LIMK1 and cofilin to increase actin dynamics (Vorster et al. 2011; Yoder et al. 2008). The cortical actin may function as an anchorage for the viral preintegration complex, and HIV may rely on active actin treadmilling to cross the cortical actin layer (Vorster et al. 2011; Yoder et al. 2008; Fig. 2). In support of this model, inhibition of HIV-mediated chemotactic signaling through pertussis toxin inhibits viral nuclear migration (Yoder et al. 2008); treatment of cells with jasplakinolide also diminishes viral DNA synthesis and nuclear migration (Yoder et al. 2008). In addition, siRNA knockdown of cofilin increases early

HIV DNA synthesis but simultaneously decreases HIV nuclear migration (Yoder et al. 2008); siRNA knockdown of LIMK1 also decreases HIV DNA synthesis and viral nuclear migration (Vorster et al. 2011). Induction of actin dynamics through transient treatment with latrunculin A, a cofilin-activating peptide S3, or with anti-CD4/CXCR4 antibody-coated magnetic beads enhances HIV latent infection of CD4 T cells (Yoder et al. 2008). In addition, spinoculation triggers both cofilin activation and actin dynamics in transformed and resting CD4 T cells (Guo et al. 2011). This leads to the upregulation of the CXCR4 receptor and a marked enhancement of HIV-1 DNA synthesis and nuclear migration (Guo et al. 2011).

In HIV-1 latent infection of resting memory CD4 T cells, pretreatment of cells with the chemokine CCL19, CXCL9, CXCL10, and CCL20 significantly increases viral DNA integration (Cameron et al. 2010). It has been suggested that chemokines such as CCL19 trigger cofilin

activation and changes in actin filaments that enhance viral nuclear localization (Cameron et al. 2010). These studies suggest that chemokine-mediated actin dynamics are required for HIV-1 nuclear migration and the establishment of viral latent infection in resting memory T cells.

Role of Actin in HIV Assembly and Release

Cytoskeletal actin is implicated in HIV assembly and release from several actin inhibitor studies (Jolly et al. 2007). Cytochalasin D only partially inhibited HIV-1 release from infected T cells, but mycalolide B potently inhibited HIV-1 release. Treatment of T cells with actin inhibitors such as latrunculin A also results in a decrease in Env-Gag colocalization at polarized actin caps on the plasma membrane, suggesting that actin is required for Env and Gag transportation to the plasma membrane for assembly. In addition, wortmannin and KT5926, a myosin light-chain kinase inhibitor, also inhibit viral release. It is suggested that myosin-actin interaction may be required to drive viral budding. HIV-1 Gag directly binds to F-actin through its nucleocapsid region. Nevertheless, viral nucleocapsid association with F-actin does not appear to be critical for viral budding; rather, active actin treadmill has been proposed to be important for driving viral budding at proper speed. HIV Gag is also found to bind to the actin cross-linking protein filamin A, suggesting that filamin A may also function to facilitate the transport and anchorage of Gag to the plasma membrane for viral assembly. Recently, the cofilin kinase, LIMK1, has been suggested to regulate HIV particle release; disrupting of LIMK1 activity led to the accumulation of virion particles on the plasma membrane (Wen et al. 2014).

Actin and Actin Cytoskeletal Proteins Present in HIV Virion Particles

Actin and several actin regulatory proteins have been found in virions purified from sucrose

gradient centrifugation (Ott et al. 1996). Three types of actin-related factors are present inside HIV virions in various molar ratios to HIV Gag: actin, 10–15%; ezrin and moesin, 2%; and cofilin, 2–10%. The packaged actin filaments are found to be specifically associated with the nucleocapsid. The packaging of cytoskeletal proteins may result from the direct involvement of actin in the viral assembly process. Nevertheless, the functional importance of cytoskeletal proteins' presence in virions remains to be studied.

Role of Actin in HIV Cell-Cell Transmission

HIV-1 cell-cell transmission is proposed to occur through a structure called virological synapse (VS), which requires both actin and microtubules for polarized viral budding and viral cell-cell transfer (Sattentau 2010). Formation of the VS involves HIV gp120-induced clustering of gp120 with CD4/CXCR4, and ICAM-1 with LFA-1 on the effector and target cells, respectively. Actin is involved in the clustering and polarization of Env-Gag on the effector cells; actin is also involved in the stabilization of the CD4/CXCR4 and LFA-1 clusters on target cells. In addition, using a cell-free lipid-bilayer system, gp120 is shown to induce signal transduction to create an F-actin-depleted zone on target cells, possibly facilitating viral transfer and post transfer events. The cofilin kinase, LIMK1, and its regulator ROCK1 have also been shown to modulate HIV cell-cell transmission (Wen et al. 2014).

Potential Role of Actin in Viral Pathogenesis

In vitro, HIV-1 gp120 is suggested to act as a viral chemokine to attract or repel CD4 or CD8 T cells through triggering actin activity. Gp120 is also shown to alter T-cell chemotactic response to CCL20, CCL21, and sphingosine-1-phosphate (SIP), which may inhibit CCL21-mediated lymphatic entry and SIP-mediated egress. In addition, gp120 has been shown to promote T-cell

non-responsiveness to SDF-1 through deregulation of cofilin. HIV-1 Nef has also been shown to inhibit SDF-1-mediated chemotaxis and to inhibit the activity of cofilin. In HIV-1-infected patients, cofilin activity in their blood resting CD4 T cells is found to be aberrantly upregulated, suggesting that dysregulation of actin modulators such as cofilin may have a profound effect on T-cell activity and functionality (Wu and Yoder 2009).

In HIV-infected patients, there is also a strong positive correlation between HIV viremia and the chemokine CCL2. Treatment of resting CD4 T cells with CCL2 leads to an increase in gp120-induced actin polymerization that enhances viral entry and viral early DNA synthesis (approximately fivefold) (Campbell and Spector 2008). Similarly, in patients, the T-cell homing and inflammatory chemokines CCL19 and CCL21 are markedly increased at early stages of infection. Treatment of resting CD4 T cells with CCL19 or CCL21 triggers cofilin and actin activities that promote high levels of HIV-1 DNA integration (Cameron et al. 2010). These studies suggest that the infection and establishment of viral reservoirs in resting T cells likely involves chemokines and chemokine-mediated actin dynamics (Wu and Yoder 2009).

Conclusion

HIV-mediated actin dynamics have recently been shown to be important for viral entry, reverse transcription, and efficient nuclear migration. In addition, actin cytoskeleton may also be involved in viral assembly and HIV cell-cell transmission. In blood CD4 T cells, HIV-mediated chemotactic signaling and actin dynamics not only facilitate viral infection but may also contribute to viral pathogenesis through disruption of normal chemotactic responses and T-cell activity. Furthermore, HIV-mediated chemotactic signaling may interact or interfere with signal transduction from chemokines, and this interaction is expected to have a profound impact on HIV infection and pathogenesis.

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AIDS-Related Primary Central Nervous System Lymphoma

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Definition

Primary central nervous system lymphoma (PCNSL) is a rare extra-nodal B-cell non-Hodgkin lymphoma, which arises in the brain, spinal cord, meninges, or eyes. In individuals infected with HIV, PCNSL occurs with advanced immunosuppression and low CD4 T-cell count. It is considered an AIDS-defining malignancy. Unlike PCNSL in immune-competent hosts, AIDS-related PCNSL

(AR-PCNSL) is almost exclusively caused by a cancer-causing herpesvirus, Epstein Barr Virus (EBV, also known as human herpesvirus-4). While PCNSL in immune-competent patients and AR-PCNSL have pathological and clinical overlap, AR-PCNSL is distinguished by its strong association with immunosuppression and viral etiology.

Introduction

Primary central nervous system lymphoma (PCNSL) is a rare extra-nodal non-Hodgkin lymphoma, which accounts for approximately 3–5% of primary brain tumors. Prior to effective therapy for HIV, a large proportion of cases of PCNSL, especially in younger individuals, occurred in severely immune-compromised patients with acquired immunodeficiency syndrome (AIDS) (Ziegler et al. 1984); therefore, PCNSL is considered an AIDS-defining malignancy. However, after the broad availability of highly activated antiretroviral therapy (HAART) in 1996, the incidence of PCNSL in HIV-infected individuals has decreased by nearly 90%. Nonetheless, the incidence of AIDS-related (AR)-PCNSL in the USA remains significantly elevated at an estimated 26 cases per 100,000 person-years among people with AIDS and continues to affect mainly patients under the age of 50 (Shiels et al. 2011). In areas where HAART is available, most patients with AR-PCNSL are not taking HAART either because they are not aware they are HIV infected or because of poor adherence to HAART. At the same time that incidence of AR-PCNSL has decreased and there has been an increase in the incidence of PCNSL in immune-competent elderly patients, highlighting a changing epidemiology of PCNSL in the USA related to differences in the underlying pathogenic processes (Shiels et al. 2011).

Histopathology and Pathogenesis

PCNSL is a B-cell lymphoma with diffuse large-cell morphology. The diagnosis should be

distinguished from systemic lymphoma with CNS involvement, dural-based low-grade lymphomas, and other rare histologies presenting in the CNS. PCNSL in HIV-infected and HIV-uninfected patients shares some clinical and morphologic characteristics; however, pathogenesis differs in important ways.

Both AR-PCNSL and PCNSL in immune-competent hosts are multifocal angiocentric tumors that express pan-B-cell markers (CD20, CD19, CD22, and CD79a). These tumors are highly proliferative with high expression of Ki-67. PCNSL generally has a post-germinal center phenotype. In immune-competent patients, immunohistochemistry suggests a majority of tumors have activated B-cell differentiation based on the lack of CD10 and common expression of IRF4 (90%), a transcription factor associated with lymphocyte activation. However, the germinal center-associated transcription factor BCL6 is also expressed in 60–80% of cases. In contrast, in AR-PCNSL, the immunophenotype is generally BCL6 negative and IRF4 positive (Carbone et al. 1998). This immunophenotype is also noted in posttransplant lymphoproliferative disorder (PTLD). Additional characteristics of AR-PCNSL include expression of the plasma cell marker CD138, the activation marker CD30, and adhesion molecules CD11a and ICAM-1 (CD54) that may contribute to homing to cerebral blood vessels.

There are several similarities between AR-PCNSL and PTLD. PTLD is a disorder of proliferating latently EBV-infected B cells that may be clonal and sometimes presents with CNS-only manifestations. PTLD can occur either after solid organ (SOT) or hematopoietic stem cell transplants (HSCT) as a result of medical immunosuppression. In HSCT it usually occurs within 6 months posttransplant and the lymphoproliferation is of donor-cell origin prior to EBV-specific cytotoxic T-cell reconstitution, while in SOT >90% is of recipient-cell origin and may have a longer latency due to long-term T-cell suppression to prevent organ rejection (Heslop 2009). Both AR-PCNSL and PTLD often have immunoblastic features (Castellano-Sanchez et al. 2004). Like PTLD, AR-PCNSL

tumor cells almost always have evidence of EBV infection, as noted by staining for EBV-encoded small RNA (EBER1) transcripts (MacMahon et al. 1991). AR-PCNSL is usually clonal based on polymerase chain reaction (PCR) evaluation of the immunoglobulin heavy-chain gene (IgH).

Acquired T-cell immunosuppression is a common risk factor for both AR-PCNSL and PTLD. In patients with HIV, the majority of cases of AR-PCNSL occur in severely immune-compromised patients with AIDS and CD4 counts <50 cells/uL. AR-PCNSL patients have been shown to lack EBV-specific CD4+ T cells, irrespective of absolute CD4 counts, supporting the lack of immune regulation of EBV-infected B cells as a critical mechanism of EBV-driven oncogenesis (Gasser et al. 2007). This is in contrast to PCNSL in immune-competent patients, in which evidence of EBV infection of the tumor cells is rare (MacMahon et al. 1991). A range of EBV viral proteins are expressed in both AR-PCNSL and PTLD, including the EBV-associated nuclear antigens (EBNAs) 1, 2, 3A, 3B, and 3C, latent membrane proteins (LMP) 1 and 2, and leader protein (LP). This pattern of EBV protein expression is referred to as latency III and is remarkable among EBV-associated tumors for the broadest expression of viral proteins. EBV-encoded genes may play an important role in lymphomagenesis, and at the same time, EBV latency III lymphoproliferations are also the most immunogenic EBV-associated tumors and responsive to immune-based therapies.

At the molecular level, a high level of aberrant somatic hypermutations has been detected both in PCNSL in immune-competent patients and in AR-PCNSL (Table 1) (Gaidano et al. 2003). In AR-PCNSL, recurrent mutations have been noted in 5' noncoding region of *BCL6*, *c-MYC*, and *TTF*. Additional insight into the molecular pathogenesis of PCNSL has mainly been evaluated in tumors from immune-competent subjects. In PCNSL not associated with AIDS, additional recurrent mutations have been identified (Table 1). Also, chromosomal abnormalities involving either *IgH* or *BCL6* breakpoints detectable by fluorescence in situ hybridization (FISH) or gains and losses of genetic materials detectable

AIDS-Related Primary Central Nervous System Lymphoma, Table 1 Molecular pathology of primary CNS lymphoma in immune-competent patients compared to

AIDS-related primary CNS lymphoma and other EBV-associated lymphomas with a latency III viral protein expression pattern

	PCNSL in immune-competent patients	AR-PCNSL and other latency III EBV-associated lymphomas
Somatic gene mutations	<i>PIMI</i> , <i>c-MYC</i> , <i>TTF</i> , <i>PAX5</i> , <i>Fas (CD95)</i> , <i>CARD11</i> , <i>MYD88</i> , <i>BLIMP1</i> , <i>TBL1XR1</i>	<i>BCL6</i> , <i>c-MYC</i> , <i>TTF</i> (Gaidano et al. 2003)
Chromosomal abnormalities by FISH or CGH	<i>IgH</i> and <i>BCL6</i> breakpoints	Unknown
	6p21 – 32 deletions	
	12q gains	
Human gene expression studies	Compared to systemic DLBCL: high expression of <i>XBPI</i> , <i>c-MYC</i> , <i>PIMI</i> , IL-4-induced genes (Rubenstein et al. 2006), as well as extracellular matrix and adhesion-related genes <i>osteopontin</i> , <i>CXCL13</i> , and <i>IL-8</i>	Unknown
Human microRNA (miRNA)	Compared to systemic DLBCL, further upregulation of miRNA associated with germinal center B-cell lymphomas (miR17-5p, miR-20a, miR-155), as well as those blocking B-cell differentiation (miR-9, miR-30b/c)	High miR-155 expression (Wang et al. 2011)
EBV–human gene interactions	Not applicable	EBV-encoded LMP1 interacts with cellular TRAFs, activating NFkB (Kung 2010)
		LMP1 induces IRF4, these proteins are co-expressed in AR-PCNSL (Xu et al. 2008)
		LMP1 upregulates adhesion molecules LFA1 and ICAM-1, leading to tumor necrosis and vascular destruction as seen in AR-PCNSL (Cherney et al. 1998)
EBV-encoded miRNA	Not applicable	EBV-encoded BHRF1 miRNA cluster enhances EBV's transforming potential

by comparative genomic hybridization (CGH) have been noted in PCNSL not associated with AIDS. One study conducted in HIV-uninfected patients comparing lymphomas limited to immune-privileged sites (IP-testis and CNS) to systemic DLBCL identified a loss in 6p21.32-p35.3 in IP-DLBCL. Analysis of candidate genes encoded in that region identified two separate clusters: one involved in apoptosis and a second involved in the immune response, including the regulation of HLA expression. More recently, L265P gain-of-function point mutation in *MYD88* has been noted to be a common recurrent mutation in PCNSL. This point mutation is also noted in a subset of activated diffuse large B-cell lymphomas and Waldenstrom's macroglobulinemia. Point mutations in the coiled region of *CARD11* have also been noted. These later two genes are implicated in NFkB dysregulation in B-cell lymphomas.

Gene expression profiling in PCNSL has been limited due to relatively small sample sizes ($n < 25$) and have been mainly performed on tumors from HIV-uninfected patients. Three published studies have demonstrated a transcriptional signature that somewhat resembles systemic DLBCL. However, several genes of interest have been identified as being significantly upregulated in PCNSL compared to systemic DLBCL, including genes encoding adhesion-related proteins (*CXCL13*), extracellular matrix genes (*SSP1*, *osteopontin*) (Tun et al. 2008), and the oncogenes *Pim-1*, *c-MYC*, and *Mina53*. Interestingly, increased expression of the transcription factors *XBPI* and *ATF6*, which are genes that regulate unfolded protein responses, were noted in one study. Transcriptional upregulation appears to be associated with paracrine interactions with CNS vasculature, which is in part driven by tumor and endothelial IL-4 expression (Rubenstein et al. 2006).

The role of microRNAs in normal lymphoid development as well as lymphomagenesis is an area of active research. miR-155, which plays an important role in germinal center biology and is associated with B-cell proliferation and lymphomagenesis, appears to be even more highly expressed in PCNSL than systemic DLBCL. Additional microRNAs that are upregulated compared to systemic DLBCL include miR-17-5p and miR-20a, which target the *c-MYC* pathway, as well as those blocking B-cell differentiation (miR-9, miR-30b/c), while several putative tumor-suppressor miRNAs (miR-199a, miR-214, miR-193b, miR-145) may be downregulated.

Further research is required to evaluate molecular similarities and differences between AR-PCNSL and PCNSL in immune-competent hosts. It remains unknown which of the genetic abnormalities seen in PCNSL also exist in AR-PCNSL, but it is likely that some molecular abnormalities in AR-PCNSL are due to interactions between EBV-encoded genes, microRNAs, and lymphomagenic human signaling pathways. For example, latency membrane protein-1 (*LMP1*) is a viral oncogene that encodes a homologue to CD40 that is constitutively activated. Interactions between the C-terminal-activating regions of LMP1 and tumor necrosis factor receptor-associated factors (TRAFs) can lead to the upregulation of NF κ B. LMP1 also upregulates IRF4, a hallmark of AR-PCNSL, while downregulating the transcription factor BLIMP1, which is required for plasma cell differentiation. LMP1 itself is upregulated by IL-4, and therefore IL-4-dependent signaling appears to be important in both types of PCNSL. Interestingly, mouse models of lymphomas expressing LMP1 are notable for a high degree of tumor necrosis and vascular destruction and in some cases tumor regression, mirroring what is noted in AR-PCNSL. These findings may be mediated by the upregulation of adhesion molecules and induction of chemokine antitumor responses (Cherney et al. 1998). Furthermore, miR-155, which is transcriptionally targeted by IRF4, is highly expressed in the latency III pattern of EBV-infected B cells as well as PTLD and is associated with cellular proliferation (Wang

et al. 2011). Additional epigenetic mechanisms of lymphomagenesis attributable to dysregulated expression of EBV-encoded proteins and microRNAs in B cells, as well as abnormal innate and acquired immune responses in AIDS patients, are also likely.

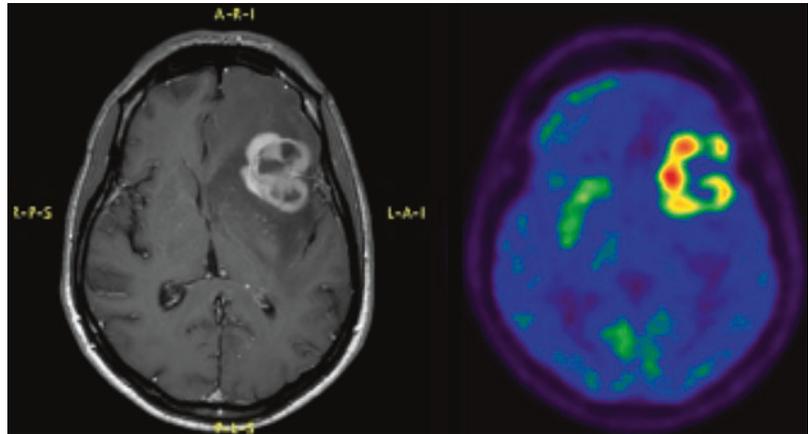
Clinical Presentation, Diagnosis, and Baseline Evaluation

Neurologic disorders are prevalent in AIDS patients and may be due to HIV-associated pathology, opportunistic infections, neoplasms, drug effects, and cerebrovascular disease. AR-PCNSL most often presents with neurologic symptoms such as headaches, lethargy, confusion, visual complaints, seizures, or focal neurologic symptoms such as cranial nerve dysfunction or hemiparesis. It occurs most of the time in patients with CD4 counts less than 50 cells/mL. CT scans usually show ring-enhancing CNS mass lesions. The main differential diagnosis of such lesions in patients with AIDS includes toxoplasma encephalitis, the most common cause, followed by PCNSL. Other causes of mass lesions are cryptococcal meningoencephalitis and tuberculosis. Before HAART, toxoplasmosis encephalitis occurred in 3–10% of patients in the USA. However, toxoplasmosis is much less frequent with HAART as well as medicines commonly used in patients with CD4 counts less than 200 to prevent *pneumocystis* pneumonia such as trimethoprim–sulfamethoxazole. In the HAART era, toxoplasmosis incidence is less than 1%, but like AR-PCNSL, it is most commonly seen in medically underserved HIV-infected populations not on HAART.

AR-PCNSL occurs primarily in the brain parenchyma and may disseminate to the leptomeninges. Spinal cord, ocular, and cranial nerve involvement are rare. Given advanced immunosuppression in this population, patients with AR-PCNSL are also at risk for concurrent opportunistic infections, including CNS infections such as toxoplasmosis, cryptococcus, or CMV. The preferred initial imaging in patients with HIV and neurologic symptoms is contrast-

AIDS-Related Primary Central Nervous System Lymphoma, Fig. 1

Left: Gadolinium-enhanced T1-weighted magnetic resonance imaging in a patient with AIDS-related primary central nervous system lymphoma. *Right:* ^{18}F -FDG-positron emission tomography in the same patient



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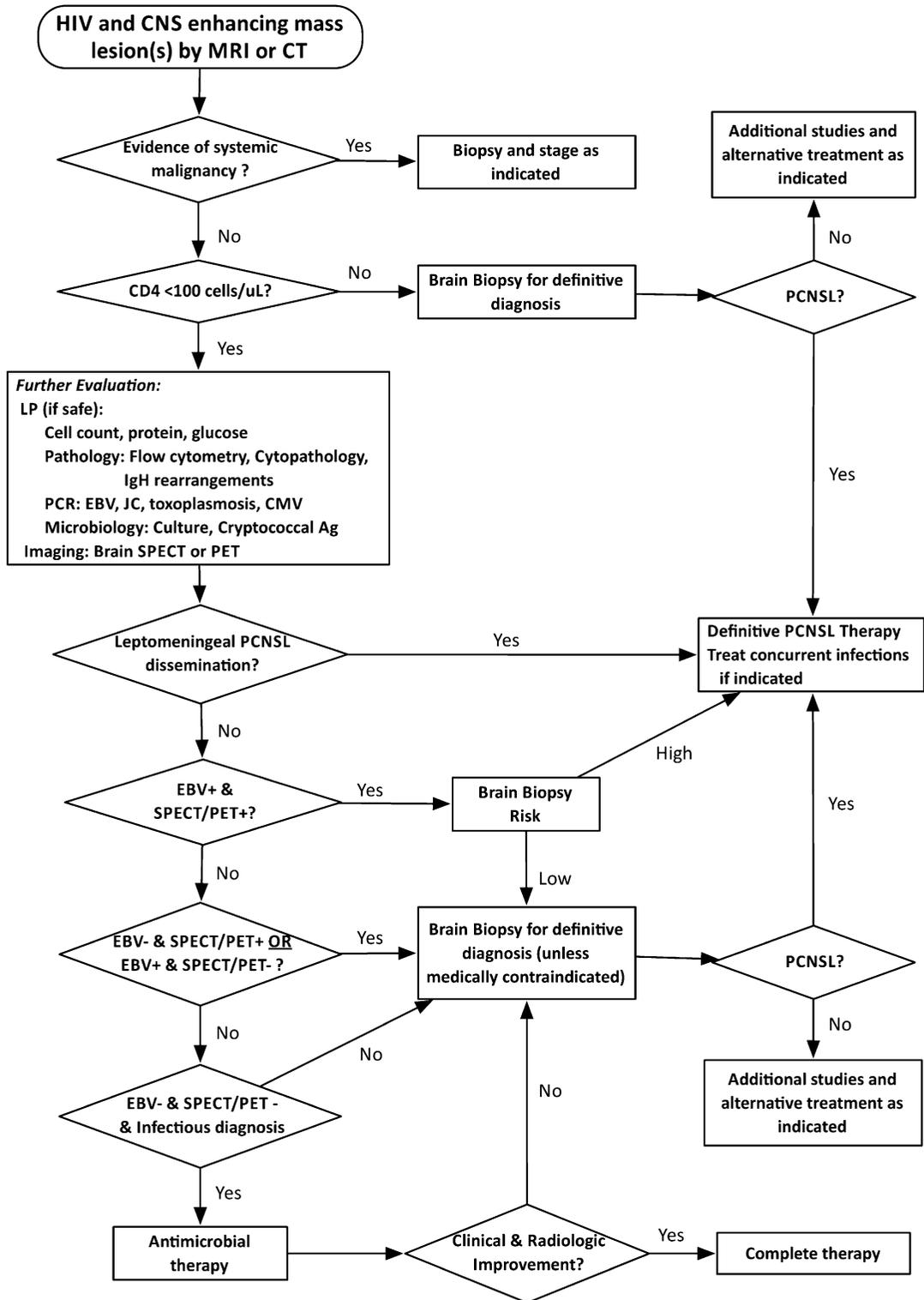
enhanced magnetic resonance imaging (MRI), although CT with contrast can be used if MRI is not available. In AR-PCNSL, these imaging studies can identify single or multiple CNS masses, which are generally ring enhancing with central necrosis, but may also have diffuse or nodular enhancement (Fig. 1). Mass-associated edema is also common and can be best noted on MRI fluid-attenuated inversion recovery (FLAIR) sequence imaging. CT and MRI are useful in excluding significant midline shift that may make a lumbar puncture unsafe. Toxoplasmosis and AR-PCNSL have similar presentations and cannot be reliably distinguished based on imaging and clinical features.

Establishing a definitive diagnosis of cerebral mass lesions in patients with known or suspected HIV (Fig. 2) requires a medical history focusing on HIV and opportunistic infections, use of prophylactic antibiotics with anti-toxoplasmosis activity, and risk factors for systemic malignancies, as well as physical exam including professional ophthalmology evaluation. Expedited evaluation should include the following studies: HIV ELISA and viral load, CD4 count, CNS and body imaging to evaluate for systemic infections or malignancies, toxoplasmosis serology, and lumbar puncture to evaluate the cerebral spinal fluid (CSF) for leptomenigeal dissemination and/or CNS infection. In cases of suspected ocular involvement, vitrectomy is indicated (Abrey et al. 2005).

Lumbar puncture with CSF studies and nuclear imaging provides important diagnostic

information to help differentiate between AR-PCNSL and CNS infection and identify concurrent pathologies. The discovery of an almost universal association of AR-PCNSL with EBV infection (MacMahon et al. 1991) has led to the use of polymerase chain reaction (PCR) to detect EBV DNA in the cerebral spinal fluid (CSF) in patients with suspected AR-PCNSL. Additional initial CSF studies to evaluate for leptomenigeal dissemination and/or infections include opening pressure, cell count, protein, glucose, gram stain, cryptococcal Ag, cytopathology, flow cytometry and PCR evaluation for immunoglobulin heavy-chain (IgH) clonal rearrangements, EBV (quantitative) viral load, JC virus, cytomegalovirus (CMV, also known as human herpesvirus-5), and toxoplasmosis (Fig. 1). Other microbiology studies may be indicated in some cases based on MRI findings and patient history.

Two nuclear imaging modalities have been evaluated in patients with AR-PCNSL, thallium-201 (Tl-201) single-photon emission CT (SPECT) and ^{18}F -fluorodeoxyglucose positron emission tomography (FDG-PET). With either nuclear imaging modality, infections usually appear as hypometabolic lesions, whereas PCNSL or other tumors are hypermetabolic (Fig. 1). In patients with HIV/AIDS and low CD4 count (generally less than 100 cells/uL), the sensitivity and specificity of nuclear imaging range between 80% and 100%. Combining imaging findings with data on toxoplasmosis serology and CSF EBV viral load can further increase



AIDS-Related Primary Central Nervous System Lymphoma, Fig. 2 An approach for evaluating HIV-infected patients with central nervous system-enhancing mass lesions

diagnostic accuracy. The combination of Tl-201 SPECT with CSF-based testing increased the specificity and positive predictive value to nearly 100% in one study (Antinori et al. 1999). However, it should be noted that EBV can be detected frequently in the CSF of patients with HIV/AIDS with systemic non-Hodgkin lymphoma or other diseases, and is not specific for AR-PCNSL. Furthermore, anti-toxoplasmosis titers can be elevated in patients with AR-PCNSL, and do not exclude concurrent pathology. For these reasons, despite technical advances in minimally invasive diagnostic tools for evaluation of intracranial masses in AIDS patients, the diagnosis of AR-PCNSL is done by pathological examination in nearly all cases.

CSF cytopathology as well as flow cytometry and IgH rearrangement studies may be useful in the diagnosis of AR-PCNSL. The impetus for using CSF cytopathology as a diagnostic tool in PCNSL comes from studies utilizing this as adjunct in diagnosis of leptomeningeal involvement. CSF cytopathology can detect leptomeningeal spread in 15–40% of untreated immune-competent PCNSL patients and is associated with higher tumor burden (Balmaceda et al. 1995; Ferreri et al. 2003; Fischer et al. 2008). Multiparameter flow cytometry is also useful for detecting leptomeningeal involvement of both systemic diffuse large B-cell lymphoma and PCNSL and is more sensitive than cytopathology. IgH PCR studies of CSF can also detect clonal IgH rearrangements in patients with PCNSL and provide important information that can be integrated with cytopathology and flow cytometric findings. It should be noted that the utility of flow cytometry and IgH PCR in AR-PCNSL remains to be fully defined; still it is reasonable to integrate these minimally invasive studies into the initial diagnostic evaluation of patients with AIDS and ring-enhancing CNS masses. In AIDS patients with ring-enhancing CNS masses, the pathological demonstration of leptomeningeal dissemination of lymphoma can establish a definitive diagnosis.

Despite these advances in minimally invasive diagnostic tools, histologic diagnosis remains the gold standard for diagnosis of AR-PCNSL, and

biopsy is required in many cases for definitive diagnosis. As AR-PCNSL is a multifocal malignancy, craniotomy with resection of a lesion is generally not performed unless there is another indication such as decompression of mass effect or concern for an abscess. Stereotactic brain biopsy has a diagnostic yield of 85–90% in the evaluation of cerebral lesions in patients with AIDS and is generally associated with low mortality, although the overall morbidity rate is approximately 8–10% mainly due to intracranial bleeding. However, thalamic or basal ganglia lesions are associated with a significantly increased risk of morbidity, often from bleeding. In AIDS patients with a CD4 count less than 100 cells/uL, positive EBV viral load in the CSF, and lesions consistent with AR-PCNSL by nuclear imaging, biopsy of such high-risk lesions may be relatively contraindicated. In such cases, diagnosis of AR-PCNSL can usually be established based on the results of these established noninvasive studies (Fig. 2).

In 1998, the American Academy of Neurology issued recommendations to guide diagnostic evaluation of AIDS patients with ring-enhancing lesions (1998). However, this algorithm does not include more recent molecular diagnostics or flow cytometry, which is sensitive for detecting B-cell malignancies in the CSF. Furthermore, this and other algorithms include a trial of empiric antibiotics for toxoplasmosis, which is no longer advisable given diagnostic advances and dramatically improved survival of patients with AIDS in the HAART era. An alternative algorithm for patients with AIDS and enhancing CNS masses that takes into account these new findings is proposed in Fig. 2. It should be noted that evaluation in some patients, for example, those with inconclusive imaging and laboratory studies in which brain biopsy is felt to be dangerous, can be challenging and the best approach will require balancing various risks. Also, this algorithm should be considered a suggested guide, and readers should be alert for new developments or new official guidelines in this area.

AR-PCNSL and other CNS infections occurring in patients with AIDS can be associated with a rapid deterioration of functional and neurologic

function. Patient performance status should be assessed using an Eastern Cooperative Oncology Group (ECOG) performance scale. Cognitive function should be monitored using serial scoring of the Mini-Mental State Examination (MMSE). Given the rapid and sometimes irreversible decline in performance status in patients with untreated AR-PCNSL or untreated CNS infections and improved long-term outcomes in AIDS patients treated with HAART, expedited evaluation is crucial. Long-term neurologic function, quality of life, and survival are likely affected by the course of action taken during the initial diagnostic evaluation (Abrey et al. 2005).

Treatment and Prognosis

The advent of effective combination antiretroviral therapy, also known as highly active antiretroviral therapy (HAART), heralded a new era for HIV-infected patients. The control of HIV replication and associated immune reconstitution leads to a decrease in deaths from opportunistic infections and dramatically improved overall survival for many AIDS patients. Studies of outcomes for HIV-related diseases are usually divided between the pre-HAART era (before 1996) and the era when HAART became broadly available in the USA and Europe (after 1996). Among populations with access to HAART, one of the great advances has been the prevention of malignancies occurring in the setting of very low CD4 counts, such as AR-PCNSL. However, despite improvement in overall survival attributable to HAART (Hoffmann et al. 2001), patients who do develop AR-PCNSL still have an overall mortality close to 90% at 2 years (Norden et al. 2011).

HAART is a necessary part of the treatment of AR-PCNSL, and immune reconstitution is considered the main driver of this effect. The control of HIV viremia allows for T-cell immune reconstitution and improved immunosurveillance against this immunogenic, virally associated malignancy. In rare cases, HAART alone has even been associated with complete resolution of PCNSL, presumably due to improved T-cell function.

Pilot and retrospective studies (Hoffmann et al. 2001) suggest further improvements in outcomes may be possible in this patient population with lymphoma-directed therapeutics and appropriate supportive care that includes prophylaxis and treatment of opportunistic infections and in some cases, short courses of steroids to manage neurologic symptoms. However, delayed diagnosis, heterogeneous approaches to diagnosis, poor performance status, and infectious or malignant comorbidities may be significant additional risk factors that affect overall survival. Unlike systemic non-Hodgkin lymphoma in patients with AIDS, for which dramatic improvements in overall survival have been achieved through prospective therapeutic studies, advances in AR-PCNSL have been limited by the lack of prospective studies. As such, there is no consensus on how to treat AR-PCNSL.

The major definitive treatment modalities for PCNSL are whole-brain radiotherapy (WBRT), chemotherapy, and the anti-CD20 monoclonal antibody, rituximab. However, there are no completed prospective lymphoma-directed studies that have been performed in patients with AR-PCNSL in the HAART era. With the exception of HAART, which is indicated based on evidence showing improved overall survival, therapeutic interventions for AR-PCNSL are based on case series and expert opinion or extrapolation from studies performed in immune-competent patients with PCNSL.

In AR-PCNSL, radiotherapy has been the predominant treatment modality since the beginning of the AIDS epidemic. Radiation has good activity against PCNSL. However, focal radiotherapy is associated with high recurrence rates and hence whole-brain radiotherapy (WBRT) is recommended. Even WBRT is unlikely to treat leptomeningeal disease outside the radiation field. In retrospective studies performed in the pre-HAART era, median overall survival with WBRT alone was less than 6 months; death was generally from either other complications of AIDS or from progressive PCNSL. A more recent study performed in the HAART era has demonstrated a 3-year survival of 64% in patients with pathologically confirmed AR-PCNSL treated

with HAART and WBRT (>30 Gy). Doses of WBRT (~40 Gy) recommended for better and more durable disease control, however, are associated with debilitating and at times life-threatening neurotoxicity (Correa et al. 2004). Therefore, radiation-sparing approaches to AR-PCNSL are highly desirable.

High-dose methotrexate, either alone or in combination regimens, is the best-studied radiation-sparing approach to PCNSL in patients without HIV. However, many regimens evaluated in PCNSL in immune-competent patients also contain high-dose cytarabine, which adds substantial toxicity and may not be appropriate for patients with AIDS. A pilot study of high-dose methotrexate 3 g/m² performed in ten patients with histologically confirmed AR-PCNSL in the pre-HAART era (Jacomet et al. 1997) demonstrated a complete response rate of 30%; however, median overall survival was only 2 months. Further evaluation of radiation-sparing approaches is required in the HAART era.

Rituximab, a monoclonal antibody directed against CD20, is an important immunotherapy that has been evaluated in the treatment of PCNSL and PTLD and is a rational agent in AR-PCNSL, in which 100% of lymphoma cells express CD20. One challenge has been determining whether a therapeutic concentration rituximab can be obtained in the central nervous system. Pharmacokinetic studies have shown that CSF levels are approximately 0.1–1% that of serum during peripheral administration. Though this may appear to be an insufficient concentration, it represents one to ten times more than needed to saturate 80–95% of CD20-positive cell surface. Furthermore, tumor-associated breakdown of the blood-brain barrier likely allows for increased accumulation at the site of the tumor. Indeed tumor accumulation of yttrium-90-labeled anti-CD20 antibodies can be demonstrated in a majority of patients with PCNSL.

Clinically, a retrospective study examined the addition of rituximab to high-dose methotrexate and ifosfamide on outcomes in newly diagnosed PCNSL in HIV-negative patients. The addition of rituximab increased the CR rate (100% vs. 68.4%; $p = 0.02$) and 6-month progression-free survival

(94.1% vs. 63.2%; $p = 0.04$) (Birbaum et al. 2012). A subsequent prospective multicenter study evaluating rituximab in combination with high-dose methotrexate and temozolomide had a 63% complete response rate. Patients went on to receive consolidation therapy with high-dose cytarabine and etoposide, and mature results from this study are forthcoming.

Overall, the results of trials in HIV-uninfected patients with PCNSL suggest that radiation-sparing modalities can achieve good results. While patients with AR-PCNSL may be more susceptible to toxicities from such regimens because of their severe underlying acquired immunodeficiency, such approaches are appropriate to consider at the HAART era. However, there are no completed studies of radiation-sparing approaches with HAART in AR-PCNSL. Curative-intent, radiation-sparing treatment of AR-PCNSL is currently under investigation in a prospective study evaluating rituximab, high-dose methotrexate, and HAART in patients with AR-PCNSL (NCT00267865).

Conclusion

AR-PCNSL is a rare AIDS-defining malignancy with a high mortality. The advent of the widespread distribution of HAART has led to a substantial decline in incidence and a modest improvement in overall survival. For AIDS patients who develop AR-PCNSL, diagnostic as well as therapeutic challenges exist. A streamlined evaluation of CNS masses (Fig. 2) may lead to earlier diagnoses and treatments and improved outcomes. All patients should be started on HAART and, given the lack of a standard therapy, should also receive curative-intent lymphoma-directed therapy, ideally within a clinical trial.

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Anal Cancer

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Definition

Anal cancer is a squamous cell cancer of the anal canal and perianal region that is very similar biologically to cervical cancer in terms of its

association with oncogenic human papillomavirus (HPV), particularly HPV 16. Like cervical cancer, anal cancer occurs most often in a squamocolumnar transformation zone and is preceded by squamous intraepithelial lesions. The incidence of anal cancer is considerably higher in HIV-infected men and women than in the general population and has increased since the introduction of highly active antiretroviral therapy. Now one of the most common cancers occurring in HIV-positive men and women, and unlike most other cancers occurring in the HIV-positive population, anal cancer is also potentially preventable through primary prevention (vaccination against HPV) and secondary prevention (screening for and removal of high-grade anal squamous intraepithelial lesions).

Introduction

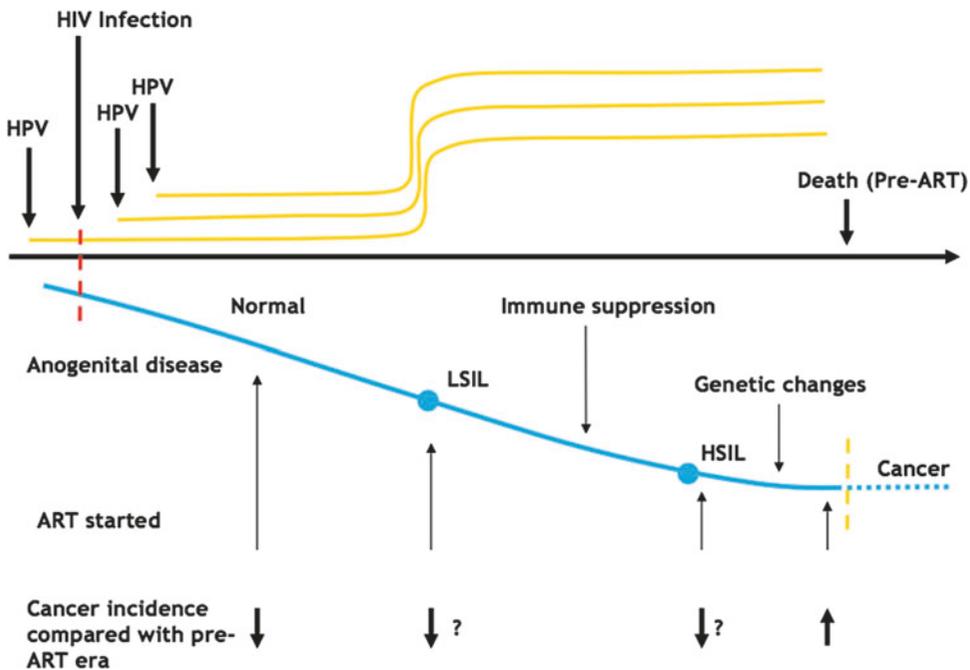
Recently, malignancies have become one of the most common causes of mortality in HIV-infected men and women. Unlike the most common HIV-associated malignancies, Kaposi's sarcoma and non-Hodgkin's lymphoma, the incidence of anal cancer has continued to increase among HIV-infected individuals well after the introduction of highly active antiretroviral therapy (HAART) (Fig. 1). HIV infection may also accelerate the natural history of anal cancer, for which the mean age of diagnosis among HIV-infected individuals is lower than among HIV-uninfected individuals. Among the more common malignancies occurring in HIV-infected individuals, anal cancer has one additional distinguishing feature – it is likely to be preventable.

Given the biological similarity between cervical and anal cancer and the well-established success of cervical cancer prevention programs, it is possible that anal cancer may similarly be preventable. Like cervical cancer programs, anal cancer prevention programs may take the form of primary prevention or secondary prevention. Primary prevention consists of prevention of infection with the underlying etiologic agent, HPV, through prophylactic vaccination. Secondary prevention efforts are focused on those who have

already been exposed to HPV and who have developed anal high-grade squamous intraepithelial lesions (HSIL), also called high-grade anal intraepithelial neoplasia (HGAIN). Identification of anal HSIL and removal of that lesion through a variety of methods is performed in an effort to reduce the risk of progression of that lesion to cancer. Secondary prevention has been shown to work very well to prevent cervical cancer and is the basis of cervical cytology screening programs, with colposcopy and biopsy to diagnose cervical HSIL, and ablate it to reduce the risk of progression to cervical cancer. A similar approach to prevention of anal cancer has been advocated by several experts in the field (Palefsky 2009). Although it seems probable that adaptation of cervical techniques to the anal canal would be successful to reduce the risk of anal cancer, there are several challenges, particularly in HIV-infected individuals. These include large lesions, multifocal lesions, high incidence of new lesions, and high recurrence rate after treatment. Well-designed clinical trials to demonstrate the efficacy of this approach have not yet been done and are urgently needed.

Incidence of Anal Cancer in HIV-Positive Men and Women

Anal cancer is biologically similar to cervical cancer in several ways. Like cervical cancer, anal cancer is associated with HPV infection, primarily HPV 16. The anal canal has a transformation zone at the anorectal junction that is similar to the main target of HPV infection in the cervix, the cervical transformation zone. At both of these anatomic sites, HPV infection leads to a series of epithelial changes that reflect various stages of HPV infection and HPV-associated transformation. HSIL includes loss of epithelial differentiation and abnormal mitotic activity and is considered to be the precursor lesion to both cervical and anal cancer. Natural history studies show that the time from HPV infection to development of HSIL is relatively short and usually occurs within just a few years. It would therefore appear that the long latency period to development of



Anal Cancer, Fig. 1 Relationship between highly active antiretroviral therapy (HAART) initiation, anal squamous intraepithelial lesions, and incidence of anal cancer. Data suggest that immune response plays an important role in preventing development of low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL). HSIL that persists accumulates genetic changes over time that are not reversible by antiretroviral therapy-induced immune reconstitution. With sufficient time and accumulation of cancer-inducing genetic changes, these lesions may progress to cancer. In the pre-HAART era, the incidence of anal cancer among HIV-infected MSM was modestly higher than among HIV-uninfected MSM. In the pre-HAART era, MSM may have died of competing causes before they had sufficient time to progress from HSIL to cancer, and this may have limited the number of cases of cancer that developed. Since the introduction of ART, the incidence of anal cancer has increased among HIV-infected MSM. Initiation of

HAART if the patient has no disease or LSIL at either a low or high CD4 level may prevent progression to HSIL and may lead to reduced incidence of anal cancer compared to those who initiate HAART after having HSIL for a long period of time. The incidence of anal cancer may or may not be higher in this group compared with the pre-HAART era. Initiation of ART if the patient has early HSIL at either a low or high CD4 level may induce regression of HSIL and may or may not lead to reduced incidence of anal cancer compared to those who initiate ART after having HSIL for a long period of time. The incidence of anal cancer may or may not be higher in this group compared with the pre-HAART era. Initiation of ART after HSIL has persisted for several years may not induce lesion regression whether the CD4 level is high or low, and the longer survival time in the absence of ASIL screening will allow the incidence of anal cancer to increase compared with the pre-HAART era

anal cancer primarily reflects the long period of time required for HSIL to progress to cancer.

Several studies have shown that anal HPV infection in women is as common or more common than cervical HPV infection (Machalek et al. 2012). Given the ease in which women acquire anal HPV infection, it is not surprising that in the general population anal cancer occurs more commonly among women than men. The incidence of anal cancer in the general population

has been rising by about 2% among both men and women. With a nearly 60% rate of anal HPV infection among HIV-uninfected men who have sex with men (MSM), it is also unsurprising that the incidence of anal cancer in this group of men is much higher than men with no history of receptive anal intercourse. The rate of anal HPV infection is even higher among HIV-infected MSM, and this group has the highest incidence of anal cancer of all.

Given the many competing causes of mortality prior to the advent of HAART and the long latency period typically required for HSIL to progression to anal cancer, the incidence of anal cancer was only modestly increased among HIV-infected MSM compared with HIV-uninfected MSM. When HAART was introduced, it was hoped that it would have the same beneficial effect on anal cancer incidence as on KS and NHL, reflecting immune reconstitution and improved immune response to the etiologic agents. However, it quickly became clear that individuals with prevalent HSIL were not undergoing regression to normal after initiating effective HAART, HPV infection was not being cleared, and the incidence of HSIL was continuing unabated. Combined with the increasing survival due to fewer competing causes of mortality and absence of organized screening or prevention programs for anal cancer, the incidence of anal cancer has increased, not decreased, in the HAART era (Shiels et al. 2012). Several HIV-anal cancer database matches showed an incidence of anal cancer of 70/100,000 or more. More recent data from the NA-ACCORD study show an incidence of 131/100,000 HIV-infected MSM (Silverberg et al. 2002) rendering the incidence of anal cancer well above the highest incidences of cervical cancer anywhere in the world. Recent data from the Swiss Cohort study suggest that the increase may be leveling off.

It is not clear whether the incidence of anal cancer will continue to increase at the same rate in the future or whether it may level off or even decrease. There are several competing factors that will ultimately determine the incidence of anal cancer in HIV-infected men and women. A factor that may reduce the incidence of anal cancer in the future is vaccination to prevent anal HPV infection with HPV 16 and HPV 18. However, it will be several decades before any reduction in the incidence of anal cancer due to vaccination is seen. Current uptake of the vaccine among males is very low, and the full benefit of the vaccine may take even longer to realize unless vaccination uptake is improved. Another factor that may lead to a lower incidence of anal cancer in the future compared with current rates is

changes in practice regarding higher threshold CD4 levels for initiation of HAART. We hypothesize that immune response may be useful to control HPV replication and progression from HPV infection to HSIL. Those whose immune systems are less damaged by HIV might have a lower incidence of HSIL and ultimately a lower incidence of anal cancer. Although not consistent from study to study, some have shown that men and women started on HAART have more clearance of existing HPV infection and lower incident of HSIL. With the current clinical standard of practice moving increasingly toward initiating HAART earlier in the course of HIV infection and at higher CD4 levels, it is possible that this will have a long-term beneficial effect in reducing incident HSIL and cancer. Unfortunately most HIV-infected individuals were initiated on HAART at CD4 levels well below current guidelines, and they may not benefit from the earlier initiation of HAART described above. As described previously, aging of the HIV-infected population may also contribute to an increase in the burden of anal cancer in the future.

Pathogenesis of Anal Cancer

Consistent with their shared etiologic association with HPV, anal cancer and cervical cancer are both preceded with a series of intraepithelial changes ranging from low grade to high grade. Low-grade changes are associated with few signs of cell transformation but instead primarily reflect cytopathic changes due to high levels of HPV replication. These changes are not believed to be precancerous. Low-grade anal squamous intraepithelial lesion (LSIL) is the most common form of ASIL associated with a wide variety of HPV types, both oncogenic and non-oncogenic (Hoots et al. 2009). Condyloma acuminatum is most often associated with HPV 6 or 11, but in HIV-infected individuals, a high proportion may be coinfecting with HPV 16 or 18. Rather than reflect coinfection of individual cells with more than one HPV type, these probably reflect separate foci of clinically subtle HSIL, with HPV 6 or 11 causing the condyloma and HPV 16 causing

the HSIL. HSIL is believed to be the true cancer precursor of anal cancer, similar to the role of cervical HSIL as the precursor to cervical cancer. Unlike anal LSIL, a high proportion of anal HSIL contain oncogenic HPV types. Infection with multiple HPV types is particularly common in HIV-infected individuals, but it is relatively uncommon to detect only non-oncogenic HPV types in these lesions. The rate at which these lesions progress to cancer is unknown and likely varies highly from person to person. Since the mean age at which HIV-infected individuals develop anal cancer is lower than the general population, it is likely that the time of progression from anal HSIL to invasive cancer is shorter in this group.

The mechanisms that trigger invasion of the underlying basement membrane are not known. Ongoing HPV oncogenic protein expression, particularly E6 and E7, is necessary for maintenance of the transformed phenotype. Induction of angiogenesis may play a role in progression to cancer, although it is not clear if it is the cause or effect. The role of HIV in the process is also not known. HIV proteins may be present in the epithelial microenvironment of HIV-infected individuals, even if they have well-controlled HIV viral loads on HAART. An inflammatory microenvironment may also be present in HIV-infected individuals, in which there may be elevations of cellular immune response proteins such as tumor necrosis factor-alpha and interferon-gamma. These proteins may be potentiating the oncogenic effects of HPV proteins, but their exact role is still unclear.

Primary Prevention of Anal Cancer Through HPV Vaccination

The quadrivalent (qHPV) and bivalent HPV vaccines have both been shown to be highly effective at preventing persistent cervical HPV infection and high-grade cervical squamous intraepithelial lesions (CSIL) due to the types of HPV in the vaccines. The quadrivalent vaccine is also effective at preventing genital warts in women due to HPV 6 or 11. Recent studies have shown early

evidence of effectiveness at the population level. Australian sexually transmitted infection clinics have reported a reduction in the proportion of women presenting with genital warts (Read et al. 2011), and in the United States, the proportion of women with high-grade CSIL that contain HPV 16 or 18 has declined (Powell et al. 2012). Based on these and other data, the quadrivalent and bivalent HPV vaccines were approved for routine use in females aged 9–26 years.

Recently the qHPV vaccine was shown to be effective at reducing the incidence of external genital warts in HIV-uninfected heterosexual men and MSM (Giuliano et al. 2011). In a sub-study of anal canal HPV infection and anal canal anal squamous intraepithelial lesions (ASIL), the qHPV vaccine was also effective at reducing intra-anal persistent infection with vaccine HPV types and anal HSIL due to vaccine types (Palefsky et al. 2011). Based on these and other data, qHPV was approved for routine use in males aged 11–21 years. Vaccination is also approved for routine use in MSM and immunosuppressed males aged 22–26 years and is approved but not for routine use in non-immunosuppressed males aged 22–26 years.

Although studies of HPV vaccination to prevent anal HPV infection, ASIL, and anal cancer have not been done in women, the qHPV vaccine was approved for this indication in women based on similarity of anal cancer between men and women and the data from the Merck 020 protocol in men. The bivalent vaccine has not been studied for prevention of anal cancer, and the qHPV vaccine is the only vaccine approved for prevention of anal cancer in men or women. However, in a post hoc analysis of Costa Rican women vaccinated with the bivalent vaccine to prevent cervical HPV infection, CSIL, and cervical cancer, there was a reduction in infection with HPV 16 and 18 in the anal canal of vaccinated women (Kreimer et al. 2011). These data provide encouragement that the bivalent vaccine may reduce the risk of ASIL and cancer in addition to CSIL and cervical cancer but studies are needed to demonstrate this.

HIV infection is not a contraindication to HPV vaccination, and given their high risk of anal HPV

infection and ASIL, HIV-infected men and women of vaccine-eligible age should be vaccinated. Several studies have shown that vaccination in HIV-infected men and women is safe (Wilkin et al. 2010). Nearly all HIV-infected individuals seroconvert in response to vaccination regardless of CD4 level. Titers may be lower in HIV-infected people and similarly aged HIV-negative individuals, but nearly all have titers well above those seen after natural HPV infection. Studies of the efficacy of HPV vaccination to prevent anogenital disease in HIV-infected individuals have not yet been reported, and the duration of protection is unknown.

The qHPV and bivalent vaccines are preventive vaccines and work to prevent initial HPV infection. Consequently HPV vaccination is most effective among those who have not been exposed to vaccine types previously, i.e., those who are naïve to a given HPV type, as defined by being DNA negative and seronegative to that type. Studies have shown that despite a large number of previous sexual partners, a high proportion of HIV-infected MSM over the age of 26 years are HPV DNA negative and seronegative to vaccine HPV types and would be considered “naïve” to these types (Wilkin et al. 2010). However, we speculate that many men who would currently be classified as “naïve” to HPV 16 or HPV 18 were previously seropositive to these types and sero-reverted to negative, a process that has been demonstrated to occur over time in healthy women.

Despite the high rate of “naivete” to vaccine types and incidence of HPV infection in HIV-positive MSM over the age of 26 years, vaccination of HIV-infected individuals over the age of 26 years is controversial. If these men were previously seropositive, the value of vaccination is not clear, and studies are needed to determine if vaccination affords these men the same protection that they would have received if they were truly naïve.

Secondary Prevention of Anal Cancer

In cervical cancer secondary prevention programs, cervical cytology, and, to an increasing

extent, adjunctive tests such as HPV DNA or RNA are used to identify women at risk of high-grade CSIL. The next step in the evaluation of these women is to visualize the cervix and vulvovaginal epithelium to localize the source of abnormal cells on cytology or positive HPV test, using a technique known as colposcopy. With the aid of the magnification provided by the colposcope and topically applied solutions such as 5% acetic acid or iodine-based Lugol’s solution, areas likely to be lesional are targeted for biopsy. Histologically confirmed high-grade CSIL is then removed using techniques such as loop electro-excision procedure or cryotherapy, depending on the setting. There is good evidence that high-grade ASIL is the precursor to anal cancer, and it is likely, although unproven, that removal of anal HSIL will reduce the incidence of anal cancer. Given the similarity between ASIL and CSIL, methods to identify those with anal HSIL are largely based on those used for cervical screening (Palefsky 2012). To identify anal HSIL, a technique known as high-resolution anoscopy (HRA) can be performed, in which patients are examined under magnification with a colposcope and with topical solutions such as 5% acetic acid or Lugol’s iodine to identify areas of ASIL visually. A biopsy of visible lesions is then performed for histologic confirmation.

Individuals at high risk of anal cancer can be considered for testing to identify anal HSIL (Palefsky 2009). These include HIV-infected men and women, regardless of mode of HIV acquisition, HIV-uninfected MSM, those with perianal HPV-related lesions, women with a history of high-grade vulvar squamous intraepithelial lesions and vulvar and cervical cancer, and those who are immunosuppressed due to causes other than HIV. In the interest of minimizing morbidity, consideration should be given to screening only after the age of 30 years given the low incidence of anal cancer among those younger than 30 years of age.

Similar to cervical screening, there remains room for improvement in screening for anal HSIL. The most direct screening method is to perform HRA. However, HRA requires extensive training and experience, and for optimal results an

interdisciplinary team is needed that includes an anoscopist, pathologist, surgeon, and counselor/educator. Currently the number of clinicians performing HRA is limited given the extensive infrastructure and training required, and there are too few well-trained clinicians performing this technique to allow it be used as a true screening tool.

One of the more easily performed screening tools that may identify individuals who would benefit from HRA is anal cytology. Anal cytology is performed in a manner similar to cervical cytology, but unlike cervical cytology, it may also be performed by patients themselves. Those with abnormal cytology are then referred for HRA. Like cervical cytology, the sensitivity of anal cytology is limited, and it tends to under-call the grade of lesion shown on HRA-guided biopsy. Given the high proportion of HIV-infected individuals expected to have abnormal anal cytology, the grade of cytology may also be used to triage patients. Ultimately all patients with any abnormality, including atypical squamous cells of undetermined significance (ASC-US), should be considered for HRA. However, the positive predictive value for anal HSIL on cytology is highest for those with anal HSIL on cytology, followed by those with atypical squamous cells – cannot rule out high-grade lesion (ASC-H) and low-grade squamous intraepithelial lesions (LSIL). Cost-effectiveness studies have shown that HIV-infected MSM should be screened annually with anal cytology if their cytology is normal, and that HIV-uninfected MSM should be screened every 2–3 years (Goldie et al. 1999). Although there are fewer data for the other at-risk groups, at the University of California San Francisco, we recommend similar screening intervals for these groups according to their HIV status.

HPV testing is being used in the cervix as an adjunct to cervical cytology or as a primary screening test to identify women who should have cervical colposcopy. Given the limitations of anal cytology, some have advocated anal HPV testing to identify those who should have HRA. However, given the high prevalence of oncogenic HPV in HIV-infected patients, HPV testing may be more useful for its negative predictive

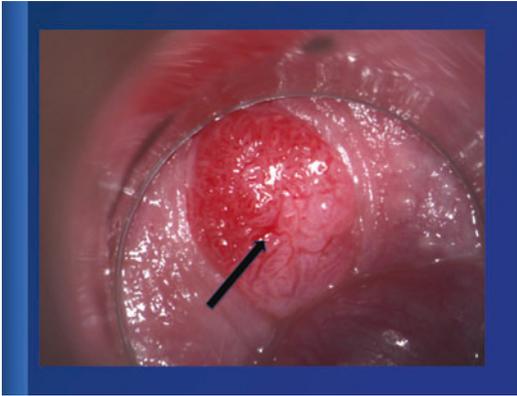
value. Further studies are needed to define the best use of HPV-based tests.

Once identified on biopsy, every effort should be made to ablate or remove the lesion with the primary goal of reducing the risk of progression to cancer. However, to date no clinical trials have been performed that define the efficacy of anal HSIL treatment to reduce the incidence of anal cancer. Currently all clinicians who choose to screen for and treat anal HSIL are doing so based on the similarity between anal cancer and cervical cancer and the proven efficacy of treatment of cervical HSIL to prevent cervical cancer. Studies to determine if this approach is effective are urgently needed.

Another goal of anal HSIL treatment is relief of symptoms. In the perianal region, anal HSIL may cause bleeding, burning, or itching, and patients may experience psychological distress due to its cosmetic appearance. Anal low-grade squamous intraepithelial lesions (LSIL) including condyloma acuminatum may also cause these symptoms. Unlike HSIL, LSIL is not believed to be precancerous. While treatment of LSIL will not likely reduce cancer risk, it is reasonable to treat it for symptom relief.

Diagnosis of Anal Cancer

Anal cytology and HRA-guided biopsy are primarily aimed at identifying anal HSIL, these should therefore be considered to be methods of anal “precancer” screening. In contrast, digital anorectal exams (DARE) with palpation for intra-anal and perianal masses is a key element of anal cancer screening. Some anal cancers may also be detected at HRA (Fig. 2). Like cervical cancer, survival after treatment of anal cancer correlates inversely with the stage of diagnosis. Five-year survival after treatment of stage I disease is as high as 70% and declines to about 20% after diagnosis of stage IV disease. In the absence of any organized anal cancer or HSIL screening program, diagnosis of anal cancer is often made when the patient presents with new anal pain that is not explained by any other obvious source of pain such as hemorrhoids, fissures, or infections.



Anal Cancer, Fig. 2 Large area of anal cancer, as seen after application of 5% acetic acid at high-resolution anoscopy, indicated by *black arrow*. Cancerous lesion is raised and has clear borders, erythema, and atypical vessels

Lesions such as HSIL are usually painless, and when patients develop pain, it may be a sign of progression to cancer that is involving pain nerve fibers. Patients may also present with new patterns of bleeding, discomfort upon defecation or anal intercourse, or rapid growth of a mass. In those areas where active screening of HSIL is in place, a new category of anal cancer is being diagnosed, i.e., very early cancers that are too small to be palpable on DARE and which are asymptomatic. These cancers are detected only on biopsy at HRA and are suspected when ulcers or highly atypical blood vessels are noted. Occasionally these may be unsuspected, with the appearance of otherwise unremarkable HSIL. This new category of anal cancer was adopted as part of the Lower Anogenital Squamous Terminology Project of the College of American Pathologists and American Society for Colposcopy and Cervical Pathology, called “Superficially Invasive Squamous Cell Carcinoma of the Anus” (SISCCA). SISCCA is defined as a lesion with less than 3 mm in depth and 7 mm in width (Darragh et al. 2012).

Treatment of Anal HSIL

Treatment for anal HSIL generally falls into two categories: (1) local treatment with clinician – or

patient-applied creams or liquids and (2) clinician-applied ablative techniques such as electrocautery, laser, or infrared coagulation (IRC) and surgery. The choice of treatments will vary with the preference of the clinician, the clinical setting, the size and number of lesions, and the location of the lesions.

Treatment of Perianal HSIL

For smaller lesions (less than 1 cm in diameter) that are also limited in number (less than 3), 85% trichloroacetic acid (TCA) or liquid nitrogen or a combination of the two may be used. These modalities may be reapplied up to four times at 2–3-week intervals, and if not successful, a different modality should be tried. Some clinicians would use hyfrecation in the office or infrared coagulation. Studies in Europe have shown modest success with imiquimod for treatment of anal HSIL, but HIV-positive patients may not respond as well to imiquimod as HIV-negative patients since the mechanism of action is immune mediated through toll-like receptors. Like other topical treatments, imiquimod has not been approved by the US Food and Drug Administration for treatment of ASIL. Recently a phase I study showed that topical cidofovir is safe for treatment of perianal HSIL and showed signs of modest efficacy that should be confirmed in phase II/phase III studies. An additional option for extensive perianal disease is application of 5-fluorouracil (5-FU) cream. This drug has been used topically for many years to treat vulvar and vaginal SIL. It may be used to reduce the size lesions to permit treatment of remaining lesional areas with more targeted therapies such as IRC or electrocautery. Its toxicities include local pain, inflammation, and ulceration, which can range from mild to severe.

In general, surgery is reserved for treating those with the most extensive disease, those who require an examination under anesthesia to permit biopsies large enough to definitely exclude invasive cancer, or rarely, treatment of complications of office-based procedures such as bleeding or infection. Larger perianal HSIL often requires more aggressive approaches in the setting of the operating room, such as IRC, electrocautery, laser, and surgical excision with skin flaps.

Treatment of Intra-anal HSIL

Treatment approaches for intra-anal HSIL are similar to those described for perianal HSIL but there are fewer options. Smaller lesions may be treated with 85% TCA, but most require IRC, electrocautery, or laser surgery. A high proportion of larger lesions may be treated with IRC, although the recurrence rate is high, as is the development of new lesions in areas that were not treated. A multicenter phase I safety study indicated that the efficacy of IRC to treat individual HSIL was about 65% within a year, with up to three treatments, similar to results of previously published retrospective chart reviews. Electrocautery appears to have similar safety and efficacy to IRC. Recently a randomized controlled trial of 156 patients with ASIL comparing imiquimod, topical 5-fluorouracil, and electrocautery showed that of the three modalities, electrocautery was the most effective for intra-anal lesions. However, each modality was associated with a high risk of lesion recurrence (Richel et al. 2013). There was little difference between the modalities for treatment of perianal ASIL. As with perianal HSIL, the most extensive disease may require surgical excision and/or fulguration in an operating theater. When performed in conjunction with HRA, surgery was effective to treat extensive anal HSIL in a retrospective chart review, particularly when combined with post-surgery IRC to treat remaining lesions or early recurrences.

Treatment of Anal Cancer

Treatment of anal cancer is based on the stage of the disease. Stages 1, 2 and 3 are typically treated with combined modality therapy (CMT) consisting of 5-fluorouracil and mitomycin, with radiation therapy. The National Comprehensive Cancer Network (NCCN) recommends 5-FU IV on days 1–4 and 29–32 with mitomycin IV on days 1–29. A minimum of 45 Gy radiation therapy is given over 5 weeks with an additional 9–14 Gy considered for patients with T3, T4, or node-positive disease or for those with residual disease after the initial 45 Gy. At some centers, intensity modulated radiation therapy is used

instead of 3-D conformal radiation in an effort to reduce radiation-associated toxicity. Cisplatin has been assessed in place of mitomycin, but clinical trials have shown insufficient benefit to recommend that it replace mitomycin as the first-line chemotherapeutic agent in combination with 5-FU (Gunderson et al. 2012).

However, cisplatin may be useful as first-line therapy with 5-FU for patients who are expected to be intolerant of the hematologic toxicity associated with mitomycin-based regimen.

Prior to the advent of HAART, HIV-infected patients had poor survival rates for anal cancer with many unable to tolerate a full regimen of CMT. In recent years, however, HIV-infected patients with good HIV control on HAART and high CD4 levels have been shown to tolerate full-dose therapy and have similar survival rates to HIV-negative individuals. The current recommendation is to treat HIV-infected patients with the standard regimen, although careful monitoring for toxicity is required and treatment breaks may be needed.

The primary role for surgery in the treatment of anal cancer is abdominoperineal resection for patients who fail CMT. In addition, local excision may be used to treat anal margin/perianal cancer provided that the surgery does not compromise anal sphincter function. With the recent recognition of SISCCA as a very early stage of invasive cancer that may develop in the perianal region or anal canal, there is substantial interest in determining whether SISCCA can be safely treated with wide local excision regardless of where in the anus it is diagnosed. Clinical trials will be needed to determine if this can become standard of care. If it does, then efforts to identify anal cancer at this very early stage will be important to effect a cure while minimizing the risk of toxicity associated with CMT.

Conclusion

Anal cancer is a problem of growing importance among HIV-infected men and women, and its incidence may continue to rise as an increasing number of HIV-infected men and women reach advanced age. Efforts need to be made to diagnose anal

cancers as early as possible given the improved morbidity and mortality outcomes associated with earlier diagnosis. It is possible that routine performance of simple techniques such as DARE will lead to earlier anal cancer diagnosis, as will performance of HRA in some at-risk individuals. Despite the high likelihood of surviving anal cancer when detected early, prevention is highly desirable given the high rates of morbidity associated with successful treatment of anal cancer.

Fortunately, unlike many other cancers that occur in HIV-infected men and women, many cases of anal cancer are potentially preventable through primary prevention in the form of vaccination against HPV 16 and 18. However, the impact of vaccination on anal cancer incidence will not be seen for several decades given the time required for those of vaccination age to reach the age at which anal cancer is typically diagnosed, and the high proportion of at-risk individuals who were exposed to HPV before vaccination became available. Further, the potential for vaccination to reduce anal cancer incidence is somewhat mitigated by relatively low vaccine uptake in the targeted age groups, especially in boys.

For those who have already been exposed to HPV, secondary prevention in the form of anal cytology screening and treatment of anal HSIL diagnosed on HRA-guided biopsy may be the best approach to reducing their risk of anal cancer. However, randomized, controlled trials of HSIL treatment to reduce the incidence of anal cancer are needed to assess the risk-benefit ratio of this approach before screening in at-risk populations will become standard of care. In the long term, reduction of the incidence of anal cancer will likely be achieved through all three approaches – DARE, anal screening for and treatment of anal HSIL, and HPV vaccination.

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Anatomic Compartments as a Barrier to HIV Cure

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Definition

Anatomic compartments as a barrier to HIV cure: organs, tissues, or tissue cells that allow persistence of HIV despite immune responses and/or antiretroviral therapy

Introduction

Anatomic compartments other than the blood represent a major site of HIV persistence on combination antiretroviral therapy (ART) and a major obstacle to efforts directed at eradication of HIV.

Though ART can block viral replication and reduce plasma HIV RNA levels below the detection limit of commercial assays (“suppression”), it does not eradicate HIV, which rebounds rapidly after interruption of ART in the vast majority of patients. Despite years of suppressive ART, HIV can usually be detected as low levels of HIV RNA in the plasma, higher levels of cell-associated HIV DNA and RNA in blood and tissues, and infectious virus in latently infected CD4⁺ T cells from the blood, which are widely regarded as a major barrier to the eradication of HIV.

However, compelling evidence also suggests that HIV persists in anatomic compartments outside of the blood (Svicher et al. 2014), which represent another barrier to HIV eradication. One of the main reasons that the tissues are an obstacle to eradication is that in both untreated and ART-treated individuals, most HIV-infected cells reside in the tissues. HIV primarily infects activated CD4⁺ T cells, but activated CD4⁺ T cells home to and are more abundant in the lymphoid tissues and gut. In addition, almost all organs contain CD4⁺ T cells, and while <2% of the body’s CD4⁺ T cells are found in the blood, most of the body’s CD4⁺ T cells reside in the gut and the lymphoid tissues. Moreover, CD4⁺ T cells from some tissues (such as gut) are more susceptible to HIV infection *in vitro*, and cell-to-cell spread in the tissues may enhance viral replication or decrease the efficacy of antiretrovirals. Consequently, infected cell frequencies are higher in certain tissues, such as gut and lymph nodes, than in the blood. In discussing HIV-infected cells and organs, some authors use the term “reservoir” to refer to a significant site of infection, while others reserve it specifically for HIV-infected tissues or cells that persist despite long-term suppressive ART (likely due to long life span or proliferative renewal) or more specifically for those that can produce virus or infectious virus. Using the second definition, the main tissue reservoirs are probably the gut, lymph nodes, spleen, genital tract, brain, lung, and kidney (Table 1).

A second major barrier to eradication posed by the tissues is that the distribution of infected cell types may differ between the tissues and blood. Aside from infecting CD4⁺ T cells, HIV can

Anatomic Compartments as a Barrier to HIV Cure, Table 2 Evidence for HIV or SIV infection in cells other than CD4+ T cells

Tissue and cell type	In vitro infection	Untreated HIV+				Treated HIV+					
		HIV DNA	HIV RNA	Infectious virus	HIV DNA	HIV RNA	Infectious virus	Protein			
Bone marrow CD34+ progenitor cells	X	X									
Bone marrow CD133+ progenitor cells								X			
Bone marrow eosinophil precursors	X										
Bone marrow mast cell progenitors	X										
Placental mast cells								X			X
Bone marrow CD4- lymphocytes			X								
Thymocytes (CD3- CD4- CD8-, CD4 + CD8+, TCR $\alpha\beta$ +, TCR $\gamma\delta$ +, CD3- CD16+)	X										
Lung CD8+ T cells		X									
Bone marrow monocytes or macrophages	X	X				X					
Lymph node macrophages		X				X		X (viral particles)			
Thymic macrophages						X (SIV)		X			
Thymic multinucleated giant cells											
Splenic macrophages		X				X					
Liver Kupffer cells or macrophages	X					X (SHIV)		X (SIV; protein and viral particles)			
Peritoneal macrophages	X										
Gut macrophages	X	X				X		X (protein)			X

Brain mononuclear cell/macrophages		X		X		X (protein and viral particles)	X (monocytoid cells)		
Brain multinucleated giant cells				X		X (protein and viral particles)			
Brain microglia	X			X		X			
Lung alveolar macrophages	X			X			X		
Renal macrophages				X (SIV, SHIV)					
Male urogenital macrophages	X			X		X		X (SIV)	
Lymph node follicular dendritic cells	X		X	X		X (protein and viral particles)	X (SIV, HIV)	X	
Gut follicular dendritic cells						X (protein)			
Testicular and epididymal dendritic cells				X (SIV)		X (SIV)			
Epidermal Langerhans cells	X			X		X	X		
Vaginal Langerhans cells						X (subtype E)			
Thymic epithelial cells	+/- (some studies but not others); can take up virus; productive infection in one study					X (SCID-hu mice)			
Gut epithelial cells	X			X		X			
Renal glomerular and tubular epithelial cells	Nonproductive (uptake and transmission)	X		X				X (3 mo. of ART)	X (viral particles)
Prostate epithelial cells						X (one but not other studies)			
Vaginal epithelial cells	Nonproductive (uptake and transmission)								
Mammary epithelial cells	Nonproductive (endosomal uptake and transmission)								

(continued)

Anatomic Compartments as a Barrier to HIV Cure, Table 2 (continued)

Tissue and cell type	In vitro infection	Untreated HIV+				Treated HIV+			
		HIV DNA	HIV RNA	Protein	Infectious virus	HIV DNA	HIV RNA	Protein	Infectious virus
Endothelial cells	Tissue specific: +brain (productive), + (liver sinusoidal), +/- (umbilical vein), - (lung)		X (brain)	X (LN, brain)					
Thymic stromal cells					X (SIV)				
Bone marrow stromal fibroblasts	X								
Gut submucosal mesenchymal cells	X								
Lung fibroblasts	X								
Cardiac myocytes		? (one report)							
Gut enterochromaffin cells		X							
Brain neurons	X		X	X					
Brain glial cells	X								
Brain astrocytes	X		X	X					
Brain oligodendroglia			X						
Testicular germ cells		X				X (some studies but not others)			
Sperm	X; uptake by EM; transmission to CD4+ T cells and oocytes	X (some studies but not others)	X (some studies but not others)	X (viral particles)					

enter or infect many other cell types (Alexaki et al. 2008), including other hematopoietic cells, endothelial cells, epithelial cells, mesenchymal cells, and germ cells (Table 2). Some cell types constitutively produce free virus (“productive” infection), while others (including some CD4+ T cells and possibly mast cell progenitors or astrocytes) produce virus only upon stimulation (“latent” infection), and other cell types do not produce free virus but can internalize viral particles and transmit infection to neighboring cells. In ART-treated individuals, CD4+ T cells are almost certainly the largest and most important reservoir, but some evidence also suggests that other cell types may be reservoirs, including hematopoietic/mast cell progenitors, T memory stem cells, tissue macrophages, dendritic cells, and possibly astrocytes or microglial cells.

A third main barrier posed by the tissues is that certain tissues may harbor virus that differs in sequence from that in the blood, which may contribute to phenotypic differences such as cell tropism, resistance to ART, or evasion of immune responses. The terms “compartment” and “compartmentalization” have been used to refer to organs or cell types containing viruses that differ significantly in sequence and/or phenotype from viruses found in the blood. The organ with the greatest evidence for compartmentalization is probably the brain, followed by male and female genital tracts and possibly the lung, kidney, liver, gut, and lymph nodes (Table 1). A fourth potential barrier posed by the tissues is that immune responses to HIV may differ in certain tissues (such as brain and testes) compared to blood. Fifth, penetration of antiretroviral drugs may be reduced in certain tissues (including brain, spleen, lymph nodes, gut, and genital tissues) compared to blood. Finally, these differences between the blood and tissues in the number and phenotype of infected cells, viral variants, immune responses, and drug levels suggest that mechanisms of HIV persistence may vary between the tissues and blood and that the tissues may respond differently to therapies aimed at treating or eradicating HIV.

The following sections review HIV infection by organ system, including the nature of the infected cell types, evidence for infection in

untreated and (where it exists) ART-treated individuals, evidence for compartmentalization, and evidence for differences in immune responses or ART levels. This entry concludes with a discussion of the cell and anatomic origins of the low-level residual plasma virus that persists on ART and the virus that rebounds after interruption of ART.

Lymph Nodes

In untreated as well as ART-treated patients, the lymph nodes (LN) are one of the largest reservoirs of infected cells. HIV infection has long been associated with lymphadenopathy and pathologic changes in the LN (Wood 1990). In 1986, HIV proteins and viral particles were detected in the germinal centers of LN in follicular dendritic cells (FDCs) and the extracellular network of immune complexes, leading to the first suggestion that FDCs may be a reservoir for antigen (Tenner-Racz et al. 1986) and may spread virus to new T cells or nodes (Spiegel et al. 1992). Though one study did not find convincing evidence of viral replication in sorted FDCs (Tenner-Racz et al. 1994), human FDC can be productively infected *in vitro*, and FDCs from infected rhesus monkeys show budding virus and can infect donor cells (Sprenger et al. 1995). Moreover, HIV particles have been detected between FDCs and in endosomes and the cytoplasm of FDCs, where they could escape recognition by CD8 + T cells (Tacchetti et al. 1997). One early study detected p24 in high endothelial cells of the paracortical postcapillary venules and in mononucleated cells of paracortex and germinal center (Baroni and Pezzella 1987), while another study found HIV RNA in FDCs, lymphocytes, and rare macrophages but not endothelial cells (Spiegel et al. 1992). In Thai patients infected with HIV subtype E, Langerhans cells and Langerhans-related dendritic cells appear to harbor most HIV RNA and protein in LN (Bhoopat et al. 2006). Several studies also suggest that the location of the virus may also change over the course of the disease (Tenner-Racz et al. 1989; Fox et al. 1991; Hurtrel et al. 1994) and that the LN may contain

large numbers of latently infected CD4⁺ T cells (Embretson et al. 1993; Peng et al. 1995).

In untreated humans and primates, the LN are infected early and contain a particularly large burden of HIV. After mucosal infection of rhesus monkeys, LN are seeded within 3 days of infection, prior to systemic viremia (Whitney et al. 2014). In a postmortem autopsy study of multiple tissues from 16 AIDS patients, HIV DNA was detected most often in LN (100%) and spleen (100%), followed by lung (93.8%) and colon (87.5%) (Sei et al. 1994). Several studies have suggested that the LN contain substantially higher copies of HIV RNA and DNA than the blood (Chun et al. 1997). One study reported that the HIV RNA level in the LN was 110-fold greater than in blood, which was attributed to higher CCR5 and CXCR4 expression in the LN (Nokta et al. 2001). The burden of HIV in the LN may also affect pathogenesis (Beer et al. 1996) and disease progression (Chakrabarti et al. 1994; Pantaleo et al. 1994).

Despite suppressive ART, HIV DNA and RNA persist in the LN. Though 3-drug therapy results in an exponential 3-log decrease in HIV RNA in LN, with a half-life only slightly longer than the blood (6 vs. 1.9 days) (Lafeuillade et al. 1996), HIV RNA and DNA can still be detected in the LN after years of ART (Wong et al. 1997a; Gunthard et al. 2001; Lafeuillade et al. 2001). One study also detected abundant amounts of HIV p24, p17, and gp120/gp41 in the germinal centers of LN after 5–13 months of suppressive ART, although HIV RNA was not detected in this study (Popovic et al. 2005). Several studies of ART-treated macaques have demonstrated that viral DNA and/or RNA levels are highest in LN and spleen (North et al. 2010; Horiike et al. 2012; Kline et al. 2013). In one study, SIV RNA levels in LN and spleen decreased much less than in plasma and gut, which was attributed to lower drug levels in these secondary lymphoid tissues (Bourry et al. 2010). Studies from humans also suggest that levels of some antiretrovirals may be low in LN (Cohen 2011).

It is unclear whether there is compartmentalization of virus between LN and blood. Some studies found differences between LN and blood

in viral sequences and drug resistance mutations (Haddad et al. 2000; Gunthard et al. 2001), while other studies have shown that HIV sequences in lymphoid tissues are similar to blood (Ball et al. 1994; van't Wout et al. 1998).

Spleen

Several studies from ART-treated primates suggest that the spleen is among the organs with the highest burden of residual infection. HIV DNA can be detected in CD4⁺ T cells and macrophages of spleens removed from HIV⁺ patients (McIlroy et al. 1996) and autopsied spleens of patients who die from AIDS (van't Wout et al. 1998), while HIV RNA can be detected in splenic macrophages of rhesus monkeys infected with SHIV (Igarashi et al. 2001). In one study of ART-suppressed, RT-SHIV-infected macaques, the highest levels of viral RNA and DNA were detected in the spleen, lymph nodes, and gut (North et al. 2010). In another study of macaques treated with ART after peak viremia, SIV RNA persisted in the spleen and lymph nodes despite significant decreases in the gut, and levels of lamivudine and indinavir were much lower in spleen compared to gut (Bourry et al. 2010).

Bone Marrow

HIV can infect various cells in the bone marrow, and HIV DNA can be detected in the bone marrow of ART-treated individuals. CD34⁺ progenitor cells (Folks et al. 1988), eosinophil precursors (Freedman et al. 1991), stromal fibroblasts (Scadden et al. 1990), and stromal macrophages (Gill et al. 1996) from human bone marrow can be infected with HIV-1 *in vitro*, leading to the first suggestion that the bone marrow may be a reservoir for HIV (Folks et al. 1988). During acute infection of rhesus macaques, bone marrow samples from days 3–14 showed SIV infection in marrow monocyte/macrophages of all animals, whereas low numbers of infected marrow CD3⁺ T cells were detected in some animals (Mandell et al. 1995).

In untreated HIV+ humans, infectious virus has been cultured from the bone marrow, although the primary source appeared to be the nonadherent fraction containing CD4+ T cells (McElrath et al. 1989). HIV DNA has been detected in different bone marrow populations, including CD4+ T cells, monocytes, CD4– lymphocytes, and rarely CD34+ progenitor cells (von Laer et al. 1990; Davis et al. 1991; Stanley et al. 1992) but not colony-forming cells of the granulocyte–macrophage lineage (Davis et al. 1991). In another study, HIV RNA was detected in bone marrow biopsies of 6/37 individuals, but localized to cells of the macrophage lineage (Weiser et al. 1996).

In humans on suppressive ART, HIV has been detected in the marrow but it is unclear whether progenitor cells constitute a reservoir. In ART-suppressed individuals, three studies detected HIV in cells from the bone marrow, but two studies did not detect HIV DNA in CD34+ progenitor cells (Durand et al. 2012; Josefsson et al. 2012), while another detected HIV DNA in CD133+ bone marrow progenitor cells from 6/11 patients (McNamara et al. 2013). Bone marrow mast cell progenitors can be latently infected with HIV, and one study detected infected mast cells in the placenta of ART-treated women (Sundstrom et al. 2007), although another study found no evidence of infected mast cells in multiple other organs (Nelson et al. 2009).

Thymus

Many studies in untreated individuals have demonstrated that HIV and SIV can infect different cells in the thymus. As early as 1987, HIV antigens were detected in the thymus of AIDS patients in cells that included multinucleated giant cells (Pekovic et al. 1987). In vitro, HIV can infect different human thymocyte populations (De Rossi et al. 1990; Schnittman et al. 1990) and possibly thymic epithelial cells (Numazaki et al. 1989a, b; Braun et al. 1996).

In untreated SIV-infected primates, viral DNA and RNA have been detected in the thymus (Baskin et al. 1991), and infectious virus can be

cultured from thymic tissue (Baskin et al. 1991), including thymic stromal cells (Muller et al. 1994); in one study, the main infected organs at 2 weeks were the thymus and spleen (Lackner et al. 1994). In untreated humans, HIV DNA (Mano and Chermann 1991), RNA (Burke et al. 1995) and infectious virus (Mano and Chermann 1991; Calabro et al. 1995) have also been detected in thymic tissue, and several studies found tissue-specific variants in the thymus (Calabro et al. 1995; Scinicariello et al. 2010). Thymic infection may also play a role in evolution of CXCR4-tropic virus (Salemi et al. 2007), disease progression (Rosenberg et al. 1994), and CD4 depletion or reconstitution on ART (Douek et al. 1998).

However, little evidence exists to support persistence of HIV or SIV infection in the thymus of ART-suppressed individuals. In one study of ART-suppressed macaques, neither SIV DNA nor replication-competent virus was detected in the thymus, although SIV DNA was detected in spleen and LN, and infectious virus was isolated from LN (Shen et al. 2003). In another study of SHIV-infected macaques on suppressive ART, Gag RNA was detected in the thymus in only 1 of 6 animals, and multiply spliced HIV RNA was not detected in the thymus (Deere et al. 2014).

Liver

Studies in untreated individuals have shown that HIV can infect cells in the liver, including Kupffer cells. Human liver sinusoid endothelial cells and macrophages express CD4 (Scoazec et al. 1990), and both can be infected with HIV-1 in vitro, while human Kupffer cells can be productively infected in vitro (Schmitt et al. 1990a, b). In rhesus monkeys infected with SHIV or SIVmac251, SHIV RNA can be detected in liver macrophages (Igarashi et al. 2001), and SIV protein as well as lentiviral particles can be detected in hepatic Kupffer cells and lymphocytes; in animals with advanced disease, Kupffer cells were the main cell type with HIV protein (Persidsky et al. 1994). In humans, one study detected HIV in the liver of some patients who died of AIDS (van't

Wout et al. 1998), while a second detected HIV RNA in 9/16 HIV+ participants (Blackard et al. 2011); the latter study also found evidence of compartmentalization between plasma and liver.

Gastrointestinal Tract

The gut may be among the earliest targets of HIV infection and one of the organs with highest numbers of infected cells, even in ART-treated individuals. It has long been recognized that HIV infection is associated with gastrointestinal symptoms, and as early as 1988, infectious virus was cultured from the gut of AIDS patients (Mathijs et al. 1988; Nelson et al. 1988). *In vitro*, HIV can productively infect colorectal carcinoma cell lines (Adachi et al. 1987), human ileal and colonic epithelial cells (Moyer et al. 1990), and submucosal mesenchymal cells (Moyer and Gendelman 1991). Intestinal epithelial cells express CCR5 and can transfer CCR5-tropic virus to other cells (Smith et al. 2003). The gut also contains a large proportion of the lymphoid tissue (up to 85%) and lymphocytes (up to 90%) in the body (Cerf-Bensussan and Guy-Grand 1991; Mowat and Viney 1997). Compared to CD4+ T cells from the blood, primary gut mucosal CD4+ T cells show increased susceptibility to *in vitro* infection with HIV (Lapenta et al. 1999; Poles et al. 2001a) and support higher levels of viral replication (Anton et al. 2000; Poles et al. 2001a), which has been attributed to greater CCR5 expression (Lapenta et al. 1999; Anton et al. 2000), T cell activation (Lapenta et al. 1999), and expression of CTLA-4. Human jejunal macrophages can also be infected with HIV and can produce virus, although they produce 2–3 logs less p24 than blood monocytes (Smith et al. 1997).

In untreated HIV+ patients, HIV has been detected in multiple different gut cell types. HIV RNA has been detected on cells that appeared to be enterochromaffin cells (Mathijs et al. 1988; Nelson et al. 1988; Levy et al. 1989), macrophages (Fox et al. 1989; Heise et al. 1993), giant cells (Heise et al. 1993), T lymphocytes (Fox et al. 1989; Heise et al. 1993), jejunal villus

enterocytes (Heise et al. 1991), and gastric epithelial cells (Liu et al. 2013). HIV proteins have been detected in gastric epithelial cells (Liu et al. 2013) and in duodenal and rectal cells (Ullrich et al. 1989; Jarry et al. 1990), including lymphocytes, macrophages, dendritic cells, and, in two patients, rectal gland cells that could be epithelial cells or immune cells (Jarry et al. 1990). Esophageal Langerhans cells are depleted in patients with stage 4 disease, suggesting that they also may be targets for HIV (Charton-Bain et al. 1999). In ART-suppressed patients, HIV DNA and RNA have been detected in gut CD4+ T cells and cells other than CD4+ T cells (Yukl et al. 2013), including CD13+ myeloid cells (Yukl et al. 2014); HIV DNA and p24 have also been detected in duodenal macrophages (Zalar et al. 2010).

Studies in untreated humans or primates have suggested that HIV infection of the gut occurs earlier and to a greater extent than infection in the blood or even the lymphoid tissues. In HIV-infected patients, CD4+ T cell depletion in the gut precedes that in the blood and is more pronounced (Lim et al. 1993; Schneider et al. 1995). In SIV-infected rhesus macaques, profound and rapid (within 7 days) loss of CD4+ T cells was observed in the lamina propria of the small and large bowel, prior to CD4+ T cell depletion in the blood, lymph nodes, or spleen, and more SIV RNA+ cells were observed in the intestine than in lymph nodes (Veazey et al. 1998). One study found that levels of HIV p24 were much higher in intestinal biopsies compared to serum, although HIV DNA (normalized to total cells) did not appear to differ between the intestine and blood (Fackler et al. 1998). In contrast, a study of patients with acute/early infection demonstrated that colonic CD4+ T cells harbor on average 13 times more HIV DNA and 10 times more HIV RNA than CD4+ T cells in peripheral blood (Mehandru et al. 2007), and a study of chronically infected patients found that HIV DNA levels are higher in the rectum compared to blood (d’Ettorre et al. 2011).

In ART-treated patients, multiple studies have documented persistence of HIV in the gut, and some have suggested differences from blood in responses to ART, levels of HIV-infected cells,

and mechanisms of persistence. One early study showed that ART did not seem to reduce the frequency of detection of HIV DNA, RNA, or infectious virus from rectal biopsies (Di Stefano et al. 2001). Another study found that early initiation of ART resulted in comparable reduction in HIV RNA in blood and rectum at 6 months, although clearance of HIV DNA was substantially reduced in rectum (Tincati et al. 2009). Two other studies detected HIV RNA in the gut (duodenum or rectum) of some ART-suppressed patients and found that HIV levels were not substantially different from those of untreated patients (Belmonte et al. 2007; Lafeuillade et al. 2009). Poles et al. (2001a, b) showed that HIV RNA and DNA levels in rectal biopsies appeared stable over 1 year of ART and calculated that the gut contained two times more HIV DNA+ cells than the blood (Poles et al. 2006). In patients suppressed on ART for up to 10 years, Chun et al. reported that HIV DNA levels per million CD4+ T cells were on average five to six times higher in the ileum compared to blood (Chun et al. 2008). A subsequent study of four different regions of the gastrointestinal tract determined that levels of HIV DNA and unspliced HIV RNA per million CD4+ T cells were higher (up to 12-fold) in all four gut sites compared to blood; differences in average transcription and the relation to immune activation suggested that different mechanisms control HIV persistence in the blood and gut (Yukl et al. 2010a). Based on the average level of HIV DNA across the four gut sites, this study estimated that the gut harbors 1.2×10^9 infected CD4+ T cells, or 83–95% of all HIV-infected cells in the body. Another study of 1–2 drug ART intensification suggested that the ileum, but not blood or other gut sites, might be a site of ongoing replication in some patients on ART (Yukl et al. 2010b). Finally, a recent study of RT-SHIV-infected, ART-suppressed macaques showed that gut tissues had the highest levels of multiply spliced HIV RNA and the ratio of multiply spliced to Gag RNA, while Gag RNA tended to be highest in the mesenteric lymph nodes (Deere et al. 2014).

Evidence suggests decreased penetration of certain antiretrovirals into the gut (Cohen 2011),

but it is unclear whether there is compartmentalization between virus in the gut and blood. Several studies have suggested compartmentalization between gut and blood (Poles et al. 2001a, b; Katzenstein et al. 2010; Lewis et al. 2013) and even between different regions of the gut (van Marle et al. 2007; McElrath et al. 2013), while other studies found no compartmentalization between gut and blood (Chun et al. 2008; Avettand-Fenoel et al. 2011; Imamichi et al. 2011).

Nervous System

HIV can infect different cell types in the brain and nervous system, and multiple studies suggest compartmentalization from blood. The frequent association between HIV infection and neurologic impairment has been recognized since the early days of the epidemic, and as far back as 1985, infectious HIV was cultured from cerebrospinal fluid (CSF) and brain of HIV+ men (Ho et al. 1985; Levy et al. 1985). In vitro, HIV can infect many different types of brain cells, including astrocytes (Rytik et al. 1991; Tornatore et al. 1991) and fetal neural cells (Harouse et al. 1989); productive infection has been demonstrated in fetal glial cells (Christofinis et al. 1987), human brain microglia (Watkins et al. 1990), and human brain capillary endothelial cells (Moses et al. 1993). One study found that astrocytes could not be productively infected (Sharpless et al. 1992), while another found that human fetal brain astroglial cells produce virus for a few days, but then revert to a latent state that can be reactivated to produce virus (Tornatore et al. 1991). Astrocyte infection has also been associated with disruption of the blood–brain barrier (Eugenin et al. 2011).

Infectious virus has been cultured from the CSF, brain, spinal cord, and peripheral nerves of untreated patients (Ho et al. 1985; Levy et al. 1985; Gabuzda et al. 1986); in one case, monocytoïd cells from the brain produced virus for as long as 100 days (Gartner et al. 1986). HIV DNA has been detected in the brain of AIDS patients (Pang et al. 1990), and HIV RNA has

been detected in brain macrophages, microglia, giant cells, astrocytes, oligodendroglia, and rarely neurons (Stoler et al. 1986; Rostad et al. 1987). One study detected HIV RNA and protein in brain capillary endothelial cells, mononuclear inflammatory cells, giant cells, and (in one patient) astrocytes and neurons (Wiley et al. 1986), while in other studies, HIV protein localized primarily to monocyte/macrophages (Gabuzda et al. 1986) and cells of the macrophage/microglial lineage (Kure et al. 1990). Several studies found that astrocytes harbored HIV nucleic acids and Nef or Rev protein but no Gag/Env (Saito et al. 1994; Tornatore et al. 1994) or rare mRNA for Gag/Pol (Ranki et al. 1995), suggesting a nonproductive infection limited to early regulatory genes, while Env protein could also be detected in macrophages and multinucleated giant cells.

One early study noted that isolation of infectious HIV from CSF did not correlate with isolation of virus from serum, suggesting that the brain may be a site of preferential viral replication (Hollander and Levy 1987). Many other studies have reported compartmentalization of HIV infection in the brain from that in the blood (Koyanagi et al. 1987; Cheng-Mayer and Levy 1988; Chiodi et al. 1989; Korber et al. 1994; Wong et al. 1997b; Strain et al. 2005; Harrington et al. 2009; Schnell et al. 2009). Moreover, HIV antibody levels and activity differ between the CSF and serum (Diederich et al. 1988).

HIV DNA and RNA have also been detected in the brain of ART-treated individuals. In one study, ART reduced the CSF HIV RNA to or near the limit of detection in nearly all patients, although the viral decay in CSF was slower than that in plasma (Spudich et al. 2005). However, HIV RNA has been detected in the CSF of patients on suppressive ART (Spudich et al. 2006; Kumar et al. 2007; Canestri et al. 2010), and both HIV DNA (Smit et al. 2004) and HIV RNA (Langford et al. 2006; Kumar et al. 2007) have been detected in brain tissue obtained at autopsy from individuals on ART. In two of these studies, viruses from brain (Smit et al. 2004) and CSF (Canestri et al. 2010) contained drug resistance mutations not found in the blood, further suggesting

compartmentalization in the nervous system. Finally, HIV persistence in the brain is suggested by several small case series of patients presenting with new neurologic symptoms (including encephalitis) associated with rebound of CSF virus despite up to 8 years of suppressive ART (Bingham et al. 2011; Peluso et al. 2012).

Lung

HIV DNA and RNA have been detected in lung cells from both untreated and ART-suppressed individuals, and limited evidence suggests compartmentalization from blood. *In vitro*, human embryo lung fibroblasts (Dolei et al. 1994) and alveolar macrophages can be infected with HIV (Coffey et al. 1997). In untreated individuals, HIV has been detected in lung CD8 + T cells (Semenzato et al. 1995) and macrophages obtained by bronchoalveolar lavage (BAL) (Sierra-Madero et al. 1994), and infection of alveolar macrophages has been associated with impairments in phagocytic and/or proteolytic function (Jambo et al. 2014; Cribbs et al. 2015). Another study detected HIV DNA in cells from BAL in 86% of HIV+ patients and found that levels of cell-free HIV RNA in BAL could be higher than serum, particularly in patients with opportunistic lung infections (Koziel et al. 1999). In one autopsy study of AIDS patients, HIV DNA was detected in the lung tissues and Env sequences showed compartmentalization from blood (van't Wout et al. 1998). HIV DNA and RNA have also been detected in alveolar macrophages from some ART-treated patients (Cribbs et al. 2015).

Vascular System and Heart

HIV can also enter endothelial cells from some tissues. *In vitro*, HIV can infect endothelial cells from human brain (productive) (Moses et al. 1993) and liver sinusoids (Steffan et al. 1992), but not lung (Kanmogne et al. 2001); umbilical vein cells are less permissive to infection (Lafon et al. 1993) but can

harbor HIV DNA and transmit infectious virus to CD4+ T cells (Scheglovitova et al. 1993). Human endothelial cells can enhance HIV replication in CD4+ T cells by 50,000-fold and can promote HIV replication in minimally activated (Choi et al. 2005) or resting CD4+ T cells (Shen et al. 2013). In untreated patients, one study detected HIV p24 in high endothelial cells of lymph node postcapillary venules (Baroni and Pezzella 1987), while another found no evidence of HIV RNA in lymph node endothelial cells (Spiegel et al. 1992). HIV DNA has also been detected in cardiac tissue obtained at autopsy from some patients with AIDS, and the infected cells resembled cardiac myocytes (Grody et al. 1990).

Kidney and Urine

HIV-infected cells can be found in the kidney, and limited evidence suggests compartmentalization as well as persistence on ART. HIV can enter HK2 cells and primary human renal proximal tubular epithelial cells in vitro, and these cells can transmit infectious virus to CD4+ T cells (Hatsukari et al. 2007; Mikulak et al. 2009). In rhesus macaques infected with SHIV or SIVmac251, viral RNA can be detected in renal macrophages (Igarashi et al. 2001), and SIV DNA can be detected in cultured primary kidney cells, which do not produce free virus but can transmit infectious virus to lymphoid cell lines (Lena and Luciw 2001). In small case series of untreated HIV-infected humans, HIV DNA and RNA were detected in renal glomerular and tubular epithelial cells as well as infiltrating leukocytes (Bruggeman et al. 2000; Marras et al. 2002). In two patients, HIV DNA Env sequences from infected renal epithelial cells showed clustering from viral sequences in the blood (Marras et al. 2002), and in one patient, HIV RNA was detected in tubular epithelial cells and glomerular podocytes after 3 months of suppressive ART (Winston et al. 2001). In one study of ART-suppressed individuals, HIV DNA was detected in the urine of 23% of patients (Chakrabarti et al. 2009). In another study of 19 ART-suppressed patients who

received renal transplants, HIV nucleic acids and viral particles were detected in podocytes and tubular cells of renal biopsies, and HIV DNA and RNA were detected in the urine (Canaud et al. 2014).

Male Reproductive Tract

HIV can infect different cells in the male reproductive tract, and some but not all studies suggest compartmentalization from the blood. HIV particles have been detected in the cell-free semen of AIDS patients at up to 10^8 particles/ml, levels higher than the plasma, leading to the suggestion that the male reproductive tract may be a reservoir (Borzy et al. 1988). Viral-like particles have also been detected in the prostate and testicle (Lecatsas et al. 1985) and in spermatozoa (Baccetti et al. 1991) of AIDS patients. HIV antigens have been detected in the testis, epididymis, and prostate of some AIDS patients in lymphocytic/monocytic cells, macrophages (Pudney and Anderson 1991), and, in one study (da Silva et al. 1990) but not others, rare positive foci that were thought to be degenerating germ cells and prostate glandular epithelial cells. HIV DNA has been detected in seminal mononuclear cells and in germ cells at all stages of differentiation (Muciaccia et al. 1998a, b; Shevchuk et al. 1998), including sperm (Bagasra et al. 1994), while HIV RNA (genomic, multiply spliced) has been detected in spermatogonia, spermatocytes, and rare spermatids and macrophages (Nuovo et al. 1994).

In macaques, SIV RNA and protein have been detected in the testes, epididymis, prostate, and seminal vesicles (Miller et al. 1994; Shehu-Xhilaga et al. 2007; Le Tortorec et al. 2008) within 14 days of infection; infected cells included macrophages, dendritic cells, T cells, germ cells (including spermatogonia), and, in one animal, Langerhans cells in the foreskin. Interestingly, immune responses varied by tissue and the testes showed little evidence of inflammation (Le Tortorec et al. 2008). HIV antibody levels are lower in semen compared to blood (Wolff et al. 1992), and testicular SIV-specific CD8+ T cells have reduced cytokine responses to

mitogens (Winnall et al. 2015), suggesting local immunosuppression in the testis.

In humans and primates on suppressive ART, several studies have demonstrated that HIV and/or SIV can still be detected in genital tissues and secretions. Despite suppression of HIV RNA in plasma, HIV RNA can be detected in the semen in 2–48% of men (Lafeuillade et al. 2002; Marcelin et al. 2008; Sheth et al. 2009; Halfon et al. 2010; Politch et al. 2012), sometimes in association with drug resistance mutations (Lafeuillade et al. 2002). Similarly, a subset of ART-treated, SIV-infected macaques continued to shed SIV in semen despite suppression in the plasma (Matusali et al. 2015). In this study, ART resulted in large decreases in SIV RNA in the prostate and vas deferens, smaller decreases in SIV RNA in the epididymis and seminal vesicle, and no decrease in SIV RNA in the urethra, where SIV RNA⁺ macrophages could be detected. However, ART had little to no effect on SIV DNA in any genital tissue, suggesting the presence of nonproductively infected cells.

It is unclear whether seminal fluid HIV RNA comes from genital tissues or blood, but multiple studies have suggested a local source and/or compartmentalization from plasma (van't Wout et al. 1998; Paranjpe et al. 2002; Smith et al. 2004; Coombs et al. 2006), suggesting a distinct reservoir. In contrast, one study in macaques showed that SIV RNA sequences were evenly distributed between the blood, LN, and genital tract (Fieni et al. 2013).

Female Reproductive Tract

HIV DNA and RNA can be detected in female genital secretions from untreated and ART-treated women, and some but not all studies suggest compartmentalization from the blood. The endometrial epithelial cell line HEC-1A can endocytose viral particles and infect underlying cells (Saidi et al. 2007), suggesting that epithelial cells may be involved in transmission. In vaginal biopsies of untreated Thai women infected with HIV subtype E, HIV p24 was detected in all patients; infected cells included Langerhans cells, Langerhans-related dendritic cells, and T lymphocytes (Bhoopat et al. 2001).

HIV can be detected in female cervical cells (Iversen et al. 2004) and genital secretions (Kovacs et al. 2001) of most untreated patients. In addition, HIV can be detected in genital secretions of 8–87.5% women on suppressive ART (Kovacs et al. 2001; Nunnari et al. 2005; Launay et al. 2011; Wahl et al. 2011; Fiscus et al. 2013), suggesting that some women may have a separate reservoir of HIV in the genital tract, perhaps due to reduced penetration of certain antiretrovirals (Taylor and Davies 2010; Else et al. 2011). Multiple studies have found evidence for compartmentalization between blood and female genital organs (Overbaugh et al. 1996; Tirado et al. 2004; Chomont et al. 2007; Bull et al. 2009), although another study found that compartmentalization in drug resistance mutations was not sustained over time (Kelley et al. 2010).

Breast and Breast Milk

It has long been known that HIV can be transmitted through breast feeding, though it is unclear whether HIV-infected cells in the breast milk arise from blood or breast tissue. Human mammary epithelial cells can internalize viral particles and transmit infection to activated CD4⁺ T cells (Dorosko and Connor 2010). Breast milk CD4⁺ T cells produce more HIV virus than CD4⁺ T cells from blood (Becquart et al. 2006), and the frequency of resting CD4⁺ T cells that can be induced to express HIV antigen is higher in breast milk than blood (Petitjean et al. 2007). In another study using breast milk from both untreated and ART-treated women, breast milk CD4⁺ T cells showed spontaneous expression of HIV antigens and could be induced by activation to produce supernatant HIV RNA (Valea et al. 2011), suggesting that spontaneous reactivation of latently infected breast milk CD4⁺ T cells may allow HIV transmission despite ART. Short courses of ART have been shown to reduce cell-free and cell-associated HIV RNA, but not HIV DNA, in breast milk (Lehman et al. 2008). One study of breast milk Env sequences demonstrated limited or no compartmentalization from plasma (Salazar-Gonzalez et al. 2011).

Skin and Adipose Tissue

In untreated HIV+ individuals, spliced HIV RNA (Giannetti et al. 1993), HIV antigens, and viral particles (Rappersberger et al. 1988) have been detected in epidermal Langerhans cells, though one study found equivocal evidence for HIV in these cells (Kalter et al. 1991). HIV DNA has been detected in the epidermis (Dusserre et al. 1992) and dermis, and virus has been cultured from skin (Rappersberger et al. 1988). A recent study also detected HIV DNA in CD4+ T cells from adipose tissue of ART-treated individuals (Couturier et al. 2015).

Origin of the Residual Virus and Rebound Virus

Two very important questions in understanding HIV persistence are the cellular and anatomic origins of the low-level plasma virus that persists on ART and the virus that rebounds after interruption of ART. One study found that the residual plasma HIV RNA sequences were rarely found in resting CD4+ T cells in blood, raising the question of whether the residual virus arises from other cell types or tissues, or whether it may have arisen from resting CD4+ T cells not sampled (Bailey et al. 2006). Some studies have suggested that the rebound virus arises from blood cells (Imamichi et al. 2001), while other studies have shown that in some patients, the rebounding plasma HIV RNA was distinct from sequences found in circulating latently infected cells (Chun et al. 2000; Zhang et al. 2000) and closest to virus in lymphoid tissues (Zhang et al. 2000). Another study found that the rebounding plasma HIV RNA did not match HIV DNA sequences found in a limited number of gut biopsies, although it is difficult to exclude an origin from elsewhere in the gut (Lerner et al. 2011). In one recent study, multiple foci of HIV RNA+ cells were detected in the gut or lymph nodes in half of the participants (often in more than one tissue) as soon as plasma HIV RNA was detectable, and sequence analysis of the blood revealed a large number of rebounding/founder viruses, suggesting the rebound virus arises simultaneously from many

cells at multiple sites (Rothenberger et al. 2015). However, the observation that different viral clones dominate in rebounding plasma virus during successive treatment interruptions (Joos et al. 2008) would require that, in the face of multicentric viral reactivation, stochastic or incompletely understood selective forces favor outgrowth of one or another viral population. It is unclear whether there is a dependence on the anatomic origins of individual clones and how well those particular tissues can amplify local viral populations.

Conclusion

Tissue reservoirs of HIV in the setting of suppressive ART are important both for their size and the diversity of the latently and chronically infected cell populations found in different sites, but their assessment is hampered by limited access to many tissues. Important differences in types of infected cells, viral genetic composition, local immune surveillance, drug levels, and mechanisms of persistence in different tissues are suggested by some studies but require confirmation and perhaps more systematic study. The development of new tools and approaches to quantify and characterize these tissue reservoirs would greatly facilitate development of future strategies for HIV eradication and permit more comprehensive assessment of responses to interventions aimed at cure.

Cross-References

- ▶ [Immunological Responses to Antiretroviral Therapy](#)
- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [Inhibition of HIV-1 Spread: Cell-Free Versus Cell-Cell](#)
- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [Macrophage-Specific Aspects of HIV-1 Infection](#)
- ▶ [Overview of HIV CNS Infection](#)
- ▶ [SIVmac Infection of Macaques, Immunopathogenesis of](#)
- ▶ [Thymic Function](#)
- ▶ [Transcription \(Initiation, Regulation, Elongation\)](#)

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bNAbs and have a greater magnitude of the intratype antiviral activity of ADCMI.

Introduction

Many HIV-2-infected patients are able to control the progression of the infection more effectively than most HIV-1-infected subjects. In many HIV-2-infected patients, the viral load remains below limits of detection and CD4 T-cell counts remain normal; therefore, the disease progression may take decades (Marlink et al. 1994). Although HIV-1 and HIV-2 share several biological features, such as the ability to bind to CD4 molecules, T-cell and mononuclear cell tropism, and a high degree of genetic variability (Huang et al. 1991), the two viruses have differences in immunogenicity and evolution. Most HIV-2-infected patients generate high titers of broadly neutralizing antibodies (nAbs), whereas this is relatively rare in HIV-1 infection. This could be due to structural differences of the envelope (Env) between HIV-1 and HIV-2 and their effect on the antibody responses, consequently their impact on Env evolution, control of viral replication, and clinical outcome.

Similar to HIV-1 infection, there is a correlation between B-cell activation and depletion with CD4 cell loss in HIV-2 infection (Marcelino et al. 2008). However, in HIV-2-positive patients, polyclonal activation and total IgA antibody production do not increase (Marcelino et al. 2008). Furthermore, in HIV-2 infection, most of the IgA-producing B cells are activated in the intestinal lymphoid tissue and the production of total and HIV-2-specific IgA functions at a normal level, suggesting that in HIV-2 infection the gastrointestinal immune system is not as severely infected as in HIV-1 infection (Gordon et al. 2007).

In HIV-2 infection IgG1 and IgG2 are the predominant responding antibody subclasses (Marcelino et al. 2008). In most chronically HIV-2 patients, IgG1, IgG3, and IgA responses are generated against gp36 and the C2V3C3 region (Marcelino et al. 2008). The C2V3C3 region of HIV-2 envelope can exert an

Antibody Response to HIV-2

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Definition

Neutralizing antibodies (nAbs) inhibit or neutralize the infectiveness of a virus or bacteria. **Broadly neutralizing antibodies (bNAbs)** are neutralizing antibodies, which are able to neutralize multiple strains of HIV. **Antibody-dependent complement-mediated inactivation (ADCMI)** is a cell mediated mechanism whereby an effector cell actively lyses a target cell, which has been bound by a specific antibody. It is one of the mechanisms through which antibodies control HIV infection. Compared to HIV-1-infected patients, more HIV-2-infected subjects develop

immunosuppressive activity on CD4 and CD8 T cells (Cavaleiro et al. 2007), which may result in the lower rates of immune activation and CD4 cell loss in most HIV-2-infected patients.

Neutralizing Antibody (nAb) Responses to HIV-2 Infection

A much greater proportion of HIV-2-infected patients develop nAbs, particularly broadly neutralizing antibodies, than do HIV-1-infected patients, and the pattern of neutralization over time differs between the two viruses (Bjorling et al. 1994). In HIV-1 patients the optimal titers of nAbs capable of neutralizing the autologous infecting HIV-1 strain were observed only briefly, approximately 5 weeks after primary HIV-1 infection. However, later in the infection most plasma samples fail to neutralize the patients' own circulating virus. In contrast, the neutralizing activity of HIV-2-infected patients against contemporaneous isolates is maintained at different stages of infection (Bjorling et al. 1994). One study showed that 68% of HIV-2-infected patients elicited nAb against R5 isolates, compared to 10–31% of HIV-1-infected patients (van Gils et al. 2009).

Potent neutralizing antibody activity against primary HIV-2 envelopes is mediated by IgG antibodies (Kong et al. 2012). It was demonstrated that there are significantly fewer glycosylation sites in the V4 region of several HIV-2 isolates, suggesting the reduced glycan shielding is an important factor in increasing the accessibility of HIV-2 envelope to neutralizing antibodies. In a cohort study, heterologous and autologous nAb responses were assessed over the course of 15 years. In this cohort, all the patients possessed high heterologous nAb titers, which were several log units higher than similar antibodies in HIV-1 infection, and a positive association between the nAb titer and viral load was observed. Furthermore, 78% of the patients exhibited high autologous nAb titers, suggesting high levels of antigenic stimulation (Bailey et al. 2006).

To this date only very few epitopes targeted by nAbs in HIV-2 have been identified and their sensitivity to neutralization verified. Many of the

HIV-2-specific monoclonal antibodies were shown to react potently with epitopes on the base of V3, V4, and the CD4-binding site (Kong et al. 2012). These nAbs frequently exhibited IC50s of 0.1 g/ml or less, suggesting that these epitopes in HIV-2 envelopes are more readily accessible to nAbs. Antibodies with these specificities may contribute significantly to the broad and potent neutralization observed in HIV-2-infected individuals. The V3 region of HIV-2 has only two glycosylation sites, whereas the same region in HIV-1 has four to five glycosylation sites to shield the virus from nAbs (Shi et al. 2005).

Antibody-Dependent Complement-Mediated Inactivation (ADDCMI) in HIV-2 Infection

Only very few studies have been conducted on antibody-dependent complement-mediated inactivation of HIV-2. A greater magnitude of the intratype antiviral activity of samples from HIV-2-infected subjects was contributed by antibody-dependent complement-mediated inactivation (ADDCMI) than seen in HIV-1 infection (Ozkaya Sahin et al. 2012). In the presence of complement, the antiviral activity was increased by 32-fold in HIV-2 intratype reactions, presumably due to the number and accessibility of Env spikes on HIV-2 virions. Compared to HIV-1, the spike on HIV-2 is more stable after budding (Ozkaya Sahin et al.); its Env is more accessible and has a thinner glycan shield (Shi et al. 2005). Furthermore, the coreceptor-binding site of the HIV-2 Env spike was shown to have a more open configuration, regardless of CD4 binding, compared to HIV-1 (Thomas et al. 2003). More efficient ADCMI in HIV-2 infection could be one of the main contributing factors to better host control of this infection.

Effect of Antibodies on Diversification of HIV-2 Envelope

Despite high evolutionary rates, there are more structural and functional constraints placed on

the HIV-2 envelope than for HIV-1. The mutations in gp125 and the C2V3C3 region (Skar et al. 2010; Barroso and Taveira 2005) mostly occur in synonymous sites in HIV-2, and they are rarely associated with the escape of the virus from nAbs (Skar et al. 2010). This could be related to weaker positive selection or even to negative selection (Barroso and Taveira 2005). Genetic diversification within HIV-2 occurs predominantly in the gp120 variable loops, V1/V2, V3, and V4. V1/V2 and V4 are the most prone to mutation. The variation in V3 is more prominent among isolates that utilize CXCR4 as a coreceptor (Lin et al. 2007). Unlike V1/V2 and V4 regions, which are able to tolerate insertions and deletions (Huang et al. 2005), the V3 length is highly conserved, i.e., 34–35 amino acid long (Ren et al. 2005), due to its critical role in HIV-2 interaction with the chemokine receptors (Lin et al. 2007; Huang et al. 2005). X4 isolates, which have a longer V3 loop by 1–3 amino acids, are more resistant to nAbs compared to R5 isolates (Marcelino et al. 2010). Amino acids FHSQ, located at V3 loop positions 315–318, interact with residues WCR, located at V3 stem positions 329–331, to form a conformational neutralizing epitope (Bjorling et al. 1994). The insertion of the 1–3 amino acids immediately after the FHSQ sequence results in the inappropriate assembly of this target epitope and consequently resistance to nAbs (Marcelino et al. 2010). Thus during the R5 to X4 transition in HIV-2 infection, significant changes occur in the charge, size, and conformation of the HIV-2 V3 loop, resulting in prevention of nAb binding to target epitopes (Shi et al. 2005). Since the C2 and C3 regions are well exposed in HIV-2, they are under strong selection for diversification and hence may be presumed to harbor nAb epitopes (Barroso and Taveira 2005). Observations suggested that 22% of the amino acids in C2, 24% in V3, and 36% in C3 were exposed to antibody recognition. Among amino acids 319/320 and 328 in the V3 loop, which are involved in coreceptor binding, only amino acid 319 was reasonably well exposed. Generally, C2 and C3 diversification in HIV-2 are subject to more structural and functional constraints, and consequently, mutations are thought to have a

negative effect on virus fitness (Barroso and Taveira 2005). This could be one of the essential differences in the biology of the two HIVs and their interactions with the host immune system and consequently the outcome of the disease.

Conclusion

Production of bNAbs and ADCMI response are higher in HIV-2-infected patients than in HIV-1 positive subjects. Furthermore, many mutations in HIV-2 envelope occur in synonymous site, resulting in either weaker positive selection or even negative selection, which could have an adverse effect on the fitness of the virus. All these factors can contribute to an effective immune control of viral replication in many HIV-2 positive individuals.

Cross-References

- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [HIV-2 Envelope: Structure, Diversity, and Evolution](#)
- ▶ [HIV-2 Infection: The Role of Immune Activation in Pathogenesis](#)
- ▶ [Immunogenetics of HIV-2 Infection](#)
- ▶ [Molecular Biology of HIV-2](#)
- ▶ [Natural History and Clinical Features of HIV-2 Infection](#)

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Antiretroviral Drug Penetration into Lymphoid Tissue

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Definition

Drug distribution through the body is not homogenous. Restricted penetration of antiretroviral drugs into anatomic compartments, such as the secondary lymph nodes and gut-associated lymphoid tissue that are primary sites of HIV replication, may be a mechanism of viral persistence in HIV-infected persons despite potent suppression of HIV replication in plasma with antiretroviral therapy. Strategies to increase antiretroviral drug delivery to reservoir compartments that improve pharmacokinetics and enhance pharmacodynamics may achieve the full suppression of HIV production, which is a prerequisite to long-term remission or eradication of HIV infection.

Introduction

In the fourth decade of the HIV epidemic, and despite remarkable advances in the treatment of HIV, the ability to eradicate the virus from an

infected individual still evades us. Combination antiretroviral therapy (ART) can achieve long-term suppression of plasma viral load to <50 copies/mL, the current goal of ART, and has reduced mortality and dramatically improved quality of life for HIV-infected persons. But immune reconstitution is incomplete, and these HIV-infected individuals continue to have abnormal vaccine responses, a higher-than-normal incidence of non-AIDS cancers and an increased risk of clinical diseases associated with chronic inflammation (e.g., cardiac disease) (Fletcher et al. 2014). The secondary lymph nodes (LN) and gut-associated lymphoid tissue (GALT) are the primary sites of HIV replication and where the latent pool of virus is maintained. This chapter will describe the physiology and HIV-associated pathophysiology of the lymphoid system, mechanisms of drug penetration into lymphoid tissues, present current human data on antiretroviral (ARV) penetration into lymphoid tissues, and discuss strategies to enhance ARV concentrations into lymphoid tissues.

Physiology and HIV-Associated Pathophysiology of the Lymphoid System

The lymphoid organs, lymphatic vessels, and the lymph comprise the lymphatic system. The bone marrow and thymus are the two primary lymphoid organs where lymphocytes originate or mature. Secondary lymphoid organs are LN, spleen, and mucosal-associated lymphoid tissues (MALT), of which the GALT is the largest and best defined component, and bronchial-associated lymphoid tissues (BALT). These secondary lymphoid organs maintain mature naïve lymphocytes and are where acquired immune responses are initiated. They are located in areas more likely to be invaded by pathogens, such as the gastrointestinal tract, respiratory tract, and genitourinary tract. The lymphatic vessels comprise a one-way transport system for fluid and proteins by collecting them from the interstitial space and returning to the blood circulation (Yanez et al. 2011). Lymph forms as interstitial fluid moves into the lymphatic capillaries and then drains into the collecting

vessels, which pass through at least one but usually several clusters of LN. Lymph nodes are small encapsulated organs located along the pathway of lymphatic vessels. They serve as filters through which lymph percolates on its way to the blood. Collecting vessels drain into larger trunks, which lead into the lymphatic ducts. The lymphatic ducts return the lymph back to the bloodstream, completing the circuit of fluid transport. The major functions of the lymphatic system are maintenance of normal tissue fluid balance, absorption of lipids and fat-soluble vitamins from the intestine, and attraction and transport of immune cells.

HIV causes a persistent, chronic infection in LT, which becomes a significant reservoir where virus is produced, stored, and persists in latently infected cells capable of virus production upon reactivation (Fletcher et al. 2014). The LT compartment is also the major site where CD4⁺ T cell depletion occurs and pathologic changes from virus replication significantly limit the ability of ART to fully reconstitute the immune system. Normal immune function is dependent on the anatomic structure of secondary LN. The anatomy of the paracortical T cell zone of secondary LN is of particular importance. Viral replication primarily occurs in resting and activated CD4⁺ T cells of the paracortical T cell zone in LN and GALT and triggers an inflammatory response. The persistent state of immune activation initiates and maintains collagen formation in the paracortical T cell zone. Collagen deposition into the parafollicular T cell zone and the resulting fibrosis are a significant cause of CD4⁺ T cell depletion in the untreated individual and a significant cause of impaired immune reconstitution despite good control of viral replication. Collagen deposition and immune dysfunction occur in a disease stage-dependent fashion; increasing levels are directly correlated with progression toward AIDS and inversely with the degree of immune reconstitution that can be expected when ART is implemented.

Lymphoid Tissues as Reservoirs for HIV

The secondary LN and GALT are the primary sites of HIV replication and where the latent pool of

virus is maintained (Fletcher et al. 2014). The reservoir of latently infected resting memory CD4⁺ T cells is established very early in the course of HIV infection. These resting cells do not actively produce virus, are not susceptible to the mechanisms of action of current antiretroviral drugs, and are a major barrier to a cure of HIV infection. Intermittent virus production from reactivation of latently infected CD4⁺ T cells is thought to be a source of low-level persistent or “blips” of virus detected in blood in well-suppressed patients on treatment. However, emerging evidence suggests it may be possible for cells in tissues such as the secondary LN and GALT to continue to produce low levels of HIV, despite ART suppression of plasma HIV RNA to undetectable levels (Chun and Fauci 2012). For example, in aviremic HIV-infected persons who had received long-term ART, there was incomplete recovery of CD4⁺ T cells in the GALT, and significantly higher levels of HIV-1 DNA normalized to CD4⁺ T cell count in GALT tissue than in either resting or activated CD4⁺ T cells in the blood (Chun et al. 2008). Additionally, there was a high degree of sequence similarity between virus in the GALT and virus in PBMCs, suggesting cross infection between the GALT and PBMCs. Others have shown similarity between HIV-1 sequences in the guts and plasma in HIV-1-positive individuals, suggesting that even with undetectable HIV-1 RNA in plasma, subjects still have ongoing replication in the gut and that there is equilibrium between these two compartments (Imamichi et al. 2011). Viral concentrations were higher along the entire GI tract as compared with the blood of HIV-infected subjects treated with ART, and ratios of unspliced RNA to DNA were lower in the colon and rectum of these subjects, compared with PBMCs, suggesting a low rate of HIV transcription (Yukl et al. 2010). Studies in SIV-infected macaques have identified mesenteric LN as a sanctuary site. For example, while effective suppression of plasma viral RNA was achieved in SIV-infected macaques treated with ART, detectable levels of virus in the spleens and LN were found (Horiike et al. 2012). Detectable vDNA and vRNA in LT, including mesenteric, axial, inguinal, iliac, and cervical LNs,

collected at necropsy have also been shown in aviremic ART-treated macaques infected with RT-SHIV and treated with efavirenz, emtricitabine, and tenofovir for 26 weeks (North et al. 2010). Recently, a sensitive immuno-PET radiotracer and whole-body imaging have been used in chronically SIV-infected, ART-suppressed macaques to detect and visualize areas of ongoing virus replication (Santangelo et al. 2015). After treatment, imaging studies revealed residual virus remained in the colon, spleen, male genital tract, nasal-associated LT, and individual LNs. qRT-PCR on these tissue samples confirmed the presence of residual virus or infected cells. Collectively, these human and animal data suggest there is persistence of viral replication in LT despite long-term, potent suppression of plasma viremia, and that such low-level continued viral replication could be sufficient to re-seed the reservoir and maintain a state of immune activation that impairs immune reconstitution.

Drug Penetration in Lymphatic Tissues

Drugs do not distribute evenly throughout the whole body. The reasons for this are complex and multifactorial. Absorption and distribution vary by drug and by location depending on the physicochemical properties of the drug, the size and rate of perfusion of the target site, and protein binding of the drug. Additional factors include whether a drug is a substrate of influx or efflux transporters, metabolic enzymes, and patient-specific polymorphisms for transporters and metabolic enzymes. These complex factors can lead to significant differences in drug concentrations in different compartments, as well as different drug concentrations in these compartments between patients.

Following oral administration, drugs enter the systemic circulation either through the portal vein or the intestinal lymphatic system. The majority of absorbed drugs are preferentially diverted into the portal blood because the rate of fluid flow in the portal blood is approximately 500-fold higher than the intestinal lymph. Drugs that enter the

systemic circulation through the portal vein are subject to hepatic first-pass metabolism, which can substantially reduce bioavailability. To penetrate into the lymphatic system, a drug appearing first in the blood must cross blood-lymphatic barriers. The fraction of drug that enters the LN can diffuse out with little resistance. In contrast, drugs successfully transported in the lymph avoid hepatic first-pass metabolism, and therefore lymphatic system transport can enhance oral bioavailability. Factors associated with greater intestinal lymphatic system absorption are higher molecular weights, particle size, lipophilicity (log octanol/water partition coefficients [log P values]), and long-chain triglyceride solubility (Yanez et al. 2011). Factors described in the literature particularly associated with greater absorption via the intestinal lymphatic system are higher molecular weights, particle size, and a log P value greater than five. All current oral ARVs are low molecular weight drugs (ranging from 247–776 Da.), and most have an experimental log p value <5. A series of studies with the protease inhibitor indinavir are illustrative of the importance of physicochemical properties in LT penetration. Indinavir (low molecular weight, log P value <5) is more rapidly cleared from lymph than plasma following oral administration to rats. LN mononuclear cell concentrations in three HIV-infected patients were only 25% those in blood and very similar to cerebrospinal fluid (CSF) (Freeling et al. 2015). Administration to macaques of a lipid-associated indinavir formulation with higher molecular weight, greater lipophilicity, and a larger particle size, all factors that favor distribution into lymph flow and accumulation in LN, increased concentrations in LN to >250% in those of blood and increased *in vitro* anti-HIV potency by twofold (Freeling et al. 2015).

Membrane transporters and drug-metabolizing enzymes play a significant role in drug disposition, clinical therapeutic responses both desired and adverse, and drug interactions. The small intestine (duodenum, jejunum, and ileum) is the primary site of oral absorption for most drugs and has been studied for the expression of drug influx and efflux transporters (Bruyere et al. 2010).

Various drug efflux transporters [e.g., P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)] and drug influx transporters (e.g., OATP1B1) have been identified in intestinal tissues. Considerable differences in expression for transporters including P-gp, BCRP, and MRP2 exist among patients and different sections of the gut. P-gp expression, for example, has been shown to increase from the duodenum to the ileum. The colon has higher expression of MCT1, OCTN2, and MRP3 than the small intestine. Human studies have shown considerable variation in cytochrome P450 (CYP) drug-metabolizing enzymes, including CYP3A4 expression, along the length of the gastrointestinal tract with the amount of CYP3A4 decreasing from the duodenum to the ileum and to the colon. Taken together, these studies show the expression of drug transporters and metabolizing enzymes is not uniform within the small intestine and along the gastrointestinal tract. Furthermore, a number of genetic polymorphisms in the drug transporter and drug-metabolizing enzyme genes have been identified. These data, therefore, provide a pharmacologic basis for inter-patient variability in ARV concentrations in LN and GALT, as well as regional differences in ARV concentrations such as between the ileum and rectum.

Multiple ARVs have been studied in various LT in humans. The available data, however, are sparse, and confirmatory studies of reported concentrations are generally lacking. This arises in part because while measurements of drug concentrations in plasma are easy to obtain, the concentrations in LT for a given drug are considerably more difficult to obtain and more complex to quantify. The lack of agreement on the best tissue matrix for quantitation of an ARV and the lack of standardization of analytical methodology for quantifying ARVs in LT among laboratories are additional challenges in interpreting the data and comparing reported values for the same drug of interest in tissue of similar anatomic origin. There are two commonly used strategies for measuring ARVs in LT: first, measuring drug concentrations within homogenates of tissue biopsies and, second, measuring ARV concentrations in mononuclear cells extracted from LT biopsies. Tissue

Antiretroviral Drug Penetration into Lymphoid Tissue, Table 1 Lymphoid tissue penetration ratios of antiretroviral drugs

Compartment	Drug	Tissue-to-peripheral blood ratio	Subject HIV status	Dosing	References
Lymph node					
	TFV-DP	0.21 ^a	HIV infected	Multiple	(Fletcher et al. 2014)
	FTC-TP	0.22 ^a	HIV infected	Multiple	ibid
	ATV	0 ^a	HIV infected	Multiple	ibid
	DRV	0.02 ^a	HIV infected	Multiple	ibid
	EFV	0.07 ^a	HIV infected	Multiple	ibid
Ileum					
	TFV-DP	26.7 ^a	HIV infected	Multiple	(Fletcher et al. 2014)
	FTC-TP	0.07 ^a	HIV infected	Multiple	ibid
	ATV	0.03 ^a	HIV infected	Multiple	ibid
	DRV	0.22 ^a	HIV-infected	Multiple	ibid
	EFV	0.48 ^a	HIV infected	Multiple	ibid
	RAL	156 ^b	Uninfected	Multiple	(Patterson et al. 2013)
Colon					
	TFV-DP	2.5 ^{a, c}	Uninfected	Single	(Louissaint et al. 2013)
Rectum					
	TFV-DP	7.6 ^a	HIV infected	Multiple	(Fletcher et al. 2014)
	TFV-DP	71.2 ^d	Uninfected	Single	(Yang et al. 2014)
	TFV	34 ^b	Uninfected	Single	(Patterson et al. 2011)
	FTC-TP	0.08 ^a	HIV infected	Multiple	(Fletcher et al. 2014)
	ATV	0.52 ^a	HIV infected	Multiple	ibid
	DRV	0.7 ^a	HIV infected	Multiple	ibid
	DRV	2.7 ^b	Uninfected	Multiple	(Brown et al. 2012)
	RTV	12.8 ^b	Uninfected	Multiple	ibid
	EFV	0.12 ^a	HIV infected	Multiple	(Fletcher et al. 2014)
	ETV	7.5 ^b	Uninfected	Multiple	(Brown et al. 2012)
	RPV	0.5–1.3 ^b	Uninfected	Single ^e	(Jackson et al. 2014)
	DTG	0.17 ^b	Uninfected	Multiple	(Greener et al. 2013)
	RAL	231 ^b	Uninfected	Multiple	(Patterson et al. 2013)
	GSK 744 (GSK1265744, cabotegravir)	<0.08 ^b	Uninfected	Single ^f	(Spreen et al. 2014)
	MVC	26.2 ^b	Uninfected	Multiple	(Brown et al. 2011)

Abbreviations *TFV-DP* tenofovir-diphosphate, *FTC-TP* emtricitabine-triphosphate, *ATV* atazanavir, *DRV* darunavir, *EFV* efavirenz, *RAL* raltegravir, *ETV* etravirine, *RPV* rilpivirine, *DTG* dolutegravir, *MVC* maraviroc

^aRatio is tissue mononuclear cells (MNCs) to peripheral blood mononuclear cells (PBMCs)

^bRatio is tissue homogenate to blood plasma

^cCalculated from C₂₄ PBMC and tissue MNC median concentrations reported

^dCalculated from median C₂₄ TFV-DP concentrations reported in CD4⁺ rectal cells and C_{max} of PBMC

^eAdministered as RPV-LA 600 mg IMx1

^fAdministered as GSK1265744 as either 400 mg IMx1 or 200 mg IMx2

homogenates are easier to obtain and to prepare for sample analysis. Cells extracted from tissue biopsies allow measurement of drug at the pharmacologic site of action within the target cell. Table 1 summarizes current LT concentration data of

ARVs obtained from human studies with orally administered ARVs that have been published. Data are from both single-dose and multiple-dose studies in HIV-infected persons as well as volunteers not infected with HIV. Ratios of tissue

(or cellular) concentrations of ARVs to blood plasma (or PBMC) concentrations widely vary. Nonetheless, some general observations can be drawn from these data. First, the penetration of ARVs into LT appears to be drug specific and not class specific as seen, for example, with the finding of substantially higher tenofovir-diphosphate (TFV-DP) concentrations in the ileum and rectum compared with emtricitabine-triphosphate (FTC-TP). Second, ARV penetration into LN appears to be lower than the ileum and rectum. With the exception of TFV-DP, tissue-derived MNC ARV concentrations have not been shown to exceed those in PBMC for any of the ARVs studied. Many of the tissue-to-blood plasma ratios derived by analyzing tissue homogenates for ARVs yield ratios >1 ; however, caution must be used when interpreting tissue homogenate concentrations as tissue homogenate may overestimate the amount of drug present at the pharmacologic site of action. Despite methodologic limitations, pharmacodynamic correlates with LT ARV concentrations exist. Multiple pharmacokinetic studies have found high concentrations of tenofovir (TFV) and in rectal cells and tissue homogenates (Table 1). Clinical studies have shown that orally administered tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) are highly effective in the prevention of HIV transmission among men who have sex with men, where the tissue of greatest pharmacodynamic relevance for prevention of HIV transmission is rectal (Grant et al. 2010).

The different physicochemical properties of the ARVs that affect penetration into the intestinal LT provide a pharmacologic basis to expect differences in concentrations compared with peripheral blood. The relevant physicochemical properties would generally predict poorer penetration into LN. Patient-specific factors, such as host genetic variability in drug-metabolizing enzymes or transporter function, are quite likely to be determinants of drug penetration as well. Finally, LN fibrosis as a pathophysiologic consequence of HIV infection might also contribute to ARV penetration into the LN. The emerging evidence that low-level replication of HIV may persist in tissues such as the secondary LN and GALT, despite ART suppression of plasma HIV

RNA to undetectable levels, and data that shows poorer penetration of ARVs into these tissues allow the hypothesis that virus might continue to replicate because ARV concentrations are insufficient to suppress replication fully.

Strategies to Enhance Drug Delivery to Lymphatic Tissues

The unique anatomy and physiology of the lymphatic system provide a number of drug delivery advantages over drug absorption via the portal blood. Some advantages include the ability to enhance bioavailability and systemic concentrations by bypassing hepatic first-pass metabolism, the delivery of sustained-release drugs, and, because of the role the lymphatic system plays in certain cancer metastasis, the ability to improve effectiveness of antineoplastic and immunomodulatory agents by achieving high concentrations and targeted delivery. The treatment of HIV infection might also be advanced by improved LT delivery of ARVs because of the role the secondary LN and GALT play in HIV pathogenesis. Strategies to enhance drug delivery to LT include (but are not limited to) structural modification of existing drugs, transporter or metabolism blockade, and targeted drug delivery with nanoformulations.

Structural Modification of Existing Drugs

Modifying the chemical structure of existing ARVs that have established safety and efficacy is an attractive approach to improve pharmacokinetic/pharmacodynamic properties in sanctuary sites. Tenofovir alafenamide (TAF; GS-7340) is a prodrug of tenofovir presently in clinical development that has improved penetration into LT. The drug displays variable absorption, with larger doses resulting in a greater percentage of the dose being absorbed than with lower doses, and is very stable in intestinal tissue. This is believed to be due to the ability of the drug to saturate intestinal transporters, including P-glycoprotein. After a single oral dose to beagle dogs, LN concentrations were 5–15-fold higher than after oral administration of TDF. TAF

achieved concentrations in PBMCs that were approximately 25-fold higher than a dose of 300 mg of TDF after oral administration to HIV-infected persons at a dose of 40 mg (Ruane et al. 2013). The significantly increased concentrations in PBMCs and in LT in animals (which is yet to be demonstrated in humans) observed with TAF appear to provide a clear illustration of the promise of structural modification of existing drugs to enhance antiviral activity in tissue compartments.

Transporter and Metabolism Blockage

Strategies to increase concentrations in sanctuary sites include use of drugs to block influx and efflux transporters or the metabolism of ARVs (Cory et al. 2013). Ritonavir and cobicistat have established roles in HIV pharmacotherapy as CYP3A enzyme inhibitors. *In vitro* research suggests that ritonavir and cobicistat also enhance intestinal absorption. Studies with Caco-2 monolayers (a cell line derived from human epithelial colorectal adenocarcinoma cells, commonly used in *in vitro* absorption studies) show increased transport of antiretrovirals including atazanavir, darunavir, and TAF across the monolayers. This is accomplished in part by inhibition of P-gp and BCRP. Similar findings have been observed for ritonavir. This inhibition of efflux transporters in the gut may lead to increased concentrations in plasma, but little is known if this will increase drug concentrations in compartments. The data discussed above for TAF and its ability to saturate intestinal P-gp efflux and achieve increased LT concentrations seems to provide such evidence. An abacavir prodrug dimer that can inhibit P-gp efflux has been designed to increase abacavir CNS penetration. *In vitro* assays, as well as a brain capillary model, found this molecule was able to inhibit P-gp in the cell culture system and is converted back to abacavir inside cells. This approach may be utilized for other ARVs as a means to increase penetration past the blood–brain barrier and perhaps other blood–tissue barriers. P-gp blockers have been studied as adjunctive treatment in cancer, but no clinical therapeutic benefit has been established to date.

Targeted Drug Delivery with Nanoformulations

The targeted delivery of drugs to increase therapeutic efficacy and limit off target toxicities is one of the most important objectives in modern pharmaceutical research. One approach is the incorporation of drugs into nanocarriers or nanoformulations. Nanoscale antiretroviral therapies, usually defined as ARVs formulated into particles with a size of less than 100 nm, have been proposed as an approach to increase concentrations and sustain delivery (i.e., increase the half-life) of ARVs in tissues and sanctuary sites. Human monocyte-derived macrophages (MDM) exposed to nanoformulations consisting of atazanavir, efavirenz, and ritonavir show rapid uptake of the ARVs and slow release over an extended time period (15–20 days) (Nowacek et al. 2010). These ARV nanoformulations can be utilized to establish drug depots for a sustained response, allowing for weekly and even less-frequent administration. Significant concentrations of ARVs are found in a variety of tissues, including the liver, spleen, kidneys, and lungs of animals for a number of days post-administration. As circulating monocytes and macrophages distribute throughout the body, including to the brain and secondary LT, this has been proposed as a means to overcome barriers preventing access of antiretrovirals (Nowacek et al. 2010). A folic acid-coated nanoformulation of atazanavir and ritonavir achieved higher and more sustained plasma and certain tissue concentrations of atazanavir at lower doses compared with a non-folic acid-coated nanoformulation (Gautam et al. 2014). A lipid nanoformulation containing lopinavir, ritonavir, and tenofovir has been evaluated in macaques (Freeling et al. 2015). Plasma and PBMC concentrations were higher and sustained for up to 7 days beyond that with free drug formulations. Importantly, the LN concentrations of lopinavir and ritonavir were substantially increased: lopinavir and ritonavir LN concentrations increased 1200-fold and 50-fold, respectively. In contrast, there was no difference in LN concentrations of tenofovir when given in the nanoformulation compared with the administration as free drug, probably because of limited encapsulation of this hydrophilic drug. No serious adverse

reactions were reported over 7 days of administration of this triple-ARV nanoformulation to macaques. The development of ARV nanoformulations may play an important role in overcoming barriers to achieving adequate concentrations of some ARVs in LT, and further study is clearly warranted.

Conclusions

Latently HIV-infected, resting CD4⁺ T cells are clearly a major impediment to eradication of HIV. However, sensitive HIV-1 RNA assays show viral production persists in some individuals with plasma viral load <50 copies/mL. LT have been shown to be viral reservoirs in nonhuman primate studies, and HIV RNA has been detected in human rectal samples and HIV DNA detected in GALT tissue of well-suppressed patients. Collectively, these findings indicate the existence of a reservoir of low-level viral replication in peripheral blood and LT. The different physicochemical properties of the ARVs that affect penetration into the intestinal LT provide a pharmacologic basis to expect differences in concentrations compared with peripheral blood. The relevant physicochemical properties and pharmacologic characteristics would generally predict poorer penetration into LT. Studies in HIV-infected persons and in animals have shown low concentrations of some ARVs in LT. These data provide a pharmacologic basis to hypothesize that inadequate concentrations of ARVs in LT allow low-level viral replication in LT of HIV-infected persons despite plasma viral load <50 copies/mL. Therefore, improving the pharmacokinetic and pharmacodynamic conditions of ARVs in LT seems to be a necessary antecedent to achieve the goals of complete remission or eradication of HIV infection.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Collagen Deposition and Fibrosis in the Lymphatic Tissues of HIV-1 Infected Individuals](#)

- ▶ [Immunological Responses to Antiretroviral Therapy](#)
- ▶ [Long-Acting Nanoformulated Antiretroviral Therapy](#)
- ▶ [Prevention Clinical Trials: Highlights of Evidence and Research](#)

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Antiretroviral Drug Penetration into the CNS Compartment

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Definition

The central nervous system is reached and infected a few hours after HIV infection. Viral replication occurs in perivascular macrophages, in microglia, and, although restricted, in astrocytes: neuronal damage is believed to be a consequence of neurotoxin production by the aforementioned cells of the immune system. Such cells are reached by antiretroviral drugs either directly (crossing blood–brain and blood–cerebrospinal fluid barriers) or through cerebral extracellular fluid (drained into cerebrospinal fluid); for several drugs, cerebrospinal fluid concentrations have been shown to reflect cerebral interstitial fluid concentrations. The penetration of several compounds into the central nervous system has been shown to be highly variable and to depend on drugs’ (molecular weight, lipophilicity, ionization, plasma protein binding, transport mechanisms) and patients’

characteristics (age, blood flow, blood–brain barrier permeability). Although the exact amount of drug necessary to inhibit viral replication in the central nervous system is currently unknown, antiretrovirals have been ranked according to their concentration/penetration effectiveness: higher scores have been repeatedly associated with lower cerebrospinal fluid HIV viral loads.

Introduction

HIV RNA has been recovered from the cerebrospinal fluid (CSF) few days after systemic infection and central nervous system (CNS) involvement parallels this event (Valcour et al. 2012). The presence of viral replication in perivascular macrophages, microglia, and astrocytes (restricted) is eventually associated with neuronal damage due to persistent immune activation and cytokine production. Inflammatory cytokines and chemokines have been found to be abnormally elevated in HIV-positive patients and they have been linked to the alteration of the blood–brain barrier (BBB) (Gannon et al. 2011). An impaired BBB might facilitate the penetration of HIV-infected monocytes, thus increasing the viral biomass in the CNS. Furthermore, CNS has been recognized as a site of compartmentalized viral replication: HIV may harbor different resistance-associated mutations in the CSF as compared to plasma (Canestri et al. 2010). Approximately 10% of treated patients have detectable HIV RNA in the CSF despite plasma viral control; the clinical and immunological consequences of this event in the absence of resistance-associated mutations are currently unclear (Edén et al. 2010).

The clinical endpoint of untreated CNS infection is the appearance of HIV-associated dementia (HAD). With the introduction of highly active antiretroviral therapy (HAART), the incidence of dementia significantly declined; nevertheless, cognitive impairment (asymptomatic and moderate according to the impact on everyday life and globally defined as HIV-associated neurocognitive disorders, HAND) remains highly

prevalent (Clifford and Ances 2013). Furthermore, 19–22.7% of subjects may progress to symptomatic neurocognitive impairment over a two-year follow up (Heaton et al. 2015).

Mechanisms of Drug Passage and Drug Measurement in the CNS

To be efficacious, drugs must reach adequate concentrations at the site of action: in the case of CNS infection by HIV, the targets are macrophages, microglia, and astrocytes within the brain parenchyma. After intestinal absorption, orally administered antiretrovirals (ARVs) (the vast majority of available drugs, with the exception of intravenous zidovudine and subcutaneous enfuvirtide) are transported by plasma proteins in the bloodstream and distributed to organs and tissues. The CNS receives approximately 14% of cardiac output, but two anatomical barriers can be found that prevent the free passage of drugs into the brain: the blood–brain barrier and the blood–CSF barrier. The first one is characterized by endothelial cells connected by tight junctions and by the presence of astrocytes end feet: several substances are restricted from crossing the BBB (Varatharajan and Thomas 2009). Nevertheless, in some areas in the brain (hypothalamus, area postrema, subfornical organ), tight junctions are not present and direct diffusion is possible.

The study of antiretroviral pharmacokinetics in the CNS has several methodological obstacles. For instance, data on tissue and intracellular drug concentrations are limited and most of the knowledge derives from cerebrospinal fluid measurements. Cerebrospinal fluid is believed to be produced by filtration from blood plasma (two thirds) and from brain extracellular fluid (one third). Several animal studies have suggested that cerebrospinal fluid is a surrogate reliable marker for most of the studied drugs although a significant variability in tissue levels prediction was observed. For instance, data in nonhuman primates suggest a good correlation between zidovudine CSF and brain parenchyma concentrations, while data for other ARVs are scarce.

Factors Affecting Antiretroviral Penetration into the CNS

Patient-related factors include age (older age may affect the passage of several drugs into the CNS due to reduced blood efflux, permissive BBB, and altered CSF flow) and BBB permeability. BBB impairment is often observed in HIV-positive patients: a permissive barrier may allow the passage of both drugs and plasma proteins, thus increasing the CSF total concentration but reducing the free drug levels, thus questioning its clinical impact; however, it has been shown to be significant for tenofovir, emtricitabine, and raltegravir.

Four chemical characteristics that affect drug passage have been identified: molecular weight (the smaller, the higher), lipophilicity (the higher, the higher, measured as octanol water distribution coefficient, LogP), ionization (the higher, the lower), and plasma protein binding (the lower, the higher). Several data confirm the effect of such drug-related features: a near direct linear correlation has been shown between plasma protein binding and CSF-to-plasma drug concentration ratios.

Several transporting proteins have been shown to be expressed at the BBB and at the blood–cerebrospinal fluid barrier (BCB) and to be involved in drug transport into the CNS: p-glycoprotein (P-gp); organic anion transporter 1, 2, and 3 (OAT1, 2, and 3); breast cancer resistance protein (BCRP); and others. Single nucleotide polymorphisms affecting such enzymes may affect drug penetration into the CNS although data showing such influence are very limited.

Antiretroviral Penetration in the CNS

Antiretrovirals' molecular weight, plasma protein binding, CSF-to-plasma ratios, and concentration/penetration effectiveness score (CPE, discussed below) are shown in Table 1 (Yilmaz et al. 2012; Eisfeld et al. 2013).

- NRTIs are small, poorly bound, hydrophilic molecules reaching very variable CSF-to-

plasma ratios. Tenofovir is ionized at physiological pH and this limits its uptake by membrane transporters. NRTIs are transported by organic anion transporters (OATs) that have been shown to be present at the choroid plexus (OAT1 and OAT3); the modulation of their activity (either by other drugs such as probenecid or by genetic polymorphisms in the encoding genes) may be relevant for zidovudine, stavudine, lamivudine, and tenofovir passage. With the exception of didanosine (whose CSF exposure has been found to be undetectable or very low), the other NRTIs have been associated with therapeutic CSF concentrations. Animal data suggested a good tenofovir CSF passage (through the blood–CSF barrier and independently of OATs) but a poor penetration into deep brain tissue; CSF tenofovir levels in humans are low and often undetectable.

- NNRTIs are small, lipophilic, highly protein-bound (with the exception of nevirapine) compounds. Efavirenz CSF levels are low (very close to the limit of detection of the instruments, thus suggesting undetectable concentrations) but frequently above 50% inhibitory concentrations (IC_{50}). While data on rilpivirine and etravirine are still limited, nevirapine high CSF-to-plasma ratios have been confirmed: the compound properties as well as the *in vivo* data suggest that nevirapine is one of the ARVs with the best CSF penetration.
- PIs are large, lipophilic, highly protein-bound (with the exception of indinavir) compounds with CSF-to-plasma ratios around 1%; they have been recognized as substrate of p-glycoprotein as well as OAT1A2 and this may limit drug accumulation into the CNS (as well as into other key tissues such as lymph nodes). Nowadays, the comparison among the three most used protease inhibitors favors darunavir and lopinavir since atazanavir was associated with low or undetectable CSF concentrations.
- Enfuvirtide is a synthetic 36-amino-acid oligopeptide (interacting with viral gp41) with a very large molecular weight: a single study and a case report confirmed CSF is very

Antiretroviral Drug Penetration into the CNS Compartment, Table 1 Antiretrovirals' molecular weight, plasma protein binding, cerebrospinal fluid ("CSF")-to-plasma ratios, and concentration/penetration effectiveness scores ("CPE")

	Molecular weight (Da)	Protein binding (%)	CSF-to-plasma ratio (%)	CPE score
NRTIs				
Abacavir	286	50	36	3
Didanosine	236	<5	Negligible	2
Emtricitabine	247	<4	43	3
Lamivudine	229	16–36	12–22	2
Stavudine	224	Negligible	27	2
Tenofovir	287	<7	4	1
Zidovudine	267	30–38	2–674	4
NNRTIs				
Efavirenz	315	99.5–99.7	0.5	3
Etravirine	435	99.9	1–4.3	2
Nevirapine	266	60	62.6	4
Rilpivirine	366	>99	1.4	n.a.
PIs				
Amprenavir	505	90	1.6	2/3
Atazanavir	704	86	0.9	2
Darunavir	547	95	0.6–1.4	3
Fosamprenavir	585	90	1.2	2/3
Indinavir	613	60	9.9	4
Lopinavir	628	98–99	0.2–0.5	3
Saquinavir	670	98	Negligible	1
Tipranavir	602	>99.9	n.a.	1
EI and R5I				
Enfuvirtide	4491	92	Negligible	1
Maraviroc	513	76	2.2–29	3
ISTI				
Elvitegravir	448	98–99	n.a.	n.a.
Dolutegravir	419	>98.9	0.4	n.a.
Raltegravir	444	83	3–20	3

NRTIs nucleos(t)ide reverse transcriptase inhibitors, *NNRTIs* non-nucleoside reverse transcriptase inhibitors, *PIs* protease inhibitors, *EI* entry inhibitors, *R5I* R5 attachment inhibitors, *ISTI* integrase strand transfer inhibitors, *n.a.* not available

low or undetectable in majority of patient samples.

- Maraviroc is a small, lipophilic, intermediately protein-bound compound that targets the human co-receptor CCR5 and is effective in preventing R5-tropic HIV virus entry. It is substrate of both cytochrome P450 3A4 and p-glycoprotein and drug-to-drug interaction, potentially affecting CSF concentrations, have been reported. The available data have been obtained with twice-daily different dosages (150 mg with PIs, 300 mg with NRTIs and nevirapine, and 600 mg with efavirenz or
- Integrase inhibitors are the latest ARV drug class and they are somehow heterogeneous: while all are small molecules and highly bound to plasma proteins, lipophilicity varies considerably (raltegravir is hydrophilic, while elvitegravir is lipophilic). So far, no data has been released on elvitegravir CSF exposure (a study is currently ongoing: ClinicalTrials.gov Identifier: NCT02251236) while a single study reported low dolutegravir CSF-to-plasma ratios (0.4%) but CSF concentrations

above IC_{50} in all samples (Letendre et al. 2014). Given raltegravir peculiar pharmacokinetics properties such as very wide inter- and intraindividual variability and an unclear concentration-dependant efficacy, its effectiveness in the CNS is still unknown.

Target Levels

The study of the pharmacodynamic effect of ARVs in the CNS is complicated by the absence of a clear pharmacodynamic target. The optimal marker would be the inhibition of HIV tissue replication in the whole brain parenchyma, although such marker is not currently available. Several other markers of CNS efficacy are currently being studied: CSF biomarkers, magnetic resonance imaging, astrocytes targeted positron emission tomography, and electroencephalographic rhythms. CSF HIV RNA and neurocognitive testing are currently used in clinical practice (Garvey et al. 2014).

The use of CSF HIV RNA as a marker of antiviral activity is the most commonly used marker since it is dramatically affected by HAART introduction and since the decrease in CSF replication parallels cognitive improvement in patients with HAD. Nevertheless, HIV replication may differ in different brain areas not accurately reflecting CSF levels. Furthermore, commercial kits for measuring HIV RNA have not been validated in the CSF and the threshold is unclear: second-generation methods can quantify as low as 20 copies/mL; very sensitive experimental techniques (quantifying 0.3–1 copies/mL) have been assessed showing residual CSF HIV RNA in a high proportion of subjects. This low-level HIV RNA in the CSF did not change after treatment intensification. However, subjects having the lowest viral load in the cerebrospinal fluid had the lowest levels of biomarkers of immune activation (such as neopterin, produced by macrophage-derived cells and believed to be a sensitive marker of CNS immune activation) (Dahl et al. 2014).

Cognitive function is analysed and monitored with neurocognitive tests; nevertheless, complete testing is time-consuming and it may be influenced by the choice of the control group and by learning effect (patients repeating slightly modified tests may perform better).

Given the inaccessibility of *in vivo* brain tissue, CSF inhibitory concentrations (IC_{50} , IC_{90} , and IC_{95}) have been used to compare the adequacy of ARVs exposure: these concentrations represent the level at which 50%, 90%, or 95% of viral replication is inhibited *in vitro* using wild-type viruses. However, these *in vitro* protein-free values have significantly variable values, and the same drug has been judged to reach optimal or insufficient concentrations in different studies when compared to different thresholds. Using standardized ICs, 95% inhibitory quotients (as CSF exposure divided by IC_{95}) have been described for several antiretrovirals: they represent how many times a drug overcomes the target level in a single patient. Patients with higher inhibitory quotients and detectable NRTIs had a better CSF viral control (Calcagno et al. 2015).

The CPE Score

The CNS penetration effectiveness score (CPE score) has been proposed by a large collaborative study group in the USA (the CHARTER group): in the revised 2010 version, ARVs were scored 1–4 (where 4 is the most neuro-effective drug, Table 1) according to drug characteristics, pharmacokinetics, and pharmacodynamic properties. In the last available analysis, several other demographic (race), clinical (current depression, current adherence to medications), and virological variables (plasma HIV RNA and duration of current HAART) have been added to CPE strata (<5, 5–10, ≥ 10), thus creating a “cerebrospinal fluid HIV risk score” (Hammond et al. 2014).

While in the original study it nicely correlated with the prevalence of CSF HIV RNA above 50 copies/mL, several other investigators have studied the composite CPE (obtained adding single drug scores to obtain a treatment score). Most

of the studies found a lower CSF HIV RNA with higher CPE score, while the effect on immune activation, MRI cerebral metabolite levels, and neurocognitive testing were less concordant among studies. Furthermore, only one study (out of three) found a correlation with CSF escape and CPE cutoffs are still debated.

Some limitations of the CPE score must be highlighted: the limited amount of evidence regarding PD data and regarding drugs' standard dosages, the absence of a clear cutoff, and the validation in patients receiving triple therapies and with fully sensitive viruses. As an example, a CPE corrected for plasma resistance-associated mutations was a better predictor (compared to standard CPE) of HAND in a cross-sectional study. For these reasons, some authors prefer not to use the aggregate CPE, but they suggest that treatment optimization in patients with CNS diseases may include drugs with individual elevated CPE score.

The CPE score may be a useful tool for choosing neuroactive antiretroviral drugs although with some limitations. Nevertheless, a recent review using rigorous methods found that neuro-HAART (i.e., a combination including ARVs with high CPE scores) was effective in improving neurocognitive function and decreasing CSF viral load (although only two of the included studies were adequately powered): this confirms the possible optimization of CNS treatment and calls for prospective, randomized, adequately powered studies (Cysique et al. 2011). The only randomized controlled trial trying to answer this question was prematurely interrupted for slow accrual (326 patients screened and 59 enrolled): CNS-targeted HAART was not associated with neither virological nor neurocognitive improvements although in patients with baseline-suppressed viral load, a trend for improved cognitive performances over time was observed (Ellis et al. 2014).

Efficacy in Monocytes, Macrophages, and Astrocytes

In vitro data suggest that the endogenous nucleoside pool in resting macrophages is smaller than

the one in activated lymphocytes and therefore that the effective phosphorylated NRTI levels required to inhibit HIV replication are lower. Some authors used in vitro effective concentration in acutely infected macrophages (EC_{50}) to calculate a "monocyte efficacy score": the obtained composite score was associated with neurocognitive performances and with the presence of HAND or minor motor cognitive disorders (Shikuma et al. 2012). Recent data challenging infected astrocytes with several NRTIs, NNRTIs, and raltegravir found that some drugs (zidovudine, lamivudine, and stavudine) may have inadequate inhibitory activity in astrocytes, with 90% inhibitory concentrations (EC_{90}) exceeding those achievable in the CSF.

These preliminary observations warrant further studies on the differential efficacy of ARVs according to target cells. Furthermore, the repeated association between HIV reservoir size (measured as PBMC- or monocyte-associated quantitative HIV DNA) and the prevalence of HAND support the implementation of specific drug strategies in selected patients (those with low CD4+ cells nadir, high HIV RNA zenith, and high cumulative viremia, for instance) (Valcour et al. 2013).

Conclusion

The infection of central nervous system immune cells by HIV is associated with severe long-term consequences. With the widespread use of highly active antiretroviral therapy, HIV-associated dementia is currently a rare AIDS-defining condition; less severe forms are however still common with multifactorial pathogenesis. Incomplete antiretroviral penetration into the central nervous system might be one of the reasons for compartmentalized infections and residual indirect neuronal damage. Several data has now associated lower central nervous system exposure (either measuring cerebrospinal fluid concentrations, ranking drugs according to the CPE, or monocyte activity scores) with detectable cerebrospinal fluid HIV RNA.

Cross-References

- ▶ [Comorbidity: Opioids](#)
- ▶ [Global NeuroAIDS](#)
- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [HIV Reservoirs in the Central Nervous System](#)
- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [Medication Adherence and HIV-Associated Neurocognitive Disorders \(HAND\)](#)
- ▶ [Neuro-AIDS, Immunopathogenesis of](#)
- ▶ [Overview of HIV CNS Infection](#)

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Antiretroviral Medications, Adult Care, and Treatment

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Definition

Antiretroviral medication(s): There are currently five main groups of antiretroviral (ARV) medications used to treat HIV. They are called ARV medications because they act against the life cycle of the retrovirus HIV. The five groups are as follows (of note, they are categorized by how they interfere with the HIV life cycle with the sixth group/designation “multi-class combination products” containing different combinations of ARV medications from the main groups working against various components of the HIV life cycle):

1. *Entry inhibitors*: ARV medications in this class of ARVs effectively interfere with the receptor-mediated entry of the HIV virus into a cell. One subclass of entry inhibitors is the CCR5 antagonists; and the one medication approved for use currently in this subclass is maraviroc, with *viroc* standing for *virus occupying*. A new medication in this class, cenicriviroc, is currently in preclinical studies and may be available soon.
2. *Fusion inhibitors*: ARV medications in this class of ARVs effectively interfere with the receptor-mediated entry of the HIV virus into a cell. One subclass of entry inhibitors is the fusion inhibitors; and the one medication approved for use currently in this subclass is enfuvirtide (or T-20, Fuzeon); but this specific medication is only available in parenteral (subcutaneous injection) form.
3. *Reverse transcriptase inhibitors* (of which there are two types):
 - (i) *Nucleoside reverse transcriptase inhibitors (NRTIs)*: ARV medications in this class of ARVs are based on molecules that are converted in the cell to structures analogous to DNA bases, which are incorporated into the DNA strand transcribed by HIV polymerase (or reverse transcriptase) from HIV RNA but block the DNA from further elongation.
 - (ii) *Non-nucleoside reverse transcriptase inhibitors (NNRTIs)*: ARV medications in this class of ARVs are molecules that block the action of HIV reverse transcriptase by binding a pocket of the reverse transcriptase and distorting the shape of the molecule.
4. *Protease inhibitors (PIs)*: ARV medications in this class of ARVs inhibit the activity of the HIV protease, which is required by HIV to cleave the precursor HIV proteins produced by the infected cell to the smaller proteins required for the assembly of new HIV virions.
5. *Integrase strand inhibitors (ISIs)*: ARV medications in this class block the integration of viral DNA into the nucleus (host genome) of cells.
6. *Multi-class combination products*: multiple classes of ARV medications are available in

these multi-class combination products, with are widely utilized in resource-replete settings as follows:

- Genvoya™ (tenofovir alafenamide + emtricitabine + cobicistat + elvitegravir)
- Triumeq™ (abacavir + lamivudine + dolutegravir)
- Stribild™ (tenofovir + emtricitabine + cobicistat + elvitegravir)
- Odefsey™ (tenofovir alafenamide + emtricitabine + rilpivirine)
- Complera™ (tenofovir + emtricitabine + rilpivirine)
- Atripla™ (tenofovir + emtricitabine + efavirenz)

Combination antiretroviral therapy (commonly referred to as ART): The use of three or more active ARV medications that act on different stages of the HIV life cycle. ART promotes immunologic recovery (CD4+ cell count increase) and virologic control (markedly reducing/suppressing viral burden as measured/quantified by circulating plasma HIV-1 RNA or viral load levels) and helps prevent the development of potentially debilitating and life-threatening opportunistic infections (OIs).

NOTE: The summary text that follows is based on care and management of HIV-infected adults residing in resource-replete settings and is based on the most current “Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents (July 14, 2016)” (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016).

HIV-infected adults residing in resource-limited settings (RLS) receive comprehensive care and treatment, and the summary text that follows is based on the World Health Organization’s (WHO) “Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection; Recommendations for a Public Health Approach” (release date June 2016) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). Of note, in these guidelines, based largely on published data from the recently completed START and TEMPRANO clinical

trials (TEMPRANO ANRS Study Group et al. 2015; INSIGHT START Study Group et al. 2015), the recommendation for care of HIV-infected persons residing in resource-limited settings is to provide lifelong ART to all children, adolescents, and adults, including all pregnant and breastfeeding women living with HIV, regardless of CD4+ cell count (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). This is a significant change, and it is important to note that although numerous countries within sub-Saharan Africa and other resource-constrained settings have adopted *test-and-start* (also referred to as *test-and-treat*) strategies (specifically the initiation of ART in HIV-infected children, adolescents, and adults regardless of immune status/CD4+ cell count), some countries still recommend ART initiation based on a person's immune status and/or clinical disease staging, i.e., HIV-infected adult having a CD4+ cell count less than 500 cells/mm³ and/or having a WHO clinical stage 3 or 4 condition. Within the next few years, it is anticipated that these remaining countries in RLS will all have transitioned to *test-and-start*.

In addition, in RLS, the ARV medication options, especially when it comes to second-line therapy, are considerably more limited, with few protease inhibitors (primarily lopinavir/ritonavir (provided in heat-stable Aluvia™ capsules or Kaletra™ tablets) as well as atazanavir/ritonavir being available for second-line therapy in many settings with integrase strand inhibitors just beginning to be available in some settings. Laboratory monitoring also differs in that in many RLS, plasma viral load monitoring is still not uniformly available, so response to therapy as well as suboptimal response to therapy decisions is largely based on clinical (the development of an incident WHO clinical stage 3 or 4 condition) and/or immunologic (suboptimal CD4+ cell count response) criteria.

Introduction

Without therapy, most HIV-infected adults will develop progressive immunosuppression, as evidenced by markedly reduced CD4+ cell

count values and significantly elevated levels of circulating plasma virus (i.e., very high plasma HIV-1 RNA levels), leading to the development of AIDS-defining illness(es) and premature death (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

The availability of ART for the treatment of HIV infection has improved dramatically since the advent of highly active antiretroviral therapy (formerly referred to as HAART) in 1996 (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). Due to the widespread availability of ART, millions of HIV-infected persons are now receiving lifelong ART and benefiting from significant reductions in HIV-/AIDS-associated morbidity and mortality (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). In fact, as per the most recent UNAIDS data, at the end of 2016, there were an impressive 19.5 million persons accessing ART (Cohen et al. 2011). As a result, markedly fewer proportions of HIV-infected persons are dying from AIDS-related conditions, and what we are witnessing is a burgeoning epidemic of noncommunicable diseases (NCDs), specifically cardiovascular, kidney, liver, and other end-organ complications (including non-AIDS-associated malignancies) among persons receiving these potentially life-saving ARV regimens. In addition, due to slow progress in HIV vaccine development in light of very impressive ARV medication treatment efficacy data, ARV medications are now also being recommended to prevent HIV transmission or acquisition. Landmark clinical trials originally presented in 2011 documented the impact of ARVs in preventing HIV sexual transmission (Thigpen et al. 2013; Grant et al. 2010; UNAIDS fact sheet 2017). The HIV Prevention Trials Network (HPTN) 052 study found that HIV-infected persons initiating ART with CD4+ cell counts between 350 and 550 cells/

mm³ had an unprecedented 96% reduced risk of transmitting HIV to uninfected, stable sexual partners (Cohen et al. 2011). Clinical and prevention synergy was confirmed when early ART also reduced disease progression in infected individuals. Knowledge of one's HIV status is critical for personal decision-making. If one's HIV-infected partner does not take ART or an HIV-uninfected person is potentially exposed by a non-stable partner, ARV medications can be used orally or topically (microbicide) by uninfected, vulnerable persons themselves to prevent infection, i.e., pre-exposure prophylaxis (PrEP).

“When to Start” Based on the results of these and related treatment as prevention studies, current practice in resource-replete settings of the world is to initiate ART in all adults found to be HIV-infected regardless of their CD4+ cell count (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016). There are certain priority medical conditions and/or clinical scenarios when HIV-infected adults should be offered immediate ART; these conditions/scenarios are listed below: (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2017; Zolopa et al. 2009; Makadzange et al. 2010; Boulware et al. 2014)

- *Pregnancy.*
- *AIDS-defining conditions* including HIV-associated dementia (HAD) and

AIDS-associated malignancies [**NOTE:** cryptococcal meningitis is a special case as based on published studies (Zolopa et al. 2009; Makadzange et al. 2010; Boulware et al. 2014) and on expert opinion, it is prudent to delay initiation of ART at least until after completion of antifungal induction therapy (the first 2 weeks) and possibly until the total induction/consolidation phase (10 weeks) has been completed (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2017). In addition, delay of ART may be particularly important in patients with evidence of increased intracranial pressure and/or in those patients with low

CSF white blood cell counts (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2017)].

- *Acute opportunistic infections.*
- *Lower CD4+ cell counts* (e.g., <200 cells/mm³).
- *HIV-associated nephropathy* (HIVAN).
- *Acute/early HIV infection* (if/when identified).
- *HIV/Hepatitis B co-infection.*
- *HIV/Hepatitis C co-infection.*

In terms of resource-constrained settings, numerous national public initiatives offering first-line combination antiretroviral therapy (ART) for HIV infection have commenced in sub-Saharan Africa since 2002 (Wester et al. 2009). In the African region (comprising the most current UNAIDS data from West, Central, East, and Southern Africa), which continues to bear the brunt of the HIV epidemic, more than 13.8 million persons were receiving potentially life-saving treatment at the end of 2016 compared to 50,000 persons approximately 14 years earlier (Cohen et al. 2011). Analyses from the region report favorable clinical/treatment outcomes and impressive declines in AIDS-related mortality among HIV-infected adults and children receiving ART (Wester et al. 2009).

HIV-infected adults residing in resource-limited settings (RLS) receive comprehensive care and treatment, and the summary text that follows is based on the World Health Organization's (WHO) “Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection; Recommendations for a Public Health Approach” (release date June 2016) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). HIV treatment recommendations in the current version of the WHO consolidated guidelines are largely based on published data from the recently completed TEMPRANO and START clinical trials (TEMPRANO ANRS Study Group et al. 2015; INSIGHT START Study Group et al. 2015), which showed the net benefit of initiating ART in HIV-infected adults having a CD4+ cell count of greater than 500 cells/mm³ compared to waiting until their CD4+ cell count

had declined to less than 350 cells/mm³. Specifically, in the START trial (INSIGHT START Study Group et al. 2015), a total of 4,685 patients were followed for a mean of 3.0 years. At study entry, their median viral load was 12,759 copies/ml, and the median CD4+ count was 651. Primary endpoint (serious AIDS events, serious non-AIDS events, or death) occurred in 1.8% of persons in the immediate group (immediate ART initiation – regardless of CD4+ cell count) versus 4.1% in deferred group (deferred initiation group – waiting until their CD4+ cell count declined to <350 cells/mm³), for a risk reduction of 57% (INSIGHT START Study Group et al. 2015). The protective effects/risk reduction of the immediate group was evident for both serious AIDS and serious non-AIDS events (greater for serious AIDS, TB, and cancer), and these findings were consistent regardless of age, sex, race, region of the world, as well as CD4+ cell count and viral load at entry (INSIGHT START Study Group et al. 2015) (NOTE: 54% of START trial participants were from low- and middle-income countries, and all 100% of TEMPRANO trial participants were enrolled in a West African setting, specifically Cote D’Ivoire (TEMPRANO ANRS Study Group et al. 2015; INSIGHT START Study Group et al. 2015)). In the TEMPRANO trial (TEMPRANO ANRS Study Group et al. 2015), immediate ART and 6 months of isoniazid preventive therapy (IPT) to eligible adults led to lower rates of severe illness than did deferred ART and no IPT, both overall and among patients with CD4+ cell count values of at least 500 cells/mm³. In summary, for HIV-infected children, adolescents, and adults residing in resource-constrained settings, the WHO consolidated guidelines (updated in June 2016) recommend the provision of lifelong ART to all children, adolescents, and adults, including all pregnant and breastfeeding women living with HIV, regardless of CD4+ cell count (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). This is a significant change, and it is important to note that although numerous countries within sub-Saharan Africa and other resource-constrained settings have adopted *test-and-start* (also referred to as *test-and-treat*) strategies (specifically the

initiation of ART in HIV-infected children, adolescents, and adults regardless of immune status/CD4+ cell count), some countries still recommend ART initiation based on a person’s immune status and/or clinical disease staging, i.e., HIV-infected adult having a CD4+ cell count less than 500 cells/mm³ and/or having a WHO clinical stage 3 or 4 condition. Within the next few years, it is anticipated that these remaining countries in RLS will all have transitioned to *test-and-start*. The WHO has also expanded earlier recommendations to offer PrEP to selected people at substantial risk of acquiring HIV (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). Alternative first-line ARV treatment regimens are also recommended, including an integrase inhibitor as an option in resource-limited settings and reduced dosage of a key recommended first-line drug, efavirenz, to improve tolerability and reduce costs (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). Implementing these guidelines fully will have an unprecedented impact on preventing people from becoming newly infected and reducing the number of people dying from HIV-related causes over the coming years (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). Based on these new *test-and-treat* (or *test-and-start*) strategy, initiating ART in all HIV-infected persons regardless of their immune status, the number of people eligible for ART increases significantly from 28 million to all 37 million people currently living with HIV globally (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016; UNAIDS fact sheet 2017). The new recommendations are aimed to aggressively address the lofty UNAIDS 90-90-90 goals, which aim to end the AIDS epidemic as a public health threat by 2030 (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). Specifically, the 90–90–90 goals include i) 90% of the people living with HIV knowing their HIV status, ii) 90% of the people who know their HIV status receiving ART, and iii) 90% of the people receiving ART having fully suppressed viral loads (Consolidated

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Fig. 1 Guidelines to start ART in Adults

4.3.1 When to start ART in adults (>19 years old)

Recommendation

- ART should be initiated in all adults living with HIV, regardless of WHO clinical stage and at any CD4 cell count (strong recommendation, moderate-quality evidence).
- As a priority, ART should be initiated in all adults with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4) and adults with CD4 count ≤ 350 cells/mm³ (strong recommendation, moderate-quality evidence).

Sources:

Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Geneva: World Health Organization 2015 (<http://www.who.int/hiv/pub/guidelines/early-release-arv/en>).

Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection; recommendations for a public health approach. Geneva: World Health Organization; 2013 (<http://www.who.int/hiv/pub/guidelines/arv2013/download/en>).

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guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

Please refer to the inserted Table below from the “Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach; June 2016 (World Health Organization)” (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

All patients initiating ART, regardless of setting, should be willing and able to commit to treatment and understand the benefits and risks of therapy and the importance of adherence.

“What to Start” Resource-replete settings: In resource-replete settings, the current recommendations for first-line ART in adults are as follows:

Recommended First-Line Regimens (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016):

1. **One integrase strand inhibitor (INSTI) + two nucleotide/nucleoside reverse transcriptase inhibitors (NtRTIs/NRTIs):**
 - DTG/ABC/3TC (TriumeqTM) — if HLA-B*5701 negative
 - DTG (TivicayTM) plus either TDF/FTC (TruvadaTM) or TAF/FTC (DescovyTM)
 - EVG/c/TAF/FTC (GenvoyaTM) or EVG/c/TDF/FTC (StribildTM)
 - RAL (IsentressTM) plus either TDF/FTC (TruvadaTM) or TAF/FTC (DescovyTM)

2. **Boosted PI + two NRTIs:**

- DRV/r (PrezistaTM) plus either TDF/FTC (TruvadaTM) or TAF/FTC (DescovyTM)

Alternative First-Line Regimens (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016):

1. **One non-nucleoside reverse transcriptase inhibitor (NNRTI) + two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs):**

- EFV/TDF/FTC (AtriplaTM)
- EFV plus TAF/FTC (DescovyTM)
- RPV/TDF/FTC (CompleraTM) or RPV/TAF/FTC (OdefseyTM)
(IF VL <100,000 AND CD4+ cell count >200)

2. **Boosted protease inhibitor (PI) plus two NRTIs**

- ATV/c (EvotazTM) or ATV/r (ReyatazTM) plus either TDF/FTC (TruvadaTM) or TAF/FTC (DescovyTM)
- DRV/c (PrezcobixTM) or DRV/r (PrezistaTM) plus ABC/3TC
(IF HLA-B*5701 negative)
- DRV/c (PrezcobixTM) plus either TDF/FTC (TruvadaTM) or TAF/FTC (DescovyTM)

Legend:

Integrase strand inhibitors (INSTIs)

DTG = dolutegravir

RAL = raltegravir

EVG = elvitegravir

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Fig. 2 First-line ART for Adults

4.4.1 First-line ART for adults

Recommendations

- First-line ART for adults^a should consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or an integrase inhibitor (INSTI).
- TDF + 3TC (or FTC) + EFV as a fixed-dose combination is recommended as the preferred option to initiate ART (strong recommendation, moderate-quality evidence).
- If TDF + 3TC (or FTC) + EFV is contraindicated or not available, one of the following alternative options is recommended:
 - AZT + 3TC + EFV
 - AZT + 3TC + NVP
 - TDF + 3TC (or FTC) + NVP (strong recommendation, moderate-quality evidence).
- TDF + 3TC (or FTC) + DTG or TDF + 3TC (or FTC) + EFV 400 mg/day may be used as alternative options to initiate ART (conditional recommendation, moderate-quality evidence). 
- Countries should discontinue d4T use in first-line regimens because of its well-recognized metabolic toxicities (strong recommendation, moderate-quality evidence).

^a Adults include pregnant and breastfeeding women, for whom additional guidance is found in Box 4.3.

3TC lamivudine, AZT zidovudine, d4T stavudine, DTG dolutegravir, EFV efavirenz, FTC emtricitabine, NVP nevirapine, TDF tenofovir
Source: Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. Geneva: World Health Organization; 2013 (<http://www.who.int/hiv/pub/guidelines/arv2013/download/en>).

Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs)

ABC = abacavir

3TC = lamivudine

FTC = emtricitabine

TAF = tenofovir alafenamide

TDF = tenofovir fumarate

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

EFV = efavirenz

RPV = rilpivirine

Protease inhibitors (PIs)

DRV = darunavir

ATV = atazanavir

Pharmacologic boosters

r = ritonavir (pharmacologic booster with intrinsic HIV activity but only at high doses)

c = cobicistat (pharmacologic booster without any intrinsic HIV activity)

NOTE: In terms of genetic testing, in resource-replete settings, ALL patients need to undergo HLA-B*5701 genetic testing prior to initiating abacavir to avoid the risk of potentially life-threatening hypersensitivity reactions that have been strongly associated with persons carrying the major histocompatibility complex (MHC) class I risk allele, specifically within the HLA-B serotype at position 57 (aptly named the HLA-B*5701 risk allele) (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Mallal et al. 2008; Martin et al. 2007).

Resource-Limited Settings

For a summary of “what to start” among HIV-infected adults residing in resource-limited settings of the world, please refer to the most recent Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV

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Fig. 3 First-line ART Regimens for Adults

Table 4.2. First-line ART regimens for adults (see Annex 11 for doses)

Preferred regimen	TDF + 3TC (or FTC) + EFV
Alternative regimens	AZT + 3TC + EFV (or NVP) TDF + 3TC (or FTC) + DTG ^a TDF + 3TC (or FTC) + EFV ₄₀₀ ^b TDF + 3TC (or FTC) +NVP
Special circumstances ^{c,d}	Regimens containing ABC and boosted PIs

^a Safety and efficacy data on DTG for pregnant and breastfeeding women and TB coinfection are still pending.
^b Efficacy data for EFV at a lower dose of 400 mg/day in the case of pregnant and breastfeeding women and TB coinfection are still pending.
^c Special circumstances may include situations where preferred or alternative regimens may not be available or suitable because of significant toxicities, anticipated drug–drug interactions, drug procurement and supply management issues, or for other reasons.
^d Using stavudine (d4T) as an option in first-line treatment should be discontinued.
 3TC lamivudine, ABC abacavir, AZT zidovudine, DTG dolutegravir, EFV efavirenz, FTC emtricitabine, NVP nevirapine, PI protease inhibitor, TDF tenofovir.

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Infection: Recommendations for a Public Health Approach; June 2016 (World Health Organization) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). The major “what to start” recommendations for adults are summarized in this table below:

Second-Line ART

Second-line ART for adults should consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) + a ritonavir-boosted protease inhibitor (PI) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

- After failure on a TDF + 3TC (or FTC)-based first-line regimen, use AZT + 3TC as the NRTI backbone in second-line regimens (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).
- After failure on an AZT or d4T + 3TC-based first-line regimen, use TDF + 3TC (or FTC) as the NRTI backbone in second-line regimens (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

Use of NRTI backbones as a fixed-dose combination is recommended as the preferred approach (*strong recommendation, moderate-*

quality evidence) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

- Heat-stable fixed-dose combinations LPV/r and ATV/r are the preferred boosted PI options for second-line ART (*strong recommendation, moderate-quality evidence*) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016). **NOTE:** Presently, the most widely used boosted PI for 2nd line ART is heat-stable lopinavir/ritonavir (Aluvia™).

Please see below summary for second-line ART in resource-limited settings as outlined in the WHO consolidated guidelines (June 2016 revised version) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

New Patient (Baseline Assessments)

Patients newly identified as being HIV-infected require an in-depth initial visit or two to determine their disease status (how much damage has been done to date to their immune system including identification of key comorbid medical conditions) as well as assess their overall psychological status (readiness to initiate ART).

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Fig. 4 Summary of preferred second-line ART Regimens for Adults and Adolescents

Table 4.16. Summary of preferred second-line ART regimens for adults and adolescents

Target population	Preferred second-line regimen ^a	
Adults and adolescents	If d4T or AZT was used in first-line ART	TDF + 3TC (or FTC) + ATV/r or LPV/r ^{b,c}
	If TDF was used in first-line ART	AZT + 3TC + ATV/r or LPV/r ^{b,c}
Pregnant or breastfeeding women	Same regimens as recommended for adults and adolescents	
HIV and TB coinfection	If rifabutin is available	Standard PI-containing regimens as recommended for adults and adolescents
	If rifabutin is not available	Same NRTI backbones as recommended for adults and adolescents plus double-dose LPV/r (that is, LPV/r 800 mg/200 mg twice daily) ^d
HIV and HBV coinfection	AZT + TDF + 3TC (or FTC) + (ATV/r or LPV/r) ^b	

^a ABC and didanosine (ddI) can be used as NRTI back-up options but add complexity and cost without clinical advantages.

^b DRV/r can be used as an alternative PI option.

^c RAL + LPV/r can be used as an alternative second-line regimen (conditional recommendation, low-quality evidence).

^d Standard LPV/r and RTV-boosted saquinavir (SQV/r) doses with an adjusted dose of RTV (that is, LPV 400 mg/ RTV 400 mg or SQV 400 mg /RTV 400 mg twice daily) can be used as alternative options.

3TC lamivudine, ATV atazanavir, AZT zidovudine, d4T stavudine, FTC emtricitabine, LPV lopinavir, NRTI nucleoside reverse-transcriptase inhibitor, NVP nevirapine, PI protease inhibitor, r or RTV ritonavir, TDF tenofovir.

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Fig. 5 Second-line ART in resource-limited settings

Recommendations

- Second-line ART in adults should consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a ritonavir-boosted protease inhibitor (PI).
- The following sequence of second-line NRTI options is recommended:
 - After failure on a TDF + 3TC (or FTC)-based first-line regimen, use AZT + 3TC as the NRTI backbone in second-line regimens.
 - After failure on an AZT or d4T + 3TC-based first-line regimen, use TDF + 3TC (or FTC) as the NRTI backbone in second-line regimens.
- Use of NRTI backbones as a fixed-dose combination is recommended as the preferred approach (strong recommendation, moderate-quality evidence).
- Heat-stable fixed-dose combinations of ATV/r and LPV/r are the preferred boosted PI options for second-line ART (strong recommendation, moderate-quality evidence).
- Heat-stable fixed-dose combinations of DRV/r can be used as an alternative boosted PI option for second-line ART (conditional recommendation, low-quality evidence). 
- A combination of RAL plus LPV/r can be used as an alternative second-line ART regimen (conditional recommendation, low-quality evidence). 

Source: Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection; recommendations for a public health approach. Geneva: World Health Organization; 2013 (<http://www.who.int/hiv/pub/guidelines/arv2013/download/en>).

At the time of initial evaluation, the following baseline assessments should be performed: (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Aberg et al. 2014).

Resource-Replete Settings

Baseline laboratory testing (at time of entry into care): (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Aberg et al. 2014)

- Comprehensive metabolic panel/chemistry (including blood urea nitrogen plus creatinine).
- Complete blood count (CBC).
- Urinalysis (and calculated creatinine clearance; current recommended formula is CKD-EPI (Inker et al. 2012)).
- Fasting lipid profile (total/HDL and LDL cholesterol + triglycerides).
- Fasting glucose or hemoglobin A1C testing.
- Hepatitis B screening (HBsAg, HBsAb, anti-HBc, or HBcAb) and those who are susceptible to infection should be vaccinated against HBV (**NOTE:** HBsAb should be repeated 1–2 months or at the next scheduled visit after the third vaccine was given to assess for immunogenicity; a second series of vaccine is recommended for patients whose HBsAb levels are negative/not detectable OR <10 IU/mL after the primary vaccine series) (Aberg et al. 2014).
- Hepatitis C screening (HCV antibody).
- Syphilis screening (VDRL/RPR).
- CD4+ cell count (absolute and percentages).
- Plasma HIV-1 RNA (viral load).
- HIV resistance testing (HIV-1 genotype).
- HLA B*5701 screening (**NOTE:** needs to be done before initiating ANY patient on abacavir but often routine practice to obtain in all patients at baseline).
- Toxoplasmosis prior exposure/screening (anti-toxoplasma IgG).
- Morning serum testosterone levels are recommended in adult men with decreased libido, erectile dysfunction, reduced bone mass or low trauma fractures, hot flashes, or sweats and should be considered in the setting of less specific symptoms (i.e., fatigue, depression).

Vaccine recommendations (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Aberg et al. 2014) (**NOTE:** please refer to above laboratory testing section for information pertaining to hepatitis B vaccine recommendations):

- Hepatitis A vaccination is recommended for ALL susceptible men who have sex with men (MSM), as well as other susceptible persons (e.g., injection drug users, persons with chronic liver disease, travelers to countries

with high endemicity, *or* patients who are hepatitis B and/or C infected) (**NOTE:** hepatitis A total or IgG Ab should be repeated 1–2 months or at the next scheduled visit after the second vaccine to assess for immunogenicity).

- Varicella primary vaccination may be considered in HIV-infected, VZV-seronegative persons aged >8 years with CD4+ cell count values >200 cells/mm³.
- HPV vaccination is recommended for all females aged 9–26 years and all males aged 9–21 years.
- Pneumococcal vaccine is recommended (all should receive a dose of PCV13 (Pneumovax) followed by a dose of PPV23 (Pneumovax) at least 8 weeks later (**NOTE:** if previously vaccinated with PPV23, give PCV13 at least 1 year after PPV23; a second PPV23 dose is recommended 5 years after the initial/first PPV23 dose).
- Influenza vaccine annually (inactivated recommended; DO NOT use live attenuated intranasal vaccine (FluMist))
- Polio vaccine (for travelers to endemic areas) (**NOTE:** OPV contraindicated; use IPV)
- *Haemophilus influenzae* type B vaccine (**ONLY** if asplenic)

Sexually transmitted infection (STD) screening: (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Aberg et al. 2014)

- All women should be screened for trichomoniasis, and all women aged ≤ 25 years of age should be screened for *Chlamydia trachomatis* infection.
- Men and women should be screened for gonorrhea and chlamydia infection at initial presentation and then annually if at risk for infection.

Additional screening:

- Cervical PAP smear (all HIV-infected women at time of entry into care and then repeat at 6 months and then annually if results normal): women with atypia (atypical squamous cells of unknown significance or cannot rule out high-grade intraepithelial lesion, atypical

glandular cells, low- or high-grade squamous intraepithelial lesions, or squamous carcinoma by Pap testing) should undergo colposcopy and directed biopsy, with further treatment as indicated by results of evaluation.

- Baseline chest radiography (CXR) should be done in all HIV-infected persons having a positive TB screening test result (**NOTE:** it may also be useful in other patients likely to have preexisting lung abnormalities).

Resource-Limited Settings

- Comprehensive metabolic panel/chemistry (needs to include at a minimum kidney (creatinine) and liver function (SGPT/ALT) testing)
- Complete blood count (CBC)
- Urinalysis (and calculated creatinine clearance; current recommended formula is CKD-EPI (Inker et al. 2012))
- Fasting lipid profile (total/HDL and LDL cholesterol + triglycerides) (**ONLY** when/if commencing a protease inhibitor-containing regimen)
- Hepatitis B screening
- Syphilis (RPR/VRDL) screening
- CD4+ cell count (absolute and percentage)
- Plasma HIV-1 RNA (viral load) (**when available**)
- Vaccines (pneumococcal, rotavirus, etc. when available/applicable)
- Chest radiography (optional but would consider obtaining in all patients at baseline)

Longitudinal Care/Assessments

Resource-Replete Settings

- Chemistry (every 6–2 months) (**NOTE:** includes serum Na, K, HCO₃, Cl, BUN, creatinine (including calculated estimated glomerular filtration rate (eGFR) using the CKD-EPI formula), glucose (preferably fasting))
- Complete blood count (CBC) with differential (every 3–6 months)
- Urinalysis (every 6 months if on TDF-containing ART)
- Fasting lipid panel (repeat annually if normal)

- Fasting glucose or hemoglobin A1C testing (repeat annually if normal)
- CD4+ cell count testing (every 3–6 months) (**NOTE:** reduce to every 12 months after initiated on ART in presence of consistently suppressed viral load if CD4+ cell count in 300–500 cells/mm³ range; if CD4+ cell count >500 cells/mm³ in a patient with consistently suppressed viral load, then CD4+ cell count testing is **OPTIONAL**)
- HIV-1 RNA (viral load): need to repeat 2–8 weeks after ART initiation or modification and then every 3–6 months (and also at time of treatment failure and/or when clinically indicated)
- Tropism testing (if considering a CCR5 antagonist)
- HLA*B5701 testing (if considering abacavir therapy)

Resource-Limited Settings

At the time of HIV diagnosis:

Recommended:

- HIV testing (serology for adults and children 18 months or older)
- CD4+ cell count
- TB symptom screening

Desirable (if available):

- Hepatitis B (hepatitis B surface antigen (HBsAg)) serology
- Hepatitis C virus (HCV) serology
- Cryptococcal antigen (if CD4+ cell count <100 cells/mm³)
- Screening for sexually transmitted infections (STIs)
- Pregnancy test to assess if ART initiation should be prioritized to prevent HIV transmission to the child
- Assessment for major noncommunicable chronic diseases (NCDs) and comorbidities

Follow-up (pre-ART initiation – if applicable):

- CD4+ cell count (every 6–12 months in circumstances where ART initiation is delayed)

At time of ART initiation:**Desirable:**

- Pregnancy test
- Hemoglobin test for starting AZT/ZDV
- Serum creatinine and calculated estimated glomerular filtration rate (eGFR) OR when starting TDF
- Alanine aminotransferase (ALT/SGPT) for starting nevirapine (NVP)
- Baseline CD4+ cell count

Longitudinally:**Recommended:**

- CD4+ cell count (every 6 months until clinically stable and then every 12 months thereafter)
- HIV-1 RNA (viral load): at 6 and 12 months post ART initiation and then every 12 months thereafter (and also at time of treatment failure and/or when clinically indicated)

Desirable:

- Serum creatinine and eGFR for TDF
- Pregnancy test, especially for women of child-bearing age not receiving family planning and on treatment with DTG or low-dose EFV

Opportunistic Infection Prophylaxis**Resource-Replete Settings**

- In general, all patients in resource-replete settings need to be initiated on routine prophylaxis to protect against *Pneumocystis jiroveci* (formerly *carinii*) (PCP) pneumonia and disseminated *Mycobacterium avium* complex (MAC) infection based on their level of immunosuppression (CD4+ cell count value) (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016).
 - If CD4+ cell count <200 cells/mm³

Commence prophylaxis with one double-strength (DS) Bactrim (160 mgs trimethoprim/800 mgs sulfamethoxazole) per day (**NOTE:** also protects against toxoplasmosis infection)

(**NOTE:** For sulfa allergic patients, provide prophylaxis with oral dapsone 75–100 mgs daily; however, for severely sulfa allergic patients as dapsone still contains a minor sulfa moiety, one needs to prophylax for PCP with monthly aerosolized pentamidine treatments; some care providers may also opt to prophylax with atovaquone/mepron);

PCP prophylaxis may be discontinued in persons having a sustained (two or more successive readings) CD4+ cell count value of >200 cells/mm³.)

- If CD4+ cell count <50 cells/mm³

Commence prophylaxis with azithromycin (1200 mgs orally once per week; typically prescribed every Monday morning)

MAC prophylaxis may be discontinued in persons having a sustained (two or more successive readings) CD4+ cell count value of >100 cells/mm³.

Resource-Limited Settings

***Pneumocystis jiroveci* pneumonia (PJP) prophylaxis:** Cotrimoxazole (CTX) prophylaxis is recommended for adults (including pregnant women) with severe or advanced HIV clinical disease (WHO stage 3 or 4) and/or with a CD4 count ≤350 cells/mm³ (strong recommendation, moderate-quality evidence). In settings where malaria and/or severe bacterial infections are highly prevalent, cotrimoxazole prophylaxis should be initiated regardless of CD4 cell count or WHO stage (conditional recommendation, moderate-quality evidence). Cotrimoxazole prophylaxis may be discontinued in adults (including pregnant women) with HIV who are clinically stable on ART, with evidence of immune recovery and viral suppression (conditional recommendation, low-quality evidence) (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

Continuation of CTX prophylaxis regardless of ART status, age, CD4+ cell count, or WHO clinical stage in settings with a high prevalence of malaria and/or severe bacterial infections (SBIs) is also recommended based on data from randomized controlled trials, which show significant reduction in the risk of hospitalization, malaria,

Antiretroviral Medications, Adult Care, and Treatment, Table 1 List of currently available/approved antiretroviral medications (adapted from AIDSMEDES: Your Ultimate Guide to HIV Care; <http://www.aidsmeds.com/list.shtml>)

	Drug	Abbrev.	Generic name
Single-Tablet Regimens			
	Atripla	N/A	Efavirenz + tenofovir disoproxil fumarate + emtricitabine
	Complera	N/A	Rilpivirine + tenofovir disoproxil fumarate + emtricitabine
	Genvoya	N/A	Elvitegravir + tenofovir alafenamide + emtricitabine + cobicistat
	Odefsey	R + FTC + TAF	Rilpivirine + emtricitabine + tenofovir alafenamide
	Stribild	Quad	Elvitegravir + cobicistat + tenofovir disoproxil fumarate + emtricitabine
	Triumeq	Trii	Dolutegravir + abacavir + lamivudine
E	Bictegravir + tenofovir alafenamide + emtricitabine	BIC + TAF + FTC	Bictegravir + tenofovir alafenamide + emtricitabine
E	Dolutegravir + rilpivirine	DTG + RPV	Dolutegravir + rilpivirine
E	Doravirine + tenofovir disoproxil fumarate + lamivudine	N/A	Doravirine + tenofovir disoproxil fumarate + lamivudine
Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs)			
	Combivir*	AZT + 3TC	Zidovudine + lamivudine
	Descovy	FTC + TAF	Emtricitabine + tenofovir alafenamide
	Emtriva	FTC	Emtricitabine
	Epivir*	3TC	Lamivudine
	Epzicom*	ABC + 3TC	Abacavir + lamivudine
	Retrovir*	AZT	Zidovudine
	Trizivir	ABC + AZT + 3TC	Abacavir + zidovudine + lamivudine
	Truvada	TDF + FTC	Tenofovir disoproxil fumarate + emtricitabine
	Videx EC*	Ddl	Didanosine
	Viread	TDF	Tenofovir disoproxil fumarate

(continued)

Antiretroviral Medications, Adult Care, and Treatment, Table 1 (continued)

	Drug	Abbrev.	Generic name
	Zerit*	d4T	Stavudine
	Ziagen*	ABC	Abacavir
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)			
	Edurant	RPV	Rilpivirine
	Intelence	ETR	Etravirine
	Rescriptor	DLV	Delavirdine
	Sustiva	EFV	Efavirenz
	Viramune and Viramune XR*	NVP	Nevirapine
E	Doravirine	N/A	Doravirine
Protease inhibitors (PIs)			
	Aptivus	TPV	Tipranavir
	Crixivan	IDV	Indinavir
	Evotaz	ATV/c	Atazanavir + cobicistat
	Invirase	SQV	Saquinavir
	Kaletra	LPV/r	Lopinavir + ritonavir
	Lexiva	FPV	Fosamprenavir
	Norvir	RTV	Ritonavir
	Prezcobix	DRV/c	Darunavir + cobicistat
	Prezista	DRV	Darunavir
	Reyataz	ATV	Atazanavir
	Viracept	NFV	Nelfinavir

(continued)

Antiretroviral Medications, Adult Care, and Treatment, Table 1 (continued)

	Drug	Abbrev.	Generic name
Integrase inhibitors			
	Isentress	RAL	Raltegravir
	Tivicay	DTG	Dolutegravir
	Vitekta	EVG	Elvitegravir
Entry inhibitors			
	Fuzeon	ENF	Enfuvirtide
	Selzentry	MVC	Maraviroc
E	Ibalizumab	N/A	Ibalizumab
E	Pro 140	N/A	N/A
Pharmacokinetic enhancers			
	Tybost	N/A	Cobicistat

and diarrhea among adults and children with HIV in settings with a high prevalence of malaria and/or SBIs (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016; Campbell et al. 2012; Polyak et al. 2014; Bwakura-Dangarembizi et al. 2014). In addition, the recommendation to continue CTX prophylaxis in settings with a high prevalence of malaria and/or SBIs may simplify HIV management, forecasting, and supply management issues. However, in settings where malaria is not endemic and/or the patient has not had evidence of severe bacterial infections, then CTX can be safely discontinued if the ART-treated adult has experienced immunologic recovery and/or viral suppression on ART (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016).

In settings where malaria and/or severe bacterial infections are highly prevalent, cotrimoxazole prophylaxis should be continued regardless of CD4 cell count or WHO clinical stage (conditional

recommendation, moderate-quality evidence). Routine cotrimoxazole prophylaxis should be given to all HIV-infected patients with active TB disease regardless of CD4+ cell count (strong recommendation, high-quality evidence).

Conclusions

In summary, the care and management of adults receiving potentially life-saving antiretroviral therapy (ART) is complex and requires considerable experience and training. Fortunately, comprehensive and frequently updated HIV ART-specific guidelines exist (Panel on Antiretroviral Guidelines for Adults and Adolescents 2016; Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2016) and are routinely available to healthcare providers. For complex care and management questions, it is always advisable to consult an expert/experienced HIV specialist (Table 1).

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Antiretroviral Therapy and Drug Resistance in HIV-2 Infection

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Definition

This entry reports on the susceptibility of HIV-2 to antiretroviral drugs and debates about the management of antiretroviral therapy in HIV-2-infected patients.

Introduction

Human immunodeficiency virus type 2 (HIV-2) is known to be less pathogenic and far less prevalent than HIV type 1 (HIV-1). However, HIV-2 affects an estimated 1–2 million people in West Africa (Gottlieb et al. 2008). HIV-2 has a lower infectivity than HIV-1, which is likely to be related to lower viral RNA levels. Even though its rate of progression is slower than HIV-1 infection, with a greater proportion of long-term nonprogressors (Thiebaut 2010), HIV-2-infected patients may develop advanced immunodeficiency and experience adverse clinical events.

Most antiretroviral drugs have been designed for HIV-1 and may not provide optimal viral suppression of HIV-2 infection. Furthermore, no randomized trials have assessed when and how to treat HIV-2-infected patients. The natural resistance of HIV-2 to non-nucleoside reverse-transcriptase inhibitors (NNRTIs) and the lack of commercially available viral-load assays complicate the treatment and monitoring of HIV-2-infected patients.

Susceptibility of HIV-2 to Antiretroviral Drugs

HIV-1 and HIV-2 are related but differ by ~50–60% at the nucleotide level, with significant differences in amino acid sequences. The structures of reverse transcriptase (RT), protease (PR), and integrase (IN) of the two viruses are relatively similar, especially when the catalytic sites are considered. Many studies have described the development of HIV-1 resistance to all classes of antiretroviral drugs, but there is limited information regarding HIV-2, which is naturally resistant to enfuvirtide (a fusion inhibitor) and to all currently used NNRTIs. In addition, HIV-2 exhibits a low genetic barrier to nucleoside reverse-transcriptase inhibitors (NRTI) with more frequent selection of the Q151M NRTI multidrug resistance mutation, and it is intrinsically or partially resistant to several protease inhibitors.

Entry Inhibitors

The process of HIV-2 entry involves identical steps to HIV-1, including having similar virus–cell interactions, first between the HIV-2 gp105 envelope and CD4 receptor and then between the V3 loop of gp105 and the CCR5 or CXCR4 coreceptors. HIV-2 isolates can use a broad range of coreceptors to produce *in vitro* infection including CCR1, CCR2b, CCR3, CCR8, BOB/GPR15, and BONZO/CXCR6. Genotypic determinants of HIV-2 tropism located in the gp105 V3 loop were recently identified, and a strong association between HIV-2 phenotypic tropism and V3-loop sequences, which allow the prediction of CCR5- and/or CXCR4-tropic viruses in HIV-2 infection, has been reported (Visseaux et al. 2012). The four major genotypic determinants of dual/CXCR4 tropism identified are a mutation at residue 18, the V19K/R mutation, insertions at residue 24, and a V3 global net charge.

Maraviroc works by binding to the CCR5 coreceptor to prevent HIV-1 from entering the cell, but its use is limited to patients with CCR5-tropic viruses. The activity of maraviroc against the HIV-2 CCR5 virus has been recently evaluated using a phenotypic PBMC-based test:

TZM-bl reporter cells showed that maraviroc is active *in vitro* against CCR5 HIV-2 clinical isolates and has similar 50% inhibitory concentration values as HIV-1 (Borrego et al. 2012; Visseaux et al. 2012). However, discrepancies exist between studies and cell-line models with regard to determining tropism: in the Borrego study, the 90% inhibitory concentrations were found to be significantly higher than for HIV-1.

Regarding the fusion inhibitors, HIV-2 displays natural resistance to enfuvirtide. A recent study showed that enfuvirtide was 211-fold less active against primary HIV-2 isolates, which confirms and extends the previous results based on laboratory-adapted viral isolates (Borrego et al. 2012).

Nucleoside Reverse-Transcriptase Inhibitors (NRTIs)

Although most *in vitro* studies have shown that similar concentrations of NRTIs are needed to block both HIV-1 and HIV-2 replication (Witvrouw et al. 2004), a few published studies suggest that some drugs may not be as effective against HIV-2 (Reid et al. 2005). Many of the amino acid substitutions that are associated with NRTI resistance in HIV-1 also seem to be implicated for HIV-2. Amino acids at six positions in wild-type HIV-2 are analogous to secondary or accessory drug-resistant mutations in HIV-1 (69 N, 75I, 118I, 210 N, 215S, 219E). These residues may predispose HIV-2 to distinct evolutionary pathways when responding to drug treatments.

With regard to the thymidine analogues, zidovudine (ZDV) and stavudine, most studies have reported that HIV-2 and HIV-1 are equally sensitive to these in both culture and cell-free biochemical assays, whereas two reports suggest that HIV-2 might be relatively resistant to ZDV (Boyer et al. 2012; Reid et al. 2005). HIV-1 more readily incorporates ZDV and is more susceptible to ZDV than HIV-2, and higher concentrations of ZDV are needed durably to suppress the replication of HIV-2 compared to HIV-1 (Ntemgwa et al. 2009). Resistance to thymidine analogues does not follow the usual pathway of selection of mutations at positions 41, 67, 70, 210,

215, and 219. Instead, the selection of the Q151M multidrug-resistant mutation, which confers various levels of *in vitro* phenotypic resistance to almost all NRTIs except tenofovir, is frequently observed (Descamps et al. 2004). This mutation is also easily selected by didanosine. In combination with the mutation at codon 111 (V111I) – which can also occur as a natural polymorphism – this mutation confers high-level resistance to all NRTIs. Mutations at codon 215 (S215A/C/F/L/P/Y) can also be observed with thymidine analogues. However, mutations are rarely observed at positions 67 and 70 and never at positions 41 and 210. Several years ago, it was suggested that the mechanisms responsible for ZDV resistance are different for HIV-1 and HIV-2 and that the exclusion pathway plays a more important role in HIV-2 than selective excision. Recently, additional experiments have confirmed that although HIV-1 RT can adopt an exclusion- or excision-based resistance mechanism against ZDV, HIV-2 RT can only use the exclusion mechanism (Ntemgwa et al. 2009; Boyer et al. 2012).

Non-nucleoside Reverse-Transcriptase Inhibitors

HIV-2 is naturally resistant to all NNRTIs. Natural resistance of HIV-2 to NNRTIs is thought to be due to the Y188L polymorphism, which is naturally present in all HIV-2 isolates. Reversion to Y188 renders the RT of HIV-2 susceptible to some NNRTIs, including efavirenz and delavirdine. The NNRTIs (nevirapine, delavirdine, and efavirenz) and recently approved etravirine and rilpivirine possess limited activity against HIV-2 and are needed at effective concentrations of at least 50-fold higher than those that inhibit HIV-1, which renders the use of these drugs inappropriate for HIV-2-infected patients.

Integrase Inhibitors

The integrase inhibitors, raltegravir, elvitegravir, and dolutegravir, have shown *in vitro* activity against wild-type HIV-2 strains. The phenotypic susceptibility of clinical HIV-2 isolates to integrase inhibitors is similar to that of HIV-1, and this may be because the key catalytic motifs are fully conserved in HIV-2 at the genomic

positions described for HIV-1. Several naturally occurring amino acids in HIV-2 integrase, which are equivalent to secondary raltegravir or elvitegravir resistant-associated replacements in HIV-1, have been observed.

Recent biochemical and phenotypical studies have shown that HIV-2 resistance to raltegravir involves pathways (Y143C, G140S/Q148R, G140S/Q148H, N155H) previously identified as resistance determinants in the HIV-1 coding sequences. The N155H and G140S/Q148R mutations make similar contributions to resistance in both HIV-1 and HIV-2. The G140S mutation confers little resistance but compensates for the catalytic defect because of the Q148R mutation. Conversely, Y143C alone did not confer resistance to raltegravir unless E92Q was present. Furthermore, the introduction of the Y143C mutation into the N155H-resistant background decreased the resistance level of enzymes containing the N155H mutation. In addition, it was observed *in vitro* that the Y143C, Q148R, and N155H replacements in HIV-2 ROD9 integrase compromised viral replication capacity.

Protease Inhibitors (PIs)

Compared to HIV-1, HIV-2 expresses natural polymorphisms in the PR at positions 10I/V, 20 V, 32I, 33 V, 36I, 46I, 47 V, 63E/K, 71 V, 73A, 77 T, 82I, and 93 L, which may be implicated in drug resistance. Some natural polymorphisms, such as 46I and 47 V, give HIV-2 intrinsic resistance to amprenavir. Indeed, these mutations are also associated with amprenavir resistance in HIV-1, and several studies have shown that HIV-2 wild-type isolates reduce susceptibility to amprenavir compared to HIV-1 (Witvrouw et al. 2004).

Biochemical kinetic studies have demonstrated that protease inhibitors (PIs) bind to HIV-2 with a 10- to 100-fold lower affinity than to HIV-1, depending on the inhibitor. Studies have shown that the differences in phenotypes between wild-type HIV-2 and HIV-1 may depend on the PI being tested. Two independent studies have shown that only saquinavir, lopinavir, and darunavir have similar 50% inhibitory concentration values against the wild-type HIV-2 that are

within the same range as for HIV-1. Relative to the HIV-1 reference strain, the median 50% inhibitory concentrations of the HIV-2 wild-type isolates were 31-fold higher for amprenavir, eightfold higher for atazanavir, and three- to fourfold higher for indinavir and nelfinavir. However, there is controversy regarding tipranavir, with one study reporting natural resistance and another reporting full susceptibility. These data suggest that saquinavir, lopinavir, and darunavir may be preferred therapeutic options for HIV-2-infected patients. Drug selection studies in tissue cultures have shown that PR natural polymorphisms can accelerate the time to the development of resistance to several PIs, with selection of I54M, I82L, I84V, and L90M mutations responsible for reducing phenotypic susceptibility. As regards darunavir, there are currently not enough data on its resistance pathways, which affects the possibility of it being used as a sequential therapy.

To date, only one study has investigated whether mutations at Gag-PR cleavage sites can affect HIV-2 drug resistance in the same way as for HIV-1. Indeed, in HIV-2 PI-experienced patients, the presence at the baseline of A430V substitution in the NC/p1 gag cleavage site is associated with a worse virological response to lopinavir.

When to Start Antiretroviral Therapy

The optimal time to start antiretroviral therapy (ART) still needs to be defined: for many years, recommendations have been based on those used and validated for HIV-1 infection, and no randomized trials have addressed this question for HIV-2.

To date, the following arguments have been put forward for starting ART earlier than in HIV-1 infection:

- (i) The poor immunological response to a combined ART (cART) observed in several retrospective studies. In particular, a study compared HIV-2- and HIV-1-infected patients receiving a first cART and matched for factors associated with disease progression in HIV-1 infection: gender, age, HIV

transmission group, and period of treatment initiation (Drylewicz 2010). This study reported a lower increase in CD4-cell count during the first 2 months of treatment (+25cells/mm³/month for HIV-2 vs. +60 for HIV-1) and for the first year (-3 cells/mm³/year for HIV-2 vs. +46 for HIV-1). In addition, the plasma viral-load drop was three-fold more important in HIV-1 patients: [1.56 log₁₀/ml/month versus 0.62 among HIV-2 patients ($p < 10^{-4}$)]. Of note, there was no difference in baseline CD4-cell count depending on HIV type. Other non-comparative studies have reported a poor immunological response whatever the antiretroviral regimen tested, triple NRTI or a PI combination (Matheron et al. 2006; Benard et al. 2009, 2011). This dramatic difference in response to cART could be explained by the delay in giving therapy to these infected patients: the longer infection time before experiencing a decrease in CD4 compared to those infected by HIV-1 suggests that earlier treatment is needed, even if the ability of the antiretroviral drugs to reduce plasma HIV-2 RNA seems limited.

- (ii) Even if the proportion of HIV-2 long-term nonprogressors in clinical cohorts is higher than among HIV-1-infected patients (6% vs. 0.43%), a decrease in CD4-cell count occurs during the natural history of HIV-2 (Thiebault 2011). However, the slope is probably flatter than that for HIV-1, and the CD4-cell count threshold used for HIV-1 infection to start cART is reached later during the course of HIV-2 disease.
- (iii) Plasma HIV-2 RNA, which is less frequently detected in HIV-2-infected patients and is lower when it is detected (median 2.8 log copies/ml), as well as the lack of a commercially available assay, makes this virological response difficult to monitor. Nevertheless, plasma viral load seems to be predictive of clinical progression when it reaches a peak of 1000 copies/ml, whatever the CD4-cell count.

The current recommendations state that cART should be started in patients with a CDC stage

B or C infection and when the CD4-cell count is $<500/\text{mm}^3$. In patients whose plasma RNA is >1000 copies/ml in two successive samples, a closer follow-up is recommended, and if the slope of the CD4 decrease becomes steeper (even if this remains less steep than that seen in HIV-1 progression, which may be $>50/\text{year}$), then this can be considered as an indication for cART. As a minimum, treatment should be considered earlier in cases of hepatitis B or C coinfection, if the patient is aged >50 years or if there are cardiovascular risk factors, similar to the guidelines for HIV-1 infection (DHSS, Gilleece et al. 2010; Yeni 2010).

What Treatment to Start

Randomized trials are the only way to determine effective antiretroviral combinations for both antiretroviral-naïve and experienced patients; however, most data available for HIV-2 therapy are from small, observational studies (Gottlieb et al. 2008).

Given the resistance of HIV-2 to NNRTIs, the French and British guidelines recommend starting treatment with two NRTIs and a boosted PI, either lopinavir or darunavir (Gilleece et al. 2010; Yeni 2010). The US Department of Health and Human Services (DHHS) HIV treatment guidelines suggest starting with a boosted-PI regimen, but do not specify which drugs should be used – saquinavir, lopinavir, or darunavir performed in vitro against HIV-2 with the same efficacy as HIV-1. Successful treatment has been reported in patients receiving two NRTIs plus lopinavir/ritonavir or indinavir/ritonavir (Benard et al. 2009; Jallow et al. 2009). Lopinavir may select the V47A resistance mutation (Jallow et al. 2009), which makes the virus hypersusceptible to saquinavir but does not affect the efficacy of darunavir, thus suggesting that PIs could be introduced in sequence. With regard to darunavir, there are no data on the resistance mutations selected. The NRTI backbone of this first-line antiretroviral therapy may be tenofovir/emtricitabine (or lamivudine) or zidovudine/lamivudine. As in HIV-1 infection, tenofovir/ emtricitabine may be

preferred on the basis of its tolerability. In cases of failure, tenofovir will select the K65R mutation, which seems to preclude the development of thymidine analogue mutations as described for HIV-1. Finally, a good first-line regimen option would be tenofovir/emtricitabine/boosted lopinavir, as assessed by studies reporting a response rate of 60% in up to 96 weeks, based on CD4 and HIV-2 RNA composite endpoints (Benard et al. 2009).

Although there are no data that recommend the use of raltegravir as a first-line regimen, this compound, which has been proved to be active in vitro against HIV-2, may be a preferred option in association with the NRTI backbone as an initial therapy. Given its low genetic barrier to resistance, it may perform better at an earlier stage than as a second-line therapy, when resistance to NRTIs weakens its potency.

Current WHO guidelines suggest that two triple nucleoside regimens, zidovudine/lamivudine/abacavir and zidovudine/lamivudine/tenofovir, may be considered as alternative first-line treatments. This option can be considered in individuals living in sub-Saharan African countries with concomitant tuberculosis. The PI-sparing nucleoside regimens for treating HIV-2 infection may rapidly select the development of Q151M, K65R, and M184V, resulting in resistance to pan-NRTIs (Descamps et al. 2004). Recent data from retrospective European observational studies show a worse CD4-cell response to triple NRTI cARTs compared to a PI-based regimen (Benard et al. 2011).

Monitoring Treatment Response

Given its low rate of detection, plasma HIV-2 RNA alone cannot be used to assess treatment efficacy; response to treatment is usually evaluated using a composite of both immunological and virological criteria. In practice, the lack of a commercially available viral-load assay makes measurement of viral load more difficult to determine in HIV-2. A collaborative HIV-2 infection study group (ACHIEV2E) evaluated various

HIV-2 RNA assays used in nine different centers and found considerable variation between laboratories, particularly for HIV-2 group B (Damond et al. 2011). The genome of HIV-2 group B is very variable, which leads to possible under-quantification of viral-load assays.

Compared to HIV-1 patients, fewer HIV-2-infected patients have a detectable viral load; for example, the proportion of patients with a detectable viral load at enrolment in the French ANRS HIV-2 cohort was only 37% overall, ranging from 18% in patients with CD4-cell counts $>500/\text{mm}^3$ to 51% in those with counts $<300/\text{mm}^3$. In individuals starting treatment with undetectable HIV-2 RNA, the CD4-cell count may be the only method of monitoring the efficacy of the treatment regimen. However, data from the ANRS CO5 HIV-2 Cohort Study Group showed lower than expected CD4 cell recovery in HIV-2-treated patients, despite most patients achieving virological suppression. In practice, HIV-2 RNA should be interpreted together with the CD4-cell count when both considering and monitoring a treatment. A drop from the peak or a return to a baseline CD4-cell count is indicative, as for HIV-1, of failure.

Monitoring for drug resistance is difficult as commercial assays are not available and it is advisable to send specimens to a reference laboratory. The interpretation of resistance-genotyping results is limited by the absence of clinically validated algorithms, although two publicly available websites have started to establish rules for the interpretation of HIV-2 resistance genotype assays: <http://www.geno2pheno.org/> and <http://www.hivfrenchresistance.org/>.

The transmission of drug resistance in Portuguese HIV-2-infected patients has been reported to account for 3.3% of the drug-naïve population. This result suggests that routine baseline resistance testing should be considered before initiating the treatment of HIV-2-infected patients. Nevertheless, contrary to HIV-1, a standard list of mutations that characterize the epidemiology of HIV-2-transmitted drug resistance has not yet been established.

When and What Treatment to Switch to

As for HIV-1, optimization of sequential treatments must be individualized, and the choice of a second- and third-line therapy will depend on the results of the genotypic resistance assay. The interpretation of HIV-2 mutations, as previously stated, is based on *in vitro* studies, and there are few clinical case reports.

With regard to NRTIs, the choice will depend on whether Q151M and/or K65R have been selected in treatment failure. Tenofovir retains activity against the Q151M mutation selected by zidovudine, and zidovudine may be active when the K65R mutation is developed under the pressure of an initial tenofovir regimen. Although abacavir is probably an option when only the Q151M mutation is present, this will be compromised in the presence of K65R and M184V.

Extensive resistance should be assumed to include Q151M, K65R, and M184V, depending on the NRTIs employed in the first-line ART. For a second-line therapy, tenofovir, zidovudine, or abacavir, depending on the first-line regimen, may be used as the NRTI backbone with lamivudine or emtricitabine in spite of the frequent presence of M184V. There is no evidence that this mutation has the same impact on HIV-2 as it does on HIV-1.

When the first-line therapy is based on a boosted PI, interpretation of the genotypic resistance assay is based on very few data, which makes the choice of a second sequential PI difficult. Saquinavir or darunavir may be an attractive choice for second-line therapy after lopinavir-based ART. The choice of raltegravir as a second-line regimen will depend on the potential efficacy of the NRTI backbone, which is determined from the results of the genotypic resistance assay, although there is a risk of only a transient benefit if the viral load is high and resistance to NRTIs is already present.

Although the clinical efficacy of CCR5 inhibitors is still unknown, they could be considered as part of the third-line regimen if access to an HIV-2

tropism assay is available that allows the use of this ARV class.

Foscarnet may represent a therapeutic option for salvage therapy in patients with HIV-2 infection exhibiting multiclass drug resistance.

Special Circumstances

Mother-to-Child Transmission and Prevention

Several studies have reported a natural HIV-2 mother-to-child transmission (MTCT) rate very much lower than that observed for HIV-1, ranging from 0% to 4% (EPF 1994). Nevertheless, if ART must be started in pregnant women who have predictive factors for HIV progression, in the same way as for nonpregnant individuals, treatment is also recommended to prevent MTCT in all HIV-2-infected mothers: indeed, there is no way to assess case by case whether an undetectable HIV-2 plasma viral load will lead to an absence of MTCT. Even if the risk is very low, it can be reduced still further using a MTCT regimen adapted from those who have HIV-1, using a cART of two NRTIs and a PI whose efficacy has been demonstrated for HIV-2. Maternal cART can be started at weeks 26–28 of the gestation period (or earlier in women who themselves require treatment); zidovudine infusion or repeated zidovudine tablets can be given during labor, and zidovudine can be given to the infant for 4 weeks or for 1 week, depending on the North or South setting. Single-drug prophylaxis with zidovudine alone (during pregnancy and intrapartum) can be considered in case-by-case discussions as an alternative to prevent HIV-2 MTCT, in asymptomatic patients with no predictive factors for progression and an undetectable viral load in repeat assays. Of note, two cases observed among a French perinatal cohort were caused, respectively, by an untreated primary infection and by a regimen not adapted to HIV-2: these remind us of the risk of MTCT during such circumstances and underline the importance of using drugs active against HIV-2 in PMTCT regimens.

Dual Infection HIV-1/HIV-2

Only a few studies report on the response to ART of dually infected patients with both HIV-1 and HIV-2 infection. Even though HIV-1 should be considered the dominant virus, the efficacy of drugs against both viruses needs to be considered when selecting the ART regimen. Under this condition, cART seems to be as effective, in terms of virological and immunological response, as it is for patients infected with HIV-1 alone. In cases of dual infection, a baseline genotypic resistance test for HIV-1, and if possible for HIV-2, should be performed. Immunological and virological follow-up of both HIV-1 and HIV-2 in dually infected patients is highly recommended (Landman et al. 2009).

Tuberculosis

Most HIV-2-infected people live or originate from sub-Saharan African countries where tuberculosis is endemic, and available rifamycin curative therapy is limited to rifampin, which interacts with PIs, contraindicating its concomitant use. A cART regimen that is recommended for coinfection with HIV-1 includes NNRTIs, which do not work against HIV-2. In cases of HIV-2 coinfection, the current WHO recommendation is to consider a triple NNRTI regimen. Nevertheless, this cART regimen was shown to give a poor immuno-virological response in HIV-2-infected people in a retrospective European study; other cART regimens, including integrase inhibitors, provide an alternative that is being used in developed countries, even if this is not yet validated by clinical studies.

Conclusion

Not all antiretrovirals are effective against HIV-2. NNRTIs are inactive and the virus is at least partially resistant to some PIs. NRTIs seem to be equally active against HIV-1 and HIV-2, but different resistance pathways and the apparently easy selection of some resistant mutations make them a fragile backbone of the antiretroviral regimen. The reduced response to ART in HIV-2-infected patients raises the question of an optimal

antiretroviral drug regimen and of the best time to initiate treatment in HIV-2 infection. A better understanding of the differences in pathogenicity between HIV-1 and HIV-2 infections may improve the treatment of both these viruses. In addition, randomized controlled trials are ultimately needed to determine the optimal therapy for HIV-2, given the limited range of drugs available for this virus.

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Antiretroviral Therapy for HIV-Infected Infants, Children, and Adolescents in Resource-Rich Settings

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Definition

The treatment of HIV-infected infants, children, and adolescents (ICA) mandates the use of a combination of antiretroviral drugs (ARVs) from two or more classes targeting different steps in the HIV life cycle. These classes include nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand-transfer inhibitor (INSTI), and attachment/entry inhibitors. There are now 21 Food and Drug Administration (FDA) approved therapeutic agents available for pediatric and adolescent populations ≤ 17 years of age, with several of those medications available in child-friendly formulations such as suspensions, chewable tablets, and powders. In qualifying adolescents, the use of multiple ARVs combined into a single tablet, also known as single tablet regimens (STRs), may also be considered. The goals of combination antiretroviral therapy (cART), recommendations of when and why to initiate therapy, an overview of available ARVs, and relevant management strategies in HIV-infected ICA living in resource-rich countries (RRC) will be addressed in this entry. In this review, RRC are defined as per UN criteria (UN Statistics Division 2015).

Introduction

There are at least 3.2 million children under the age of 15 who are HIV infected, with most living in resource limited settings (RLS), (UNAIDS 2013). The global epidemiology of pediatric HIV has changed dramatically in recent years. While the rate of new HIV infections has decreased in infants worldwide, owing to the expansion and success of programs to prevent maternal to child transmission, the number of children living with HIV continues to expand. Increased access to cART, coupled with the availability of newer drugs with improved formulations, has profoundly decreased the morbidity and mortality associated with this infection and has allowed perinatally HIV-infected children (PHIV) to survive well into adulthood. The penetration of modern cART into pediatrics has helped these children live longer and has fostered the hope that therapy will prevent or at least mitigate the end-organ damage inflicted by long-term HIV infection. In RRC where there are markedly fewer infected newborns, the pediatric HIV population is presently composed of a growing population of aging, treatment-experienced PHIV individuals, and, alarmingly, a burgeoning population of behaviorally infected teenagers. In the USA, many of the new infections in adolescents and young adults are occurring in men who have sex with men (MSM) (DHHS 2015a Adolescent and Adult Guidelines).

Goals of Therapy

The aim of HIV treatment is to preserve immune function and health by effectively maintaining viral suppression. Optimal viral suppression is achieved when the viral load is driven below the limit of detection of the assay employed, typically <20 to <75 copies per milliliter (mL) of plasma. Uncontrolled viral replication leads to an impaired immune system and increases the risk of various infections and malignancies; it also results in chronic immune activation which is centrally implicated in the pathogenesis of HIV-related end-organ disease. In addition to restoring and

preserving immune function and preventing HIV-related morbidity and mortality, other goals of cART include: (1) the prevention and emergence of viral drug resistance; (2) minimization of drug-related toxicity; (3) maintenance of normal physical growth and development, and (4) improvement in quality of life. In the adolescent population, HIV treatment is also important to reduce the risk of sexual transmission to partners. Similarly, cART is essential for adolescent females considering pregnancy in order to prevent perinatal transmission.

Although the pathogenesis of HIV infection and principles of treatment are similar between children and adults, certain characteristics unique to pediatric HIV infection require fundamentally different approaches and strategies. These factors include, but are not limited to, HIV infection in the setting of an immature immune system, lack of appropriate formulations and poor palatability of ARVs, limited pharmacokinetic (PK) and pharmacodynamic (PD) data to inform dosing guidelines, age-specific differences in interpreting CD4 counts, significantly higher baseline viremias in perinatally infected infants, adherence issues, and patient and caregiver resilience and coping mechanisms.

Furthermore, due to advances in treatment, therapeutic goals have changed considerably, since early in the epidemic, from preventing short-term complications of HIV infection to a more long-term view of sustaining children during their entire lifespan. Current treatment guidelines and recommendations reflect this changing philosophy and are updated regularly to remain aligned with advances in the fields of basic and clinical HIV research.

When to Start Antiretroviral Therapy

Table 1 highlights the recommendations of when to start cART in ICA, according to the Paediatric European Network for Treatment of AIDS (PENTA) and the Department of Health and Human Services (DHHS) guidelines, informative resources for pediatric HIV providers (Bamford et al. 2015, DHHS 2015b Pediatric Guidelines).

Antiretroviral Therapy for HIV-Infected Infants, Children, and Adolescents in Resource-Rich Settings, Table 1 When to initiate cART in infants, children, and adolescents

Age	DHHS 2015	PENTA 2015
0–1 year	All	All
1–3 years	CD4 count <1,000 cells/mm, or CDC stage 2	CD4 count ≤1,000 cells/mm; CD4 percentage ≤25%
	Moderate HIV-related symptoms	CDC category B/C ^a , or WHO stage 3 or 4
	HIV VL > 100,000	
3–5 years	CD4 count <1,000 cells/mm, or CDC stage 2	CD4 count <750 cells/mm, CD4 percentage ≤ 25%
	Moderate HIV-related symptoms	CDC category B/C, or WHO stage 3 or 4
	HIV VL > 100,000	
6–17 years	CD4 count <500 cells/mm, or CDC stage 2	CD4 count <350
	Moderate HIV-related symptoms	CDC category B/C, or WHO stage 3 or 4
	HIV VL >100,000	

VL viral load

^aCDC categories B and C have been replaced by “moderate HIV-related symptoms” and “opportunistic illnesses in HIV infection,” respectively

The decision to initiate cART in the pediatric and adolescent population depends on age, clinical status, and immunologic parameters. Clinical status is determined by the presence of HIV-related symptoms which are classified as either moderate or severe in nature (Table 2). Immunological parameters considered in the algorithm of when to begin cART include CD4 count and HIV viral load (VL), which is partially captured in the Center for Disease Control’s (CDC) staging of HIV infection. The staging of HIV infection was revised and simplified in 2014 to include 5 stages: 0 (or early infection), 1, 2, 3 or unknown (Selik et al. 2014). Staging is based primarily on CD4 count for age (with CD4 % as alternative), but patients with a severe opportunistic illness (see Table 2) are automatically classified as stage 3. In the USA, a preference exists for CD4 counts over CD4 percentage since the former is considered to be a more reliable prognostic marker in children of all ages (Selik et al. 2014). The PENTA guideline places less emphasis on HIV viral load in the decision to treat. For those with HIV viral loads greater than 100,000 copies/mL plasma, the DHHS would recommend initiating treatment, whereas according to PENTA, treatment should be considered.

Both PENTA and DHHS recommend urgent initiation of treatment for all children less than 1 year of age due to the risk of rapid disease progression in this population. Urgent initiation,

within 1–2 weeks of diagnosis, is recommended due to the high risk of severe illness and death in infancy. A randomized clinical trial in South Africa found that early antiretroviral therapy in infants by 12 weeks of age reduced early infant mortality by 76% and HIV progression by 75% (Violari et al. 2008). Infants treated earlier also had better neurological outcomes than in those in whom therapy was deferred.

For children 1–5 years of age treatment is recommended if the CD4 count falls below 1,000 (CDC stage 2) or if there are moderate HIV symptoms. In Europe, children ages 3–5 can defer treatment until the CD4 count drops below 750. However, treatment should be started urgently, within 1–2 weeks, for any child in stage 3.

HIV disease progression in children aged 6 years and older is similar to adults. Recent studies in adults have shown that earlier initiation of cART, even before the CD4 count drops below 500, leads to better outcomes. Treatment results in improved immunologic health and decreased morbidity and mortality. The DHHS now endorses initiating cART in all HIV-infected adults, regardless of CD4 count, even if asymptomatic (DHHS 2015a Adolescent and Adult Guidelines). This approach results in improved immunologic function, decreased morbidity and mortality, and provides the added benefit of decreasing the risk of sexual transmission if all

Antiretroviral Therapy for HIV-Infected Infants, Children, and Adolescents in Resource-Rich Settings, Table 2 HIV-related symptoms

Moderate HIV-related symptoms

Anemia (hemoglobin <8/g/dl, neutropenia (white blood cell count <1,000/ μ l) and/or thrombocytopenia (platelet count <100 \times 10 ³ / μ l) persisting for \geq 30 days
Bacterial meningitis, pneumonia, or sepsis (single episode)
Candidiasis, oropharyngeal (thrush), persisting (>2 months) in children >6 months of age
Cardiomyopathy
Cytomegalovirus infection, with onset before 1 month
Diarrhea, recurrent or chronic
Hepatitis
Herpes simplex virus stomatitis, recurrent (>2 episodes within 1 year)
Herpes simplex virus bronchitis, pneumonitis, or esophagitis with onset before 1 month
Herpes zoster (shingles) involving at least 2 distinct episodes or more than 1 dermatome
Leiomyosarcoma
Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia complex
Nephropathy
Nocardiosis
Persistent fever (lasting >1 month)
Toxoplasmosis, onset before 1 month
Varicella, disseminated (complicated chickenpox)

Stage 3 defining opportunistic illnesses in HIV infection

Bacterial infections, multiple of recurrent ^a
Candidiasis of bronchi, trachea, or lungs
Candidiasis of esophagus
Cervical cancer, invasive
Coccidiomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age >1 month
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy attributed to HIV
Herpes simplex: chronic ulcers (>1 month's duration), bronchitis, pneumonitis, or esophagitis (onset at age >1 month)
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi sarcoma
Lymphoma, Burkitt (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
<i>Mycobacterium avium</i> complex or <i>Mycobacterium kansasii</i> , disseminated or extrapulmonary
<i>Mycobacterium tuberculosis</i> of any site, pulmonary, ^b disseminated, or extrapulmonary
<i>Mycobacterium</i> , other species or unidentified species, disseminated or extrapulmonary
<i>Pneumocystis jirovecii</i> pneumonia
Pneumonia, recurrent ^b
Progressive multifocal leukoencephalopathy
<i>Salmonella</i> septicemia, recurrent
Toxoplasmosis of brain, onset at age >1 month
Wasting syndrome attributed to HIV

^aOnly among children aged <6 years

^bAmong children \geq 6 years, adolescents, and adults

adults are started on cART immediately. In children ages 6–17, the CD4 count threshold at which to recommend cART initiation is unknown. At this time, guidelines recommend starting treatment in children and adolescents ≥ 6 years of age if the CD4 count is below 500 (or < 350 in Europe), in the setting of high-level viremia (VL $> 100,000$), or if there is evidence of moderate HIV-related symptoms. As mentioned previously, all children and adolescents experiencing immunologic decline that reaches CDC stage 3 thresholds should be started on cART immediately.

While these recommendations provide guidance on when to start cART, the DHHS also recommends the consideration of cART in all children aged 1 year and older, even if asymptomatic and at CDC stage 1. The risks and benefits of starting cART in asymptomatic children needs to be carefully considered. While there are many antiretroviral agents available for use in children, choices are still limited and the drugs can be more challenging to administer in children than in adults. Consideration also needs to be given to the ability of the caregiver and the child to adhere to the regimen. Strategies to enhance adherence in these populations have been met with limited success. A recent Cochrane systematic review of interventions to improve ARV adherence in children and adolescents, in which only four out of 2,566 studies met their strict selection criteria, revealed that intensive home based and peer support programs failed to demonstrate any significant impact on adherence (Bain-Brickley et al. 2011). These knowledge gaps support the need for more well-designed studies with novel approaches focused on sustaining lifelong adherence to cART in these vulnerable populations.

What Antiretroviral Therapy to Start

There are five classes of antiretroviral drugs available and two pharmacokinetic enhancers. Table 3 lists significant drug-drug interactions and potential severe adverse effects of ARVs, approved by the FDA, for use in ICA. Combination antiretroviral therapy typically includes at least 3 drugs

from 2 different classes. In children, this should consist of 2 nucleoside reverse transcriptase inhibitors (NRTI) as the backbone, and the third drug should be either a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). Regimen selection will need to consider patient characteristics, such as age, comorbidities, coinfections, previous ARV exposures, and drug resistance testing results.

Preferred Regimens for Initial Therapy

The DHHS recognizes those regimens as preferred when clinical trial data has shown that the drug combination is more durable and efficacious (viral load suppression and immunologic improvement) and exhibits acceptable safety and toxicity profiles. These determinations are preferably made on the basis of data derived from pediatric clinical trials, but extrapolation from adult studies is frequently necessary for many of the newer ARV drugs on the market. Alternative drugs or regimens are those that are less favorable than the preferred choices because of various reasons including less experience in children, decreased efficacy and durability, increased toxicity, and/or issues with dosing, administration, or drug interactions for that drug or regimen. A third category is designated for use in special circumstances, drugs or combinations that can be used if a patient cannot be treated with either the preferred or alternate drugs or regimens. Tables outlining regimens that are “not recommended” or “never recommended” due to concerns about inferior virologic responses, safety, pharmacologic antagonism, insufficient clinical data, unacceptable rates of resistance formation, and serious toxicity are also listed in the guidelines. These treatment scenarios will not be addressed in this chapter – please refer to the guidelines (DHHS 2015b Pediatric Guidelines) for more information on this subject.

The first-line regimen in full term infants ≥ 14 days old is lopinavir/ritonavir with zidovudine and either lamivudine or emtricitabine. Infants > 3 months of age can be given abacavir instead of zidovudine. Any patient to be started on

Antiretroviral Therapy for HIV-Infected Infants, Children, and Adolescents in Resource-Rich Settings, Table 3 FDA approved antiretroviral drugs for use in

infants, children, and adolescents (Adapted from Van der Linden et al. 2009)

ARV class	Generic name (abbr.)	Age FDA approval	Significant D:D:I and drug metabolism	Potentially severe toxicities
NRTI	Abacavir (ABC)	≥3 months	None	Hypersensitivity in HLA-B*5701 + individuals
	Didanosine (ddI)	≥2 weeks	TDF ↑ [ddI] – do not coadminister	Peripheral neuropathy, lactic acidosis, pancreatitis
	Emtricitabine (FTC)	Birth	None	Neutropenia, lactic acidosis
	Lamivudine (3TC)	≥3 months	None	Peripheral neuropathy, pancreatitis, lactic acidosis
	Stavudine (d4T)	Birth	ZDV (antagonism) – do not coadminister	Peripheral neuropathy, pancreatitis, lactic acidosis; lipodystrophy/atrophy
	Tenofovir (TDF)	≥2 years	TDF ↑ [ddI] – do not coadminister	Bone and renal
	Zidovudine (ZDV)	Birth	D4T (antagonism) Doxorubicin (decreases ZDV – P levels)	Anemia, neutropenia (common)
NNRTI	Efavirenz (EFV)	≥3 months	CYP3A4 inducer/inhibitor	Skin rash, hepatic transaminase elevations, CNS (abnormal dreams, insomnia)
	Etravirine (ETV)	≥6 years	Complex – multiple CYP450 interactions	Skin rash, hypersensitivity
	Nevirapine (NVP)	≥15 days	Complex – CYP450 induction	Skin rash, hypersensitivity, hepatotoxicity
	Rilpivirine (RPV)	≥18 years	CYP3A4 substrate PPIs, antacids, H2 blockers decrease plasma [RPV]	Depression, mood changes
PIs	Atazanavir (ATV)	≥3 months	CYP3A4 substrate/inhibitor PPIs, antacids, H2 receptor antagonists decrease plasma [ATV]	Indirect hyperbilirubinemia and jaundice; PR interval prolongation
	Darunavir (DRV)	≥3 years	CYP3A4 substrate/inhibitor	Skin rash, hepatic transaminase elevations, hepatic dysfunction
	Fosamprenavir (FPV)	≥6 months	CYP3A4 substrate/inhibitor/inducer	Skin rash, Steven-Johnson syndrome, lipodystrophy, neutropenia
	Indinavir (IDV)	≥18 years	CYP3A4 substrate/inhibitor	Lipid abnormalities, lipodystrophy, nephrolithiasis/urolithiasis
	Lopinavir/ritonavir (LPV/r)	≥14 days	CYP3A4 substrate/inhibitor	Diarrhea, rash, hyperlipidemia (esp. hypertriglyceridemia), lipodystrophy
	Nelfinavir (NFV)	≥2 years	Complex – multiple CYP450 interactions	Diarrhea, lipodystrophy, exacerbation chronic liver disease

(continued)

Antiretroviral Therapy for HIV-Infected Infants, Children, and Adolescents in Resource-Rich Settings, Table 3 (continued)

ARV class	Generic name (abbr.)	Age FDA approval	Significant D:D:I and drug metabolism	Potentially severe toxicities
	Saquinavir (SQV)	≥16 years	CYP3A4 substrate/inhibitor	Rash, lipodystrophy, exacerbation chronic liver disease
	Tipranavir (TPV)	≥2 years	CYP3A4 substrate/inducer	Rash, hepatic dysfunction, lipodystrophy
Entry/attachment inhibitors	Enfuvirtide (T-20)	≥6 years	No known interactions Catabolism to constituent amino acids	Local injection-site reactions, cellulitis, hypersensitivity, pneumonia (unclear association)
	Maraviroc (MVC)	≥16 years	CYP3A4 and Pgp substrate	Hepato- and cardiotoxicity
INSTIs	Dolutegravir (DTG)	≥12 years	UGT1A1 and CYP3A4 substrate	Rash, hypersensitivity
	Elvitegravir (EVG)	≥18 years	CYP3A4 substrate, CYP2C9 inducer	Diarrhea
	Raltegravir (RAL)	≥4 weeks	UGT1A1-mediated glucuronidation Antacids decrease plasma [RAL]	Elevated hepatic transaminases in HBV and/or HCV coinfecting individuals
Pharmacokinetic	Cobicistat (COBI)	>18 years	CYP3A4, CYP2D6, renal organic anion transporter inhibitor	Increased risk of renal dysfunction with concomitant TDF use
Enhancers	Ritonavir (RTV, r)	>14 days	Complex – multiple CYP450 interactions	Exacerbation of chronic liver disease, lipodystrophy

D:D:I drug-drug interactions, *NRTI* nucleoside reverse transcriptase inhibitor, *NNRTI* nonnucleoside reverse transcriptase inhibitor, *PIs* protease inhibitors, *INSTI* integrase strand-transfer inhibitor, *DC* drug concentration, *ZDV-P* phosphorylated ZDV, *CYP450* cytochrome p450 enzyme family, *PPI* proton-pump inhibitors, *Pgp* P glycoprotein, *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1

abacavir should first undergo HLA-B*5701 genetic testing to ensure that it is negative. The advantage of abacavir is the possibility of switching to once daily therapy. Recent recommendations were that children with continued viral suppression and stable CD4 counts for more than 6 months can be switched from twice to once daily dosing with abacavir. In a related development, the FDA recently approved the use of once daily lamivudine and abacavir for all children ≥3 months of age, irrespective of viral load (FDA 2015). Labeling changes such as this, which are informed by pediatric clinical trial results, provide more opportunities to simplify regimens in children and improve adherence without sacrificing potency.

Children 3–6 years of age should be treated with either efavirenz or lopinavir/ritonavir with

2 of the NRTIs mentioned above (abacavir or zidovudine with either lamivudine or emtricitabine). Children ages 6 and older have the option of being treated with the NNRTI efavirenz or the PIs atazanavir/ritonavir or lopinavir/ritonavir. In contrast to NNRTI-based regimens in which a single viral mutation can lead to high-level resistance and cross-resistance with other NNRTIs, PI-based regimens have the advantage of increased virologic potency and less drug resistance development due to the high genetic barrier to resistance that this class of drugs possess. Efavirenz should be avoided in adolescent females due to the potential for teratogenicity in the first trimester of pregnancy. The preferred 2 NRTI backbone can include tenofovir once adolescents reach Tanner stage 4 or 5, combined with either lamivudine or emtricitabine.



Alternative Regimens for Initial Therapy **Adherence to Guidelines**

Nevirapine, in addition to 2 NRTIs, is an alternative therapy for infants ≥ 15 days old and may be more palatable than lopinavir/ritonavir. Nevirapine remains an alternative ARV in older children because of the risk for rare serious adverse events (hypersensitivity reactions, potentially life-threatening hepatitis) and an increased risk of virologic failure when compared to a lopinavir/ritonavir regimen. This drug should be avoided in post-pubertal adolescent females with CD4 counts >250 because of the increased risk of hepatotoxicity in this group.

Atazanavir/ritonavir is an alternative PI for children ≥ 3 months (≥ 10 kg) to <6 years due to limited experience in this population. It is the only PI that can be given once daily in infants and children and is available in powder form. Twice daily fosamprenavir/ritonavir (for infants ≥ 6 months) or darunavir/ritonavir (for children ≥ 3 years) are other options. Once daily darunavir/ritonavir can be given to treatment naive children ≥ 12 years of age.

Raltegravir is an integrase strand-transfer inhibitor (INSTI) that can be given to children ≥ 2 years (chewable or film-coated tablet), in combination with 2 NRTIs. Dolutegravir is another option for children ≥ 12 years and has the advantage of once daily dosing in patients naïve to INSTI. It is well tolerated and has an excellent safety and toxicity profile.

The alternative dual NRTI backbone includes zidovudine, with either abacavir or didanosine, or didanosine with either lamivudine or emtricitabine in infants and young children. In adolescents ≥ 13 years of age zidovudine with either lamivudine or emtricitabine is an alternative option. Children and adolescents who are Tanner stage 1–3 can also be given tenofovir in combination with lamivudine or emtricitabine after weighing the risks and benefits. Tenofovir is considered an alternative drug in younger children in Tanner stage 1–3 because of the potential risk of decreased bone mineral density in the growing child.

Although the DHHS and PENTA guidelines provide foundations for the rational and effective treatment of pediatric HIV infection in RRC, treatment decisions must ultimately be individualized to maximize adherence, minimize toxicity, and optimize patient outcomes. A review published in 2005 examined clinician adherence to DHHS pediatric HIV treatment guidelines in the USA from 1998 to 2003, a period encompassing the introduction and expansion of PI-based cART in children (Brogly et al. 2005). The study population consisted of 766 perinatally HIV-infected (PHIV) children enrolled in the Pediatric AIDS Clinical Trials Group 219C who were prescribed first-line cART outside the confines of an ARV clinical trial. During the 5 year study period, 22% of children initiated a cART regimen not recommended by the guidelines, suggesting that clinicians caring for these children were compelled to include new ARVs that may not yet have been FDA approved or recommended by the DHHS. This theme will be expanded on in later sections describing treatment considerations in special populations.

Treatment Response and Monitoring

At the time of diagnosis, HIV-infected children should be thoroughly evaluated with a baseline assessment that includes history, physical exam, CD4 count, HIV VL, and HIV genotypic resistance testing. Evaluation for coinfections will depend on the history, exam, and specific laboratory data. If cART is initiated, the patient's adherence should be assessed and side effects discussed 1–2 weeks later. Two to 4 weeks into treatment laboratory testing should be performed to verify virologic response and to evaluate for toxicity. Children and adolescents should be assessed every 3–4 months for adherence, side effects, and response to treatment (CD4 count/%, VL). Patients with sustained VL suppression, CD4 counts above opportunistic infection (OI) risk for age, good medication adherence, and stable

clinical status for at least 2 years can have their CD4 counts monitored every 6–12 months. Pediatric patients not on cART should similarly be evaluated every 3–4 months to assess clinical course and immunological health (CD4 count/%, VL). More frequent evaluation may be needed on a case-by-case basis if there is concern for clinical or immunological deterioration.

A systematic review of the literature, that included studies through 2010, found that children residing in RLCs had higher mortality rates than those in RRC, whether cART naïve or on treatment (Peacock-Villada et al 2011). Higher VL and lower CD4% and weight for age at baseline were predictors of mortality in both settings. Children residing in RLC were older and had more advanced disease at time of diagnosis which may partially explain the higher mortality rates. Earlier diagnosis and revised WHO recommendations, to start cART in all children ≤ 5 years old, will likely improve outcomes in RLCs.

Assessment/Management of Treatment Failure

A mutually respectful and trusting relationship between the clinical team, the patient, and his/her family is essential to help establish readiness to start therapy, discuss adherence, and understand the goals of cART. Once a patient starts cART, assessment of adherence and efficacy should occur at least every 3–4 months. At each visit, it is important to reinforce how the medications should be administered (frequency, \pm food) and address any concerns the patient or caregiver may have. Caregivers and adolescent patients may benefit from support networks available at the hospital or in the community. Patients with mental health concerns should be evaluated by a specialist.

If a patient is not virally suppressed, the administration of each drug should be reviewed and adherence explored. If there are concerns with palatability or adverse effects, then other options may need to be considered even if it requires transitioning from a preferred to an alternative regimen. If a once daily regimen is possible, it

should be offered. Adherence issues frequently serve as the biggest impediment to success in children prescribed cART. Many factors, including palatability of the regimen, the child's age, and behavioral and psychosocial factors within the family, can negatively impact adherence to cART. Psychiatric illness and substance abuse can create barriers to adherence in adolescents. Denial and fear of disclosure may prevent them from seeking care or safely taking their cART at home or while in college. There may be a lack of social support, unstable housing, or food insecurity. Careful consideration of these challenges may allow clinicians to offer more personalized guidance to patients and their families on the importance of remaining engaged in care and hopefully empowering them to maintain their health.

If there is ongoing treatment failure, either due to clinical, immunologic, or virologic failure, then drug resistance testing should be performed while on the failing regimen, and consideration should be given to changing or modifying the regimen. Clinical failure is the development of a new OI or clinical progression. Immunologic failure is the inability to achieve or maintain the appropriate CD4 count or % for age. Virologic failure is defined as two or more consecutive VLs >200 after 6 months of therapy or repeated VLs above the level of detection following 12 months of cART, using the most sensitive assay available.

Selection of a new regimen should include a review of the previous treatment history and drug resistance testing. Ideally at least 2 fully active drugs should be chosen and a switch made to another class. For example, if a child was on an NNRTI and 2 NRTIs, he/she should be switched from the NNRTI to either a PI or an INSTI. At times the switch can be made within the class, if the presence of resistance is thought to be unlikely.

Treatment Consideration in Special Populations

Neonates and Infants <12 Months Old

There is very limited knowledge on the safety and dosing of ARVs in young infants, especially

neonates. Zidovudine and nevirapine are the only ARVs in which dosing is available for neonates during the first 2 weeks of life. However, they are used alone or in combination for the prevention of perinatal transmission. Since diagnostic HIV testing typically requires 1–2 weeks to complete, pharmacokinetic studies evaluating dosing and safety in neonates <2 weeks old are lacking. While there was one case of prolonged remission in an infant from Mississippi who was started on therapy at 30 hours of life, it has not been shown that early treatment leads to sustained viral suppression or results in better long-term outcomes (Persaud et al. 2013). Thus, at this time there is insufficient data to make treatment recommendations for newborns during the first 2 weeks of life, and there is the risk of significant toxicity if exposed to cART.

Once infants are 14 days or older cART needs to be initiated urgently. Data from the CHER trial (Violari et al. 2008) and the HIV Paediatric Prognostic Markers Collaborative Study (Dunn et al. 2006) were unequivocal in their documentation of high rates of disease progression and death among asymptomatic HIV-infected infants who deferred therapy until their CD4% fell below 25%. The compelling need to start cART in these infants was further confirmed by a Cochrane intervention review (Penazzato et al. 2014), outlining the significant survival benefit conferred by initiating a PI- or NNRTI-based regimen as soon as possible. Lopinavir/ritonavir-based regimens were superior to NVP-containing cART in terms of risk of virologic failure. Treatment interruption protocols in infants were found to be futile based upon the short length of treatment interruption that such infants could tolerate without having to restart cART.

Adolescents

Only 53–62% of adolescents in RRC are adherent to cART, and only 70% are successfully linked to care. There are unique challenges in caring for HIV-infected adolescents that can make it difficult to engage and retain them in regular health care and to comply with daily cART (DHHS 2015a Adult and Adolescent Guidelines). Adolescents are at a developmental stage that makes it difficult

to accept chronic illness, seek care, and focus on maintaining their health. Other obstacles to adherence includes concurrent mental illness, lack of family and social support, lack of consistent access to health care, and, in the case of behaviorally infected individuals, fear of parental disclosure if a parent's health insurance is used. PHIV commonly have more drug resistance due to exposure to serial nonsuppressive regimens, extensive treatment exposure, and chronic nonadherence, which may require the use of complex and potentially more toxic treatment regimens. Thus, the decision to start or continue cART in adolescents needs to be individualized, with barriers to treatment and adherence readiness explored at each visit. For newly infected or cART naïve adolescents who are of appropriate weight and Tanner stage, fixed dose combinations of ARVs in the form of single tablet regimens should be considered to foster good adherence (DHHS 2015a Adult and Adolescent Guidelines). If an adolescent practices erratic adherence, then treatment may need to be deferred for a short period of time until the issues compromising adherence are addressed. If a decision is made to modify or completely change the regimen, the clinician may need to avoid choosing ARVs with low genetic resistance barriers to decrease the risk of jeopardizing future treatment options.

All adolescents should be educated about pre-conception care, contraception, and decreasing risk of HIV transmission to partners. Adolescent females should be counseled about possible interactions between cART and hormonal contraceptives. They should also be encouraged to maintain compliance with cART if pregnancy is being considered.

HIV-2 Infection

HIV type 2 infection (HIV-2) originated in West Africa but has since spread to other parts of Africa, Europe, India, and the USA. It needs to be considered in individuals considered at risk based on exposure history, if presentation suggests HIV infection but HIV-1 testing is negative, and if the HIV-1 western blot has an unusual indeterminate result (Campbell-Yesufu et al. 2011). Newer HIV-testing algorithms have eliminated the

western blot as a confirmatory test and incorporate HIV-1/HIV-2 antibody differentiation immunoassays that can readily identify HIV-2 infection. HIV-2 infection tends to have a less virulent course than HIV-1, with a longer asymptomatic period and slower progression to AIDS.

Summary

The treatment of HIV-infected children and adolescents with effective cART has transformed HIV from a potentially fatal disease to, in many cases, a manageable chronic illness. In resource-rich settings, mother to child transmission of HIV has been rendered almost obsolete via the provision of cART to HIV-infected women during pregnancy and the availability of safe and inexpensive sources of infant formula. However, despite these successes, major gaps still exist in our ability to effectively treat HIV infection over the lifespan of the individual. More pediatric clinical trials testing ARVs that are safe, palatable, and potent will be one of many ways to close that gap. Reducing stigma and addressing larger societal issues such as poverty, homelessness, education, untreated mental illness, and substance abuse will also have major collateral impacts on this disease. Finally, translating the advances made in RRS to the global stage will be crucial in attempts to provide universal treatment to all HIV-infected people worldwide.

Cross-References

- ▶ [Antiretroviral Therapy: When to Start](#)
- ▶ [Antiretroviral Treatment in Resource-Limited Settings](#)
- ▶ [Children, Care and Treatment](#)
- ▶ [Children, Epidemiology of HIV/AIDS](#)

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Antiretroviral Therapy: When to Start

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Definition

Clinicians have debated the optimal time to initiate antiretroviral therapy (ART) since the availability of antiretroviral in the late 1980s. While discussions and guidelines have generally centered around the CD4 cell count, the debate over when to start has seen shifts from waiting for disease progression to AIDS to starting immediately at time of HIV diagnosis. These paradigm shifts have largely been driven by recognition of complications of older antiretroviral regimens; the availability of safer, simpler, and better tolerated antiretroviral regimens; and evidence from both cohort studies and randomized controlled trials. Treatment guidelines are currently provided by several groups and have been updated multiple times to reflect new HIV knowledge and gaps. These groups include the World Health Organization (WHO) (Consolidated strategic information guidelines 2015), the British HIV Association (BHIVA) (BHIVA guidelines for the treatment of HIV-1 2015), the European AIDS Clinical Society (EACS) (2014), the US Department of Health and Human Services (DHHS) (Guidelines for the prevention and treatment 2013; Guidelines for the use of antiretroviral agents 2015), the International Antiviral Society-USA (IAS-USA) (Gunthard et al. 2014), and the Pediatric European Network for the Treatment of AIDS (PENTA) (Bamford et al. 2015). In this entry, the historical

pendulum swings in the *when to start* debate will be discussed, the data as of August 2015 will be examined, and the current guidelines, including special populations and children, will be summarized. While current recommendations on when to start ART vary somewhat in these international and national guidelines, a uniformed approach is anticipated in the near future given newly published data demonstrating the clinical benefit of initiating ART at high CD4 cell counts.

Historical Perspective

In the 1980s, AIDS was a poorly understood, nearly universally fatal illness. When the chemotherapeutic drug zidovudine (AZT) was discovered to have antiviral activity, patients and physicians sought its use even prior to FDA approval. In 1990, the AIDS Clinical Trials Group (ACTG) study 019 of AZT monotherapy showed promise for slowing progression of disease in asymptomatic HIV-infected persons with CD4 cell counts <500 cells/mm³, leading to its widespread use in resource-rich settings. But in 1995, a randomized controlled trial (RCT) of initiating AZT in HIV-infected persons with CD4 cell counts <500 cells/mm³ compared to >500 cells/mm³ failed to find a clinical benefit, and the question of *when to start* antiretroviral therapy became contentious. As additional antiviral drugs entered the market, the therapeutic options were increasing but the optimal time to initiate ART remained unknown. The number of persons dying from HIV infection and AIDS kept growing until 1996 with the advent of protease inhibitors. Use of three-drug therapy, coined *highly active antiretroviral therapy* (HAART), profoundly changed the course of HIV disease from nearly universally fatal into a chronic and treatable illness. While not a cure, morbidity and mortality improved dramatically and shifted the landscape from loss of life to quality of life with increasing concern about side effects, pill burden and adherence, and drug resistance (Jain and Deeks 2010).

The initial set of ART treatment guidelines was published in 1996 by IAS-USA, and additional

US, European, and WHO guidelines soon followed. Each of the guidelines has been revised periodically as new information became available. The initial IAS-USA guidelines recommended ART for all HIV-infected persons with a CD4 cell count <500 cells/mm³. This CD4 cell count cutoff is considered to be in the normal range, and HIV-infected persons were often asymptomatic with this degree of immune function. Because ART was recommended at this high CD4 cell count, this era became known as the *hit hard and hit early* era. However, this period did not last long. With widespread ART use, increasing problems began to emerge with the high pill burdens, complex dosing schedules, and extensive side effects. The promise of improved survival was soon overshadowed by risks of the antiretroviral medications. With these concerns, the pendulum began to shift again to waiting to start ART until more advanced disease to avoid multiple complications.

Then, in 2006, data began to emerge from observational and randomized controlled trials showing morbidity and mortality benefits from starting ART earlier. Newer antiretroviral (ARV) medications were developed and were more tolerable with simpler dosing and frequency. Thus, the threshold of *when to start* ART shifted again to earlier and earlier in HIV infection. Campaigns to bring ART to all eligible HIV-infected persons began. The initial WHO campaign was known as *3 by 5*, a plan to treat three million persons with ART by the year 2005. Now, more than three decades into HIV infection, results from several studies attempting to answer the question of what CD4 threshold to initiate ART have been published. These studies are reviewed in the section below and inform the current treatment recommendations.

Summary of Trial Data: Randomized Controlled Trials

Five large randomized controlled trials have investigated the question *when to start* treatment among HIV-infected adults. Four of these studies evaluated different CD4 cell count thresholds for ART

initiation or continuation (Strategies for Management of Antiretroviral Therapy (SMART) Study Group et al. 2006; Severe et al. 2010; Cohen et al. 2011; INSIGHT START Study Group 2015). One study evaluated early versus delayed treatment initiation in infants (Violari et al. 2008). These studies were conducted in both resource-rich and limited settings. Each of the RCTs was powered to find differences in disease progression/AIDS or prevention of HIV transmission, and each was stopped early by the data safety and monitoring board (DSMB) because of greater risk among those with deferred treatment.

SMART

The Strategies for Management of Antiretroviral therapy (SMART) trial began in 2002. At the time, ARV regimens included high pill burdens with frequent dosing and multiple side effects. Because of the lifelong need for ARVs, this RCT was designed to compare a strategy of treatment interruption (drug conservation arm) with continued therapy (viral suppression arm). At the time of the study design, the WHO guidelines recommended reserving ART for persons with CD4 cell counts <200 cells/mm³, and the 2003 DHHS guidelines recommended treatment for all HIV-infected persons with CD4 cell counts <350 cells/mm³. The study enrolled 5472 HIV-infected persons age >13 years with CD4 >350 cells/mm³ who were currently on therapy, had previously received therapy, or who had never received ART. The study was conducted at 318 sites in 33 countries. The primary end point of this study was new or recurrent opportunistic infection (OI) or death from any cause. In January 2006, the DSMB unexpectedly stopped the study because of safety risk in the drug conservation group. The estimated hazard ratio (HR) for any OI or death was 2.6 (95% CI, 1.9–3.7; $p < 0.001$) in the drug conservation group. In a post hoc analysis focusing on the 240 participants who were treatment naïve at study entry plus 228 who had not received ART in the preceding 6 months, randomization to the drug conservation arm with deferred initiation (or reinitiation) of ART until a CD4 count <250 cells/mm³ was associated with over a threefold greater risk of

opportunistic disease and serious non-AIDS events. These data, along with other analyses from SMART, suggested that early initiation and continuation of ART provided clinical benefit (Strategies for Management of Antiretroviral Therapy (SMART) Study Group et al. 2006, 2008).

CHER

The Children with HIV Early Antiretroviral Therapy (CHER) trial began in 2005 to evaluate reduction in infant mortality with the limited use of early ART initiation as a bridge to delay lifelong continuous ART later in life. At the time of this study, baseline infant mortality rates were high in resource-limited settings and were further magnified with HIV exposure and infection. The management challenges of ART in this time included were the need for lifelong therapy, limited age-appropriate drugs, long-term toxicity, adherence issues, and limited resources. The study enrolled 377 HIV-infected infants, 6–12 weeks of age, with plasma HIV RNA >1000 copies/ml and CD4% >25% into one of three randomized treatment strategies: (1) early limited ART for 96 weeks, (2) early limited ART for 40 weeks, or (3) deferred therapy. Deferred therapy was based on WHO and CDC treatment guidelines at the time. The primary end point of this study was time to death or failure of first-line ART. Results were analyzed by combining both early treatment groups into one combined early treatment group. In June 2007, the DSMB stopped the study and mandated urgent evaluation of the deferred treatment group. In the early treatment group, 11 of 252 infants reached the primary end point compared to 21 of 125 in the deferred group (HR 0.25; 95% CI, 0.12–0.51). In the early group, 4% of the infants died compared to 16% in the deferred group (HR 0.24; 95% CI, 0.11–0.51; $p < 0.001$); 40% of deaths occurred unexpectedly at home, and the rates were higher in the first 26 weeks of life with 15 infants dying before receiving any ART. Similarly, HIV disease progression occurred in 6.3% of the early therapy groups compared to 25.6% of the deferred therapy group (HR 0.25; 95% CI, 0.15–0.41;

$p < 0.001$). In summary, this study demonstrated that early initiation of ART at a median of 7 weeks compared to deferred therapy among HIV-infected infants reduced both mortality and morbidity. The degree of difference in early mortality compared with deferred ART provided strong support for the initiation of ART in infants regardless of CD4 cell count or % (Violari et al. 2008).

CIPRA

The Comprehensive International Program of Research on AIDS-Haiti (CIPRA-HT 001) trial began in 2005 and randomized 816 treatment-naïve subjects with CD4 cell counts of 200–350 cells/mm³ to open-label early initiation of ART within 2 weeks of enrollment versus deferred initiation at CD4 cell count of <200 cells/mm³. At the time of this study, the WHO guidelines recommended waiting until the CD4 cell count ≤ 200 cells/mm³. The primary end point of this study was new or recurrent OI or death from any cause. The median CD4 cell count for the study group overall was 281 cells/mm³. In May 2009, the DSMB recommended that the study be stopped early because there was a fourfold higher risk of death (HR 4.0; 95% CI, 1.6–9.8; $p = 0.001$) among the late treatment group. Later analysis also found smaller increase in CD4 cell count after 5 years (496 cells/mm³; 95% CI 477–515 vs. 373 cells/mm³; 95% CI, 357–389; $p < 0.0001$) and a higher risk of tuberculosis (unadjusted HR 2.41; 95% CI, 1.56–3.74; $p < 0.0001$). In conclusion, earlier ART initiation led to significant reductions in mortality and morbidity, particularly in incident tuberculosis. These results played an influential role in changing the WHO treatment guidelines (Severe et al. 2010; Collins et al. 2015).

HPTN 052

The HIV Prevention and Treatment Network (HPTN) 052 study began in 2007. At that time, the WHO recommended treatment for persons with CD4 cell counts of <200 cells/mm³ and treatment consideration for <350 cells/mm³, and the DHHS suggested treatment for persons with

<350 CD4 cells/mm³. This study was the first large RCT of serodiscordant couples comparing the impact of early versus delayed ART among HIV-infected persons on transmission to their HIV-uninfected partners in stable sexual monogamous relationships. The HIV-infected partner was randomized to either start ART at a CD4 cell count of 500 cells/mm³ (early arm) or wait until 350 cells/mm³ (delayed arm). The study enrolled persons ≥18 years who had never received ART at 13 sites in nine countries, including both resource-rich and limited settings. Viral genetic sequencing was done to assess linkages of seroconversion events. The primary prevention end point of this study was linked HIV-1 transmission in HIV-negative partners. The primary clinical end point was the earliest occurrence of pulmonary tuberculosis, severe bacterial infection, a WHO stage 4 event, or death. Of the 1763 serodiscordant couples enrolled in the study, 50% of HIV-infected participants were men and 97% were heterosexual. In April 2011, the DSMB recommended that the study be stopped early because of the prevention results. Only four transmission events were seen in the early treatment group (incidence 0.3/100 person-years) compared with 35 in the delayed group (incidence 2.2/100 person-years) with resulting HR of 0.11 (95% CI 0.04–0.32; $p < 0.001$). With genetic analyses, 28 transmissions were linked, 27 in the delayed group and one in the early treatment group. Forty HIV-related clinical events or deaths were noted in the early treatment group compared to 65 in the delayed treatment group (HR 0.59; 95% CI, 0.40–0.88; $p = 0.01$). In conclusion, earlier ART initiation at a CD4 cell count of 500 cells/mm³ resulted in 96% reduction in linked HIV transmissions and 89% reduction in overall HIV transmission. In addition to prevention success, there were reductions in clinical outcomes including the cumulative probability of AIDS events over 2 years (HR 0.64; 95% CI, 0.43–0.96, $p = 0.031$) and cumulative probability of TB over 2 years (HR 0.49; 95% CI, 0.28–0.89, $p = 0.018$), but no difference was seen in mortality. This combination of transmission prevention and morbidity reduction further

supported the strategy of earlier initiation of ART (Cohen et al. 2011; Grinsztejn et al. 2014).

START

The Strategic Timing of Antiretroviral Therapy (START) trial is the most recently completed RCT comparing early initiation of ART at a CD4 cell count of >500 cells/mm³ versus delayed therapy when the CD4 cell count reaches 350 cells/mm³. As of 2011, when this study started, the threshold for ART initiation was 350 cells/mm³ according to DHHS guidelines and 200 cells/mm³ according to WHO guidelines. This study enrolled 4685 persons ≥18 years with CD4 cell count ≥500 cells/mm³ who had never received ART at 215 sites in 35 countries. The primary composite end point of this study was serious AIDS event, serious non-AIDS-related event, or death from any cause. In May 2015, the DSMB recommended that the delayed study arm be stopped early and patients be offered ART because of significant differences between the groups. In the early ART arm, 42 (1.8%) subjects reached the primary end point compared with 96 (4.1%) in the delayed arm, corresponding to a 57% reduction in risk attributed to earlier initiation of ART. The risk reductions for serious AIDS-related and non-AIDS-related events were 72% and 39%, respectively. Of note, 68% of primary end point events occurred at CD4 counts of >500 cells/mm³. Life-threatening adverse events and hospitalizations did not differ between study arms. This study provides strong evidence supporting the strategy of treating all HIV-infected persons, including those who have normal CD4 counts of >500 cells/mm³ (INSIGHT START Study Group 2015).

Summary of Cohort Studies

Two large observational studies among HIV-infected adults have also had significant impact on the question *when to start* treatment among HIV-infected adults. These studies evaluated age-related differences in disease progression/AIDS as well as various CD4 cell count thresholds and are summarized below.

NA-ACCORD

Two parallel, observational data analyses from the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) have informed the pendulum shifts of when to initiate ART. Investigators analyzed data of over 17,000 HIV-infected adults (~80% men) under medical care from 1996 to 2005 in Canada and the United States who were ART naive and had never had an AIDS-defining illness. The patients were stratified on the CD4 threshold at which they initiated ART – the first analysis compared 351–500 cells/mm³ (early) to ≤350 (deferred) and the second compared >500 cells/mm³ (early) to ≤500 cells/mm³ (deferred). At the time of this study, the WHO guidelines recommended deferring ART until a CD4 cell count ≤200 cells/mm³, and DHHS guidelines suggested starting at 350 cells/mm³. The relative risk of death for the group who deferred ART until CD4 cell count of <350 cells/mm³ (compared to 351–500) was 1.69 (95% CI, 1.26–2.26; *p* < 0.001). Similarly, the relative risk of death for those who started at >500 cells/mm³ (compared to ≤500) was 1.94 (95% CI, 1.37–2.79; *p* < 0.001). In conclusion, deferring ART initiation until lower CD4 cell counts in this observational, non-randomized study was associated with significantly greater risk of all-cause mortality. Published in 2009, this very large observational study supported the shift toward earlier ART initiation (Kitahata et al. 2009).

ART-CC

The Antiretroviral Therapy Cohort Collaboration (ART-CC) was established in Europe and North America in 2000 to evaluate clinical events and death among persons living with HIV infection. At the time of this study, the WHO and DHHS treatment thresholds were <200 cells/mm³ and <350 cells/mm³, respectively. Data on more than 12,574 adult persons starting ART were collected through 2009. One of the many questions evaluated by this group was *when to start* ART. During the follow-up period, 1094 patients developed AIDS or died and 344 patients died. Baseline CD4 cell count was strongly associated with the probability of progression to AIDS or

death. Compared with patients starting ART with the lowest CD4 cell count, <50 CD4 cells/mm³, adjusted HRs for AIDS or death were 0.74 (95% CI, 0.62–0.89) for 50–99 cells/mm³, 0.52 (0.44–0.63) for 100–199 cells/mm³, 0.24 (0.20–0.30) for 200–349 cells/mm³, and 0.18 (0.14–0.22) for ≥350 CD4 cells/mm³, showing benefit with higher CD4 initiation. Although not randomized, this large study provided additional support to starting earlier ART, including CD4 cell counts above the treatment thresholds at the time (When To Start Consortium et al. 2009).

Current Guidelines

There are several groups that promulgate HIV treatment guidelines for adults: the World Health Organization (WHO) (Consolidated strategic information guidelines 2015), the British HIV Association (BHIVA) (BHIVA guidelines 2012), the European AIDS Clinical Society (EACS) (2014), the US Department of Health and Human Services (DHHS) (Guidelines for the use of antiretroviral agents 2015), and the International Antiviral Society-USA (IAS-USA) (Gunthard et al. 2014). Each of these guidelines is available online, and some are updated in real time as new data becomes available. Table 1 summarizes the recommendations of *When to Treat* as of August 2015. The results of the START trial are expected to lead to the alignment of all guidelines in support of offering ART to all HIV-infected adults.

Summary of Recommendations for Infants and Children

When to initiate ART in infants and children has changed over the decades. The CHER trial was a landmark trial that suggested earlier treatment was better than deferred treatment, even in the face of concern over lifelong toxicity, adherence concerns, and lack of age-appropriate ARV options (Violari et al. 2008). The current recommendations for treatment of infants and children are generally in agreement across the guidelines.

Antiretroviral Therapy: When to Start, Table 1 Current treatment guidelines for *when to start*

Guideline	Last updated	CD4 cell count thresholds (cells/mm ³)			
		≤200	<350	350–500	>500
WHO (Consolidated strategic information guidelines 2015)	May 2015	Treat	Treat	Treat	Not discussed
BHIVA (BHIVA guidelines for the treatment 2012)	June 2015	Treat	Treat	Treat	Treat
EACS (2014)	November 2014	Treat	Treat	Consider ^a	Consider ^a
DHHS (Guidelines for the use of antiretroviral agents 2015)	April 2015	Treat	Treat	Treat	Treat
IAS-USA (Gunthard et al. 2014)	June 2014	Treat	Treat	Treat	Treat

Notes

^aIf symptomatic, EACS recommends treatment

The WHO recommends that all HIV-infected infants and children age <5 years be started on ART and that children age ≥5 years with CD4 cell counts ≤500 cells/mm³, WHO clinical stage 3/4, or tuberculosis be initiated on ART (Consolidated strategic information guidelines 2015). The PENTA guidelines also recommend treatment for all infants <1 year of age and for all children who are WHO stage 3/4 or CDC category B/C. Treatment is also recommended for children 1–3 years if CD4 cell counts are ≤1000 cells/mm³ or CD4% ≤25%, for children 3–5 years if CD4 cell counts are ≤750 cells/mm³ or CD4% ≤25%, and for children ages >5 years if CD4 cell counts are ≤350 cells/mm³ (Bamford et al. 2015). The current DHHS guidelines recommend that all infants <1 year of age be initiated on ARV regardless of CD4 cell count, as well as children ages 1 to <6 years with CD4 cell counts <500 cells/mm³ and children aged ≥6 years with CD4 ≤200 cells/mm³. Treatment for children older than 1 year with higher CD4 cell counts should be considered (Guidelines for the prevention and treatment 2013).

Special Populations

While there is general agreement among international and national guidelines for when to initiate ART for the treatment-naïve HIV-infected adult, there are some groups for whom initiation of ART should be prioritized to prevent mother-to-child

Antiretroviral Therapy: When to Start, Table 2 Rationale for prioritizing the initiation of ART among special HIV-infected populations

Patient group	Rationale
Pregnancy	Access to ART to prevent maternal-to-child transmission
Hepatitis C virus infection	Data on HIV and HCV coinfection have shown that each infection can accelerate progression of the other
Hepatitis B virus infection	Treatment of HBV requires the use of antivirals that dually treat HIV infection; fully suppressive ART should be initiated in this setting
Acute HIV infection	Treatment at the time of acute seroconversion may reduce the HIV reservoir size and inflammation from the virus itself
HIV-associated nephropathy	May reduce renal disease progression but usually does not reverse damage
Opportunistic infection ^a	May improve immune response and outcomes

Notes

^aFor majority of opportunistic infections. In persons with tuberculosis, exact timing of ART depends on the CD4 cell count and severity of pulmonary tuberculosis, but in general should begin within 2–8 weeks of tuberculosis treatment. In persons with cryptococcal meningitis, a short delay of 2 weeks is recommended to minimize the IRIS response and increased mortality.

transmission or reduce morbidity and mortality. Table 2 summarizes the DHSS guidance for special populations (Guidelines for the use of antiretroviral agents 2015).

Conclusion

The question of *when to start* ART for HIV-infected persons has been centered on CD4 cell count thresholds since the availability of zidovudine monotherapy. The starting threshold has swung as low as waiting until progression to AIDS at CD4 cell counts ≤ 200 cells/mm³ and as high as starting at the time of diagnosis, regardless of CD4 cell count. Several large clinical trials have informed the timing of initiation based on HIV transmission, morbidity, and mortality. The past concerns of toxicity, pill burdens, adherence, and emergence of resistance had been dampened by better ARV options and improved clinical and public health outcomes from earlier initiation of ART. Taken as a whole, the question of *when to start* seems to be definitely answered – *start now* – ART should be offered regardless of CD4 cell count threshold.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [Cryptococcosis and HIV](#)
- ▶ [Prevention Clinical Trials: Highlights of Evidence and Research](#)
- ▶ [Tuberculosis and HIV](#)

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Antiretroviral Treatment in Resource-Limited Settings

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Definition

Antiretroviral therapy (ART) treatment has been proven to slow the progression of disease and reduce the risk of transmission for individuals with human immunodeficiency virus (HIV) infection. The goal of ART treatment is to completely inhibit viral replication in vivo and sustain the effects for as long as possible.

Introduction on Impact

Access to ART in resource-limited settings (RLS) has provided opportunity for infected patients to achieve nearly normal life expectancy with clear health gains. Before 2001, many people in RLS did not have access to ART due to the high cost and lack of availability of antiretroviral drugs (ARVs). Under special terms in international trade law, manufacturers in RLS began to produce generic drugs in 2001. This production expanded ART coverage, and by 2012, 9.7 million individuals representing 61% of all eligible HIV-infected patients under the 2010 World Health Organization (WHO) HIV treatment guidelines were on treatment in RLS. These scale-up efforts resulted in remarkable declines in the annual number of AIDS-related deaths from a high of approximately

2.3 M in 2005 to approximately 1.6 M in 2012 (UNAIDS report 2013). AIDS-related deaths among children born to HIV-infected mothers also declined more rapidly due to the impact of prevention of mother-to-child transmission (PMTCT) programs. However, in order for individuals and societies to benefit optimally from the successes of ART and thereby dramatically alter the trajectory of national epidemics, it is critical to adopt approaches that enhance the continuum of HIV care. This requires enhanced strategies for HIV diagnosis, linkage to care, retention in care, ART receipt, and ultimately plasma viral suppression (IOM 2012).

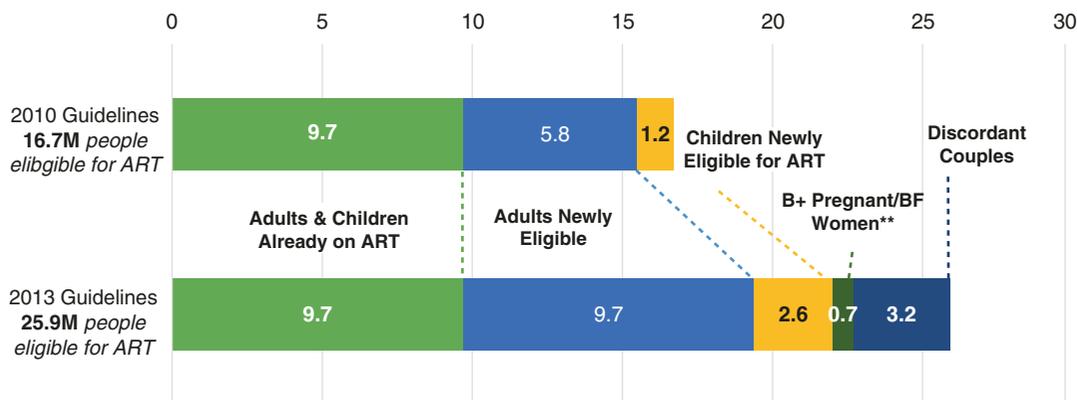
WHO Treatment Guidelines

In 2002, WHO published the first edition of the ART guidelines (WHO 2002). In 2004, guidelines for ART use for PMTCT were published (WHO 2004). The 2006 updated guidelines incorporated a “public health approach,” with simplified standardized tools for the delivery of ART (Gilks et al. 2006; WHO 2006). In 2010, WHO revised the guidelines for ART in adults and adolescents, based on a process of review and synthesis of new and emerging evidence (WHO 2010). Results from research revealed evidence that earlier initiation of ART offered significant benefits by enhancing host immune competence and reducing opportunistic infections, sustaining viral suppression, and thereby prolonging survival (WHO 2011). In 2011, the results of a randomized controlled trial demonstrated that ART can decrease HIV transmission risk in serodiscordant couples. In response to this evidence, in 2013 WHO recommended initiation of treatment when an individual’s CD4 count fell below 500 cells/mm³, immediately for all pregnant women, for HIV-infected partners in serodiscordant couples, for all children younger than 5, and for people with HIV-associated tuberculosis and Hepatitis B (Table 1).

The consolidated guidelines were formulated on the basis of the continuum of HIV prevention, treatment, and care. The recommendations incorporated the facilitation of linkage and

Antiretroviral Treatment in Resource-Limited Settings, Table 1 Consolidated guidelines on when to start ART treatment (WHO 2013)

Population	Recommendation
Adults and adolescents (≥10 years)	<ul style="list-style-type: none"> Initiate ART if CD4 cell count ≤ 500 cells/mm³ <ul style="list-style-type: none"> As a priority initiate ART in all individuals with severe/advanced HIV disease (WHO clinical stage 3 or 4) or CD4 count ≤ 350 cells/mm³ Initiate ART regardless of WHO clinical stage and CD4 cell count for individuals with; <ul style="list-style-type: none"> Active TB disease HBV co-infection with severe chronic liver disease Pregnant and breastfeeding women with HIV HIV-infected individuals in a serodiscordant partnership (to reduce HIV transmission risk)
Children ≥5 years old	Initiate ART if CD4 cell count ≤ 500 cells/mm ³ As a priority, initiate ART in all children with severe/advanced HIV disease (WHO clinical stage 3 or 4) or CD4 count ≤ 350 cells/mm ³ Initiate ART regardless of CD4 cell count for children with; <ul style="list-style-type: none"> WHO clinical stage 3 or 4 Active TB disease
Children 1–5 years old	Initiate ART in all regardless of WHO clinical stage and CD4 cell count As a priority, initiate ART in all HIV-infected children 1–2 years old or with severe/advanced HIV disease (WHO clinical stage 3 or 4) or with CD4 count ≤ 750 cells/mm ³ or $<25\%$, whichever is lower
Infants <1 year old	Initiate ART in all infants regardless of WHO clinical stage and CD4 cell count



*Includes TB and HBV co-infected
 **Only includes CD4>500; others are included under adults
 ** BF - Breast feeding

Antiretroviral Treatment in Resource-Limited Settings, Fig. 1 WHO treatment guidelines, 2010 versus 2013: Earlier initiation of treatment with expanded eligibility (Source: PEPFAR 2015)

promotion of consistency in approaches across the various settings in which ART and related services may be provided, such as specialized HIV care, primary care, community-based care, maternal and child health services, TB services, and services for people who use drugs. Finally, the guidelines enabled key clinical, operational, and programmatic implications of new science

and emerging practice in the use of ARVs to be comprehensively reviewed every 2 years across populations, age-groups, and settings (WHO 2013). With these expanded guidelines, it was estimated that 25.9 M individuals in RLS were eligible for ART compared to an estimated 16.7 M persons using the 2010 guidelines (Fig. 1).

The largest increase of newly eligible individuals was in the adult and adolescent age-groups followed by discordant couples and children. Further, the 2013 guidelines modified PMTCT requirements, ensuring continued access to ART for mothers during the pregnancy and breastfeeding period (Option B/B+). Expanding the eligibility criterion for ART in RLS creates opportunities to save lives and reduce HIV transmission. However, the expansion can also pose significant technical, operational, programmatic, and ethical challenges to policymakers and implementers. Therefore, faster scale-up, innovation, and programmatic adaptation will be required to provide care to all eligible individuals (PEPFAR 2015).

ART Regimen Choices for Adults and Adolescents

First-line Regimens in Adults and Adolescents

First-line ART should consist of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse transcriptase inhibitor (NNRTI). A fixed-dose combination of tenofovir (TDF), lamivudine (3TC) or emtricitabine (FTC), and efavirenz (EFV) is recommended as the preferred option to initiate ART in adults and adolescents. If TDF + 3TC (or FTC) + EFV is contraindicated or not available, Table 2 provides other options that are recommended.

Second-line Regimens in Adults and Adolescents

Diagnosis and treatment of the patients failing first-line ART in RLS remains challenging. The lack of routine viral load monitoring causes treatment failure to entirely depend on WHO clinical and immunological failure criteria, which have been demonstrated to correlate poorly with virological failure. Due to the potential misclassification of treatment failure resulting from using clinical and immunological failure criteria, limited availability of treatment options, poor laboratory infrastructure, lack of health care provider confidence in making switches, and the high cost of second-line ART, physicians are reluctant in deciding whether and when to switch ART. Delayed treatment switches lead to increased morbidity and mortality, and more extensive ART resistance in patients with unrecognized treatment failures.

WHO recommends the use of two classes of drugs, NRTI and protease inhibitors (PI), for adults, adolescents, and children if NNRTI-containing regimen were used in first-line ART. Unlike the NRTI, the PI may have a relatively high threshold of mutations to confer resistance, and most patients failing first-line ART do not have PI resistance mutations. The drugs most commonly used for second-line ART include tenofovir, abacavir, and lopinavir/ritonavir (LPV/r); atazanavir/ritonavir (ATV/r) can be substituted for LPV/r as needed. The second-line NRTI choice for adolescents and adults depends on the first-line ART; for patients on AZT or

Antiretroviral Treatment in Resource-Limited Settings, Table 2 First-line regimens in adults and adolescents (WHO 2013)

First-line ART	Preferred first-line	Alternative first-line
Adults (including pregnant and breastfeeding women and adults with TB and HBV co-infection)	TDF + 3TC (or FTC) + EFV	AZT + 3TC + EFV AZT + 3TC + NVP TDF + 3TC (or FTC) + NVP
Adolescents (10–19 years) ≥ 35 kg	TDF + 3TC (or FTC) + EFV	AZT + 3TC + EFV AZT + 3TC + NVP TDF + 3TC (or FTC) + NVP ABC + 3TC + EFV (or NVP)

AZT zidovudine, NVP nevirapine, ABC abacavir

Antiretroviral Treatment in Resource-Limited Settings, Table 3 Second-line regimens in adults and adolescents (WHO 2013)

Population	Preferred second-line	Alternative second-line
Adults and adolescents (≥ 10 years), including pregnant and breastfeeding women	AZT + 3TC + LPV/r AZT + 3TC + ATV/r	TDF + 3TC (or FTC) + ATV/r TDF + 3TC (or FTC) + LPV/r ABC + 3TC + LPV/r
HIV and TB co-infection	If rifabutin is available	Standard PI-containing regimens as recommended for adults and adolescents
	If rifabutin is not available	Same NRTI backbones as recommended for adults and adolescents plus double-dose LPV/r (that is, LPV/r 800 mg/200 mg twice daily) or standard LPV dose with an adjusted dose of RTV (that is, LPV/r 400 mg/400 mg twice daily)

stavudine (D4T) in first-line ART, the default second-line option is TDF combined with 3TC or FTC and LPV/r. For patients receiving TDF in first line, the second-line option will be an AZT-based regimen. For those receiving TDF during first line because of intolerance to AZT or D4T, an alternative second-line option will be abacavir (ABC) combined with 3TC or FTC and LPV/r (Table 3).

Monitoring of HIV-Infected Patients

Monitoring of HIV-infected patients is critical to assess patient progress. Due to the cost associated with routine viral load testing, patient monitoring in RLS is done mainly through clinical and WHO immunological criteria assessments, despite the shortcomings of these assessments in recognizing virological failure.

Clinical Monitoring

Assessments include observing the development of clinical events such as unexplained weight loss, herpes zoster infection, diarrhea for more than 1 month, pneumonia, tuberculosis, meningitis, or other AIDS-defining events. These clinical events are then used to classify patients into different WHO stages.

Immunological Monitoring

Periodic 6-month CD4 cell count measurements are performed to identify those with ART treatment failure. WHO immunological failure criteria

include a fall of CD4+ cell count to the pre-ART baseline value or below, a fall of $\geq 50\%$ of peak value CD4+ cell count while on treatment, and a persistently low CD4+ cell count $< 100/\text{mm}^3$.

One of the major challenges of immunological assessments is their relatively low sensitivity and specificity in predicting virological failure. The reported range of sensitivity is 16–58%, with specificity of 21–54%. These low levels of sensitivity and specificity suggest unintended misclassifications are likely to occur and some patients will receive unnecessary switches and those with unrecognized treatment failure will be prone to disease progression. Study results have confirmed these predictions but have not demonstrated a survival benefit for viral load monitoring. Although survival benefits of using viral load testing over clinical and immunological assessment are not apparent, WHO recommends the use of viral load testing whenever available since the latter does allow early detection of treatment failure.

Monitoring for Drug Toxicities

Besides clinical and immunological assessments, laboratory monitoring of drug toxicities is important. The nucleoside/nucleotide reverse transcriptase inhibitors are classes of ARVs widely used in RLS. These drugs may be associated with a variety of side effects, most of which could be monitored by routine laboratory assessments of chemistries to monitor liver and kidney function, and complete blood counts for anemia and neutropenia.

Antiretroviral Treatment in Resource-Limited Settings, Table 4 First-line regimens for children and infants (WHO 2013)

Population	Preferred first-line	Alternative first-line
Children 3 years to less than 10 years and adolescents <35 kg	ABC + 3TC + EFV	ABC + 3TC + NVP AZT + 3TC + EFV AZT + 3TC + NVP TDF + 3TC (or FTC) + EFV TDF + 3TC (or FTC) + NV
Children <3 years	ABC or AZT + 3TC + LPV/ r	ABC + 3TC + NVP AZT + 3TC + NVP

A

Monitoring for Drug Resistance

Monitoring for drug resistance in RLS is not a routine practice due to the costs associated with resistance testing and a lack of laboratory support with capacity to do resistance testing.

The availability of liquid ART formulations for treating infants and children varies between countries.

ART Regimen Choices for Pediatrics

For children infected with HIV age 3 years and older (including adolescents), EFV is the preferred NNRTI for first-line treatment and NVP the alternative. For children infected with HIV 3 years to less than 10 years old (or adolescents less than 35 kg), the NRTI choices for an ART regimen should be one of the following, in preferential order: ABC + 3TC or AZT or TDF + 3TC (or FTC).

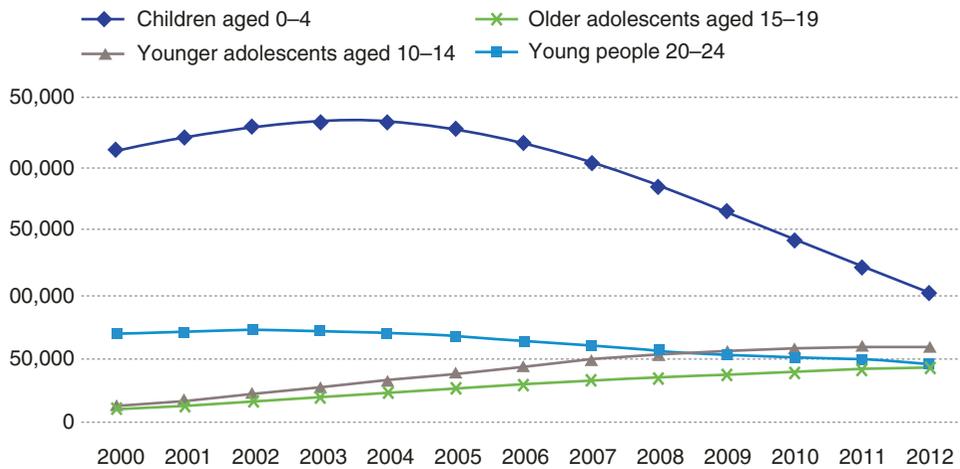
A LPV/r-based regimen should be used as first-line ART for all children infected with HIV younger than 3 years (36 months) of age, regardless of NNRTI exposure. If LPV/r is not feasible, treatment should be initiated with a NVP-based regimen. For infants and children infected with HIV younger than 3 years, the NRTI backbone for an ART regimen should be ABC or AZT + 3TC due to the potential for TDF to be associated with adverse bone effects.

For infants and children infected with HIV younger than 3 years, ABC + 3TC + AZT is recommended as an option for children who develop TB while on an ART regimen containing NVP or LPV/r to avoid drug-drug interactions with anti-TB drugs (Table 4). Once TB therapy has been completed, this regimen should be stopped and the initial regimen restarted.

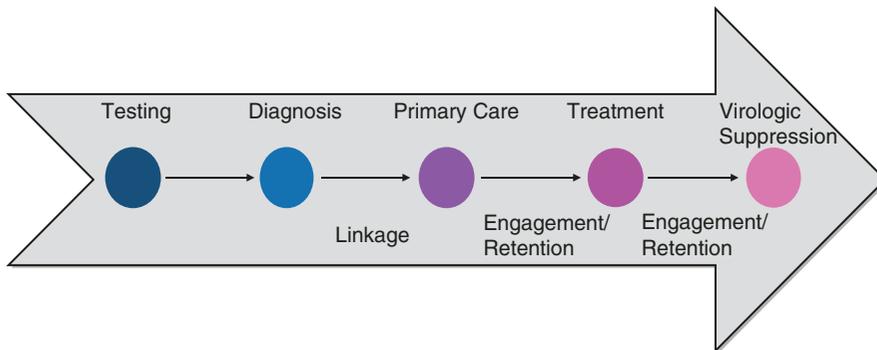
Treatment Challenges for Adolescents

In RLS, an estimated 2.1 million adolescents (10–19 years) were living with HIV in 2012 (UNAIDS 2013). While the number of global AIDS-related deaths for all ages fell by 30% between 2005 and 2012, among adolescents the number increased by 50% in that period (Fig. 2).

Adolescence is recognized as a period of life involving significant physical, physiological, and psychological changes that mark the transition to adulthood. This is also the period of life when social and gender roles are reinforced in many RLS. Adolescents infected with HIV face higher rates of depression, anxiety, and trauma than their uninfected peers. They are often exposed to parental loss and substance abuse. Most come from low-income families and subsequently face inequities in education, food security, and healthcare. Others may enter adolescence unaware of their HIV status. Inadequate access to high-quality, youth-friendly HIV treatment and sexual and reproductive health services and sexual violence against young women and girls hinder effective HIV prevention for adolescents and young people. In addition, limited protection for young people's confidentiality and right to medical privacy may hinder their ability to obtain essential services. Inadequate access to comprehensive sex education, which has been shown to effectively



Antiretroviral Treatment in Resource-Limited Settings, Fig. 2 Estimated number of AIDS-related deaths 2000–2012 (Source: UNICEF analysis of UNAIDS 2012 HIV and AIDS estimates)



Antiretroviral Treatment in Resource-Limited Settings, Fig. 3 Discrete steps in monitoring HIV care (Source: Adapted from IOM – Monitoring HIV Care in the United States)

delay sexual debut and increase condom use among young people, also undermines HIV prevention efforts (UNAIDS 2013). These mental, structural, and psychosocial challenges have negative associations with clinical outcomes. HIV-infected adolescents are more likely to be nonadherent to first- and second-line regimens, hence leading to the observed higher rates of AIDS-related deaths.

Treatment Cascade

The access to and availability of ARVs are not the only ingredients required to reduce mortality and morbidity of HIV/AIDS. In order for individuals

and society to benefit maximally from the successes of ART, it is important to adopt approaches that enhance the continuum of HIV care. Figure 3 illustrates the discrete steps along the HIV care continuum.

The cascade of care was developed to present a continuum of care, from the number of individuals within a population infected with HIV, diagnosed with HIV, linked to and retained in HIV care, on treatment, and having achieved suppression of HIV replication (Mugavero et al. 2010). It reflects the steps needed to improve an individual's health as well as identifies areas of opportunity within a population for interventions to optimize disease management and reduce ongoing HIV transmission. RLS are encouraged

to develop their country-specific national goals for testing, linkage, retention, and viral suppression. With these data, countries will be able to quantitatively track and measure the progress of their HIV programs. Recent studies from RLS have reported poor retention in pre-ART care and substantial losses to follow-up at every step, starting with patients who do not return for their initial CD4 count results and ending with those who do not initiate ART despite eligibility (Rosen and Fox 2011). Delays in diagnosis, poor engagement, and inconsistent retention in HIV care have all been shown to negatively impact both individual and public health outcomes. Therefore an emphasis should be placed on “adherence” to care and not just medications. Benefits of engagement in care for individual patients results in improvements in initiation and adherence to ART, better immunological and virological outcomes, and lower mortality. At the community level due to improvement of virological control and reduction in risky behaviors, there is reduced HIV transmission.

Adherence and Retention Interventions

Adherence

The goal of ART is to reduce the virus to undetectable levels in patients’ blood after being on treatment, and therapeutic levels of ART are essential to reach this goal. Thus adherence to ART is critical if this goal is to be realized. The main concern of interrupted medication use is the development and the spread of resistant strains of viruses leading to treatment failure. Despite the successes of ART, patients, program, and drug-related factors may each contribute to poor ART medication adherence.

Barriers to ART Adherence

HIV-related stigma, poverty, distance to care and treatment centers, alcoholism, substance abuse, being away from home, forgetfulness, depression, and a history of sexual, physical, and mental trauma are some of the notable patient-related factors that negatively impact ART adherence. In practical terms, sustained adherence to ART

requires consistent supply of ART medications. Medication stock-outs and long waiting hours due to staff shortages may impair ART adherence at the programmatic level. Despite the success of ART in reducing morbidity and mortality associated with HIV infection, their unintended adverse effects such as lipodystrophy, peripheral neuropathy, anemia, neutropenia, pancreatitis, hypersensitivity reactions, and kidney and liver toxicities may contribute to poor ART adherence.

Interventions to Improve ART Adherence

Many governments in RLS have tried to remove structural barriers of accessing ART by making ART freely available, as well as decentralizing care and treatment centers to residential areas. In most cases adherence to ART depends on personal behavior, and a number of interventions may result in improved medication adherence. A variety of health care professionals including doctors, pharmacists and nurses, and HIV-infected peers have been used successfully to deliver adherence education. Given that improved adherence may wane with time, counseling about ART adherence should be a continuous process across care and treatment centers. Establishment of peer support may be an important step in improving ART adherence. In addition, simplified treatment regimens with fixed-dose combinations and prescription of less toxic medications may also improve ART adherence.

Retention in Care

One of the greatest challenges facing HIV care and treatment programs in RLS is loss to follow-up. Loss to follow-up may result in increased morbidity, mortality, risk of HIV transmission, and development of resistant strains of viruses, all of which reduce efforts to mitigate HIV infection. Loss to follow-up is higher among those enrolled into care but not on ART than those already on ART, possibly because those not on ART perceive themselves to be clinically stable.

A number of strategies can improve retention of patients in care. For example, decentralization of care and moving treatment centers from

consultant referral hospitals to district and health centers improved patient retention by reducing the distance travel to clinics, as well as long waiting hours patients would have at consultant referral hospitals.

Establishment of community adherence support group improves patient retention into care. With this strategy, groups of 4–6 HIV-infected adults with stable HIV infection are created. Each month one group member attends clinic on behalf of others to collect ART, as well as sending blood samples for CD4 testing. In this way other group members get opportunities to be in the workplace with fewer disruptions and without interfering with ART treatment.

Empowering patients with the skills to prioritize ART use over other sociocultural activities is critical to improve patient retention in care. One of the strategies adopted by the WHO to improve patient retention is integration of health care services by providing services as “one stop shopping.” For example, integrating TB and HIV care at the TB clinic without referring TB/HIV coinfecting patients to specialized HIV clinic improves retention. Similarly antenatal care (ANC) and HIV care are integrated in most programs, and this approach has been shown to improve retention of HIV-infected pregnant mothers. Other strategies proven to improve retention include engagement of peer and family support, specialized services to those with substance abuse and mental illness, provision of reminder services, and systematic monitoring of clinic attendance.

Treatment as Prevention

In the context of an HIV serodiscordant couple where one member is HIV infected and the other is not, the viral load of the infected partner has long been identified as an important predictor of the risk of sexual transmission. Subsequently cohort studies suggested that treating the infected partner with ART could lead to a decreased risk of sexual transmission. The definitive evidence that ART is successful in decreasing the risk of transmission

came from the results of HPTN 052, published in 2011 (Cohen et al 2011). HPTN 052 studied 1763 HIV serodiscordant couples where the seropositive partner had a CD4 count 350–550, and randomized the HIV-infected partner to either immediate or delayed treatment with fixed-dose combination FTC/TDF/EFV. All participants were provided education regarding prevention of HIV transmission, and given free condoms. The primary outcome was HIV transmission to the seronegative partner, and transmitted virus was sequenced to ascertain whether transmission occurred in the original couple relationship or if a different sexual partner was responsible for transmission. The study was discontinued by the Data Safety and Monitoring Board after a median follow-up period of 1.7 years, and 90% of the couples were still retained in follow-up. The study results were striking; overall there were 39 HIV transmissions, 4 in the early therapy group and 35 in the delayed group. When linked transmission events were determined by sequencing, there was 1 transmission in the early therapy group and 27 in the delayed group, corresponding to a 96% reduction in HIV transmission when the seropositive partner received ART. These findings provide robust evidence to support the 2013 WHO Guidelines recommendation for initiating ART in the seropositive partner of a serodiscordant couple. Researchers are currently studying the impact of this recommendation outside of the context of a clinical trial to see if similar benefits are realized.

Additional uses of treatment as prevention can include pre-exposure prophylaxis (PrEP), postexposure prophylaxis (PEP), and the investigational use of microbicides containing antiretroviral compounds.

Antiretroviral Drug Resistance

Antiretroviral drug resistance is a growing challenge for successful treatment in RLS. A number of factors may be important in the development of drug resistance such as the widespread inability to measure plasma HIV RNA levels and recognize

virological failure, the lack of resistance testing to guide the optimal choice of ART regimens and therefore resulting in incomplete viral suppression, treatment interruptions due to logistical obstacles, poor adherence, and drug interactions. Antiretroviral drug resistance most commonly occurs as a consequence of incomplete suppression of viral replication in HIV-infected persons receiving ART (acquired drug resistance), and it also may occur in the context of transmitted drug resistance when HIV infection first occurs (transmitted drug resistance). These two circumstances will be considered separately.

Acquired Drug Resistance in HIV-Infected Persons Receiving ART

Drug resistance inevitably occurs over time if suppression of HIV replication is incomplete. Resistance to ART in resource-limited settings has been demonstrated in several scenarios listed below:

1. HIV-infected persons beginning ART who fail to fully suppress
2. HIV-infected persons beginning ART who do not adhere to treatment
3. HIV-infected persons who face logistical obstacles to care
4. HIV-infected mothers and infants who are exposed to incompletely suppressive regimens such as single-dose nevirapine

Many studies have addressed drug resistance among patients failing first-line ART in RLS, and nearly all have demonstrated extensive drug resistance by the time that failure was recognized (numerous studies have demonstrated that virological failure is recognized late). Most commonly three-drug regimens containing an NNRTI, 3TC, or FTC and a nucleoside or nucleotide reverse transcriptase inhibitor are employed as first-line treatment. At virological failure of first-line ART, nearly all patients have both NNRTI and 3TC or FTC resistance (Wallis et al. 2014), with consequences for potential cross-resistance in second-line ART choices. Unfortunately, resistance assays to guide second-

line ART choices are rarely available. Similarly, in HIV-infected mothers and infants with past exposure to single-dose nevirapine, NNRTI resistance is common and better treatment outcomes may be achieved when protease inhibitor-containing regimens are used following exposure to single-dose nevirapine.

Transmitted Drug Resistance

Transmitted drug resistance is increasingly recognized in resource-limited setting, especially eastern and southern sub-Saharan Africa. It is now estimated that 5–15% of transmitted HIV has drug resistance mutations, and the percentages with drug resistance are rapidly increasing (an estimated 29% per year in eastern Africa) (Hamers et al. 2013). Transmitted drug resistance does compromise the activity of ART regimens and has been linked to increased virological failure of ART and shorter time to failure (Kantor et al. 2015). These observations underscore the need to ensure successful ART with full suppression of HIV replication, and the need to improve laboratory monitoring of plasma HIV RNA levels and assessment of drug resistance mutations.

Conclusion

ART has been proven to save the lives of people living with HIV and decrease HIV transmission. However, for individuals and communities to reap these benefits, it is critical for health care systems in RLS to improve the delivery of services across the entire continuum of care. Initiatives to support active linkage, engagement, and retention in care have the potential to improve overall patient outcomes.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Children, Care and Treatment](#)
- ▶ [Clinical Ethics in HIV/AIDS Prevention, Care, and Research](#)

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APOBEC3F/G and Vif: Action and Counteractions

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Definition

The primary targets of HIV-1 (T lymphocytes, macrophages, and monocytes) are able to limit HIV-1 replication by expressing the restriction factors APOBEC3F and APOBEC3G (A3F/G). These two proteins have cytidine deaminase activity and induce mutations during reverse transcription of the genomic RNA that could be lethal for the virus. A3F/G also interfere with the reverse transcription and integration processes independently from this deaminase activity. To counteract this restriction, HIV-1 has evolved the viral infectivity factor (Vif), a multifunctional protein that is able to reduce the cellular A3F/G expression level through two major mechanisms: (1) Vif induces degradation of A3F/G by the proteasome by recruiting an E3 ubiquitin ligase complex, and (2) Vif inhibits A3F/G translation by interacting with the 5' untranslated region (UTR) of their mRNAs. These two mechanisms ultimately reduce the packaging of A3F/G into virions. The intimate relationship between Vif and A3F/G provides new therapeutic targets, and several

functional properties of Vif, such as Vif dimerization, the recruitment of the Vif-E3 ubiquitin ligase complex, and the Vif-A3F/G interaction, could be targeted by drugs.

Introduction

In addition to the three essential protein precursors (structural Pr55^{Gag}, enzymatic Pr160^{Gag-Pol}, and envelope Pr160^{Env}), the HIV-1 genome encodes six auxiliary proteins: Tat, Rev, Nef, Vpr, Vpu, and Vif. Vif (viral infectivity factor) is essential for the infection of the HIV-1 natural targets cells, such as T lymphocytes. Indeed, a *vif*-deleted HIV-1 mutant (HIV-1Δ*vif*) does not replicate in such cells, which are thus termed nonpermissive. Despite this information, the role of Vif remained unclear for a long time, until the identification of CEM15 (Sheehy et al. 2002), a natural cellular antiviral factor counteracted by Vif which has since be renamed APOBEC3G (A3G). A3G belongs to a large family of cytidine deaminases and needs to be packaged into viral particles in order to perturb the next HIV-1 replication cycle. In the first part of this review, A3G and A3F proteins and the processes by which these proteins inactivate HIV-1 replication are described. In the second part, the focus is on Vif and the mechanisms by which Vif counteracts the A3F/G restriction activities. To conclude, the importance of the relationship between A3F/G and Vif is underlined and how it could be used in new therapeutic approaches is indicated.

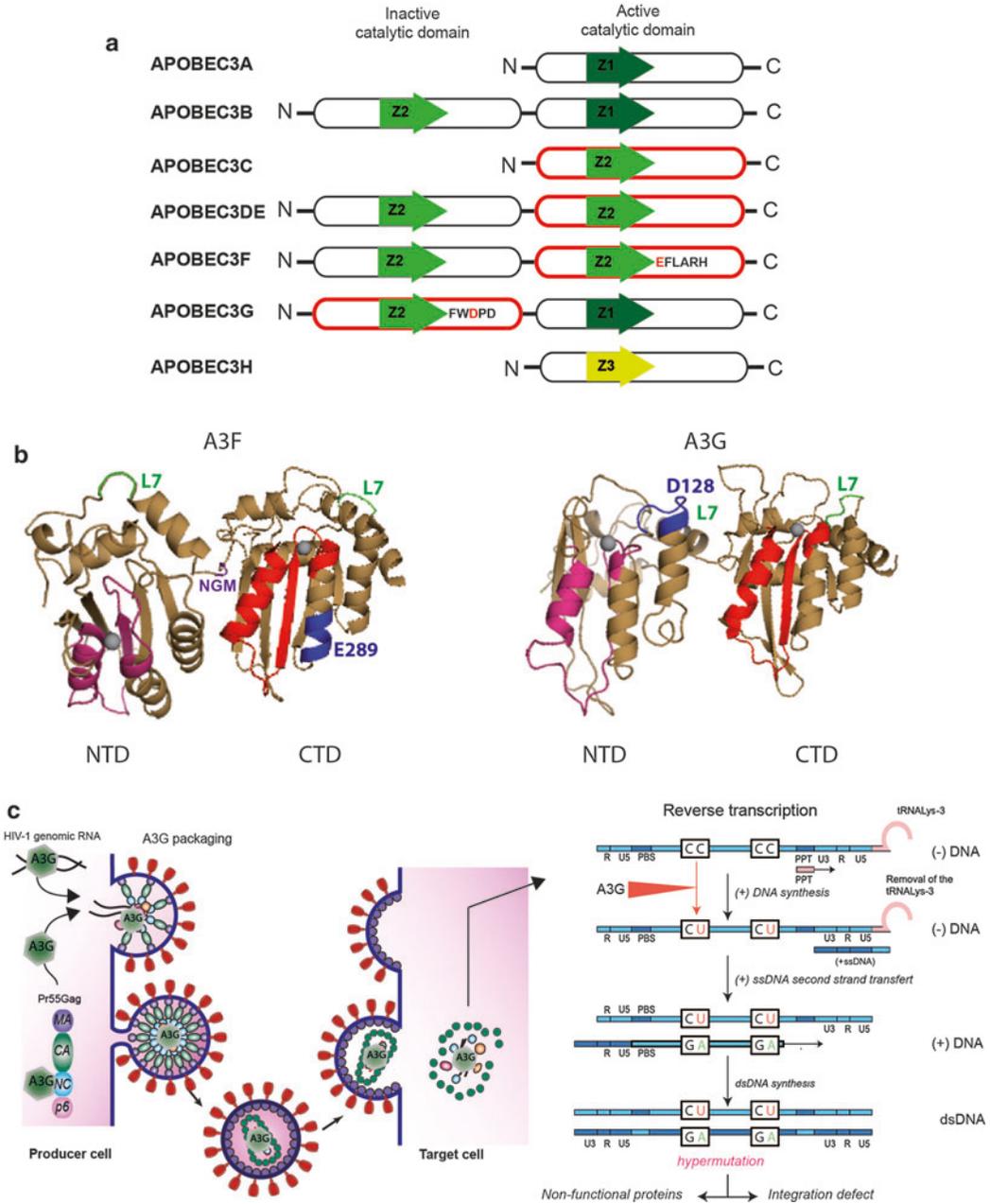
HIV-1 Restriction by APOBEC3 Enzymes

The APOBEC3 Proteins: A Potent Army Against HIV
The APOBEC3 (**apolipoprotein B** mRNA-editing enzyme, catalytic polypeptide-like 3 or A3) family is exclusively present in vertebrates; in humans, it comprises seven members (A3A to A3H), all encoded by genes located on chromosome 22 (Fig. 1a). This family of proteins plays an important role in innate antiviral immunity against retroviruses, most notably HIV-1. Each A3 protein harbors at least one active catalytic domain (CD) constituted by a zinc finger motif sharing the

consensus sequence H-X-E-X_{23–28}-P-C-X_{2–4}-C (where X is any one of the 20 amino acids). A3A, A3C, and A3H have only one zinc finger domain and hence one catalytically active domain. A3D/E, A3F, and A3G have two zinc finger motifs, but only one is catalytically active. A3B is the exception as it has two zinc finger motifs, both of which may be catalytically active. All of these enzymes are involved in the restriction of several viruses/retrotransposons. Among the A3 proteins, A3F and A3G are the most efficient in restricting HIV-1, although A3A, A3D/E, and A3H are also able to affect HIV-1 infectivity to some extent. In A3F and A3G, the active cytidine deaminase domain is located in the C-terminal zinc finger motif (Fig. 1a). The inactive N-terminal zinc finger motif is involved in different functions such as RNA binding, multimerization, or packaging into virions. A3F and A3G are both able to bind Vif, even though the binding sites do not seem to be conserved between the two proteins: the residues involved in Vif binding are located in the N-terminal CD between positions 126–132 of A3G and positions 289–294 in the C-terminal CD of A3F, respectively. Interestingly, both sites of interaction could be disrupted by a single amino acid mutation: D128K and E289K in A3G and A3F, respectively. These mutations alter the ability of the respective protein to reduce HIV-1 infectivity.

Structural information is available for the C-terminal domain of A3F (PDB: 4IOU) and A3G (PDB: 3IQS) revealing highly conserved structures between the two proteins. Both are composed of five β-sheets (β1–β5) with six α-helices (α1–α6) including a coordinated zinc ion. Models of the N-terminal domains have recently been established using the SWISS-MODEL program (Feng et al. 2014). When the two domains are combined (Fig. 1b), A3F/G appear as symmetric proteins exposing a positively charged platform on both domains, allowing binding to negatively charged DNA.

To achieve their restriction activity on HIV-1, A3F/G proteins need to be encapsidated into nascent virions to act during the next round of replication (Fig. 1c). Indeed, despite their cytoplasmic localization in target cells, A3F/G



APOBEC3F/G and Vif: Action and Counteractions, Fig. 1 APOBEC3 family: domains, structure of A3F/G, and restriction mechanisms. (a) Schematic representation of the seven APOBEC3 members. The conserved zinc (Z)-coordinating DNA cytosine deaminase motif Z1, Z2, and Z3 (phylogenetic clusters containing a SW-S/T-C-x₂₋₄-C motif for Z1 and Z2, whereas Z3 proteins have a TW-S-C-x₂-C motif) are shown in *dark green*, *green*, and *yellow*, respectively. The Vif binding domains are indicated by *red ovals*. For A3F/G, the Vif binding sequence is indicated including the crucial residues in *red*

(see text for details). (b) A3F/G structure/models: while the structures of the C-terminal domain of A3F and A3G have both been determined experimentally (PDB: 4I0U and 3IQS), experimental structures of the N-terminal domains are still missing and therefore correspond to models calculated by Feng et al. (2014). The catalytic inactive domain is depicted in *pink*, whereas the active one is in *red*. Residues in *blue* interact with Vif. Loop seven (L7) involved in the processivity and in the recognition of the target sequence to deaminate is depicted in *green* in each domain, whereas A3F NGM residues are in *purple*, at the interface of the

proteins are not able to access the neosynthesized (–) single-stranded (ss) DNA intermediate during reverse transcription of incoming viruses. This is most likely due to the protection of the reverse transcription complex by the HIV-1 capsid and/or sequestration of the A3F/G proteins in storage granules, such as stress granules or processing bodies (P-bodies). A3F/G packaging is mediated by interactions with the nucleocapsid (NC) domain of the Pr55^{Gag} precursor (Alce and Popik 2004). This interaction is also stabilized by the presence of the HIV-1 genomic RNA. Efficient binding of A3G to the HIV-1 genomic RNA requires its oligomerization. Interestingly, both RNA binding and A3G oligomerization are mediated by the N-terminal domain of A3G. Additionally, A3G packaging is favored by the co-encapsidation of 7SL RNA, which is also mediated by the NC domain of HIV-1 Pr55^{Gag}. Once packaged, A3F/G remain associated with the reverse transcription complex after virus entry in target cells where they are able to perform their antiviral activities.

Deamination-Dependent Mechanisms

Once in the cytoplasm of infected cells, A3F/G proteins interfere with reverse transcription by inducing numerous deoxycytidine to deoxyuridine mutations during the synthesis of the (–) ssDNA in a 3′–5′ processive manner (Fig. 1c; Harris et al. 2003). This process ultimately results in dG → dA hypermutations in the (+) strand proviral DNA and in the production of aberrant viral proteins, thus reducing the replicative capacities of the virus. A3F and A3G preferentially deaminate different sequencings in the (–) ssDNA: A3G preferentially deaminates the last cytidine of 5′-CC or 5′-CCC motifs, thus inducing 5′-AG or 5′-AGG mutations on the (+) strand DNA, whereas A3F deaminates 5′-TC or

5′-TTC motifs. Interestingly, the A3G target sequence is part of the unique tryptophan codon (ACC in (–) DNA; TGG in (+) DNA), and its deamination results in the introduction of stop codons (UGA) in the viral coding sequence. In contrast, A3F mainly induces missense mutations that may or may not allow the expression of the corresponding protein. This could explain why A3G has a more drastic effect on HIV-1 infectivity than A3F. Another explanation might reside in the mechanism that the two proteins use to find their target sequence on the (–) ssDNA: while A3G is able to slide very efficiently along the (–) ssDNA substrate to find its target sequence in a processive manner, A3F jumps from one (–) ssDNA substrate to another one in a distributive manner (Ara et al. 2014). The processivity determinants of A3G have been mapped in the loop 7 and helix 6 of the N-terminal non-catalytic domain, which therefore indirectly participate in the deamination activity of A3G. In A3F, the linker between the N- and C-terminal domains (¹⁹⁰NGM¹⁹²) (Fig. 1b) seems to be responsible for its inability to translocate on (–) ssDNA.

Such deamination-induced mutations in the viral genome are lethal to the virus and could lead to the degradation of the hypermutated viral DNA, the production of truncated or non-functional viral proteins, and the impairment of the integration process. Moreover, mutated/truncated proteins expressed from the viral genome may, after processing by the proteasome, serve as HIV antigens and facilitate the immune recognition of HIV-1 (Casartelli et al. 2010).

Deamination-Independent Mechanisms

Several studies using inactive mutants of the CD showed that A3F/G still have residual restriction activity and impair HIV-1 infectivity, indicating that cytidine deaminase activities are not solely

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APOBEC3F/G and Vif: Action and Counteractions, Fig. 1 (continued) domains (see text for details). Figures were drawn with the PyMOL software (PyMOL Molecular Graphics System, Version 1.5.0, Schrodinger, LLC). (c) Restriction mechanisms of A3G: to exert its

restriction activity, A3F/G needs to be encapsidated into viral particles and released in infected cells (*left panel*). Restriction of HIV-1 replication by A3F/G involves the deaminase activity (*right panel*) and deaminase-independent pathways (not shown)

responsible for their antiviral activities. Deaminase-independent activities target mainly two steps of the HIV-1 replication cycle: reverse transcription and integration. A3F/G are able to interact with reverse transcriptase and limit its efficiency, resulting in diminished viral cDNA levels (Wang et al. 2012), and can also bind HIV-1 integrase and decrease the integration efficiency by 5- to 50-fold (Luo et al. 2007). Such deamination-independent activities might be due to interactions of A3F/G with the viral genome, leading to competition and/or steric hindrance with reverse transcriptase and integrase enzymes. Although this deaminase-independent mechanism seems to contribute less than 1% to the overall restriction capability of A3F/3G, it has nevertheless been observed in primary cells (Gillick et al. 2013).

Overview and Properties of HIV-1 Vif

To counteract the restriction mediated by A3F/G, all lentiviruses except equine infectious anemia virus (EIAV) encode the viral infectivity factor (Vif), which is essential for HIV-1 replication in primary target cells (lymphocytes, macrophages/monocytes, dendritic cells). In these so-called nonpermissive cells, deletion of *vif* from the HIV-1 genome has been associated with a strong reduction of viral infectivity (100- to 1,000-fold) (Strebel et al. 1987). The nonpermissive phenotype is due to the expression of A3F/G proteins in these cell types (Sheehy et al. 2002).

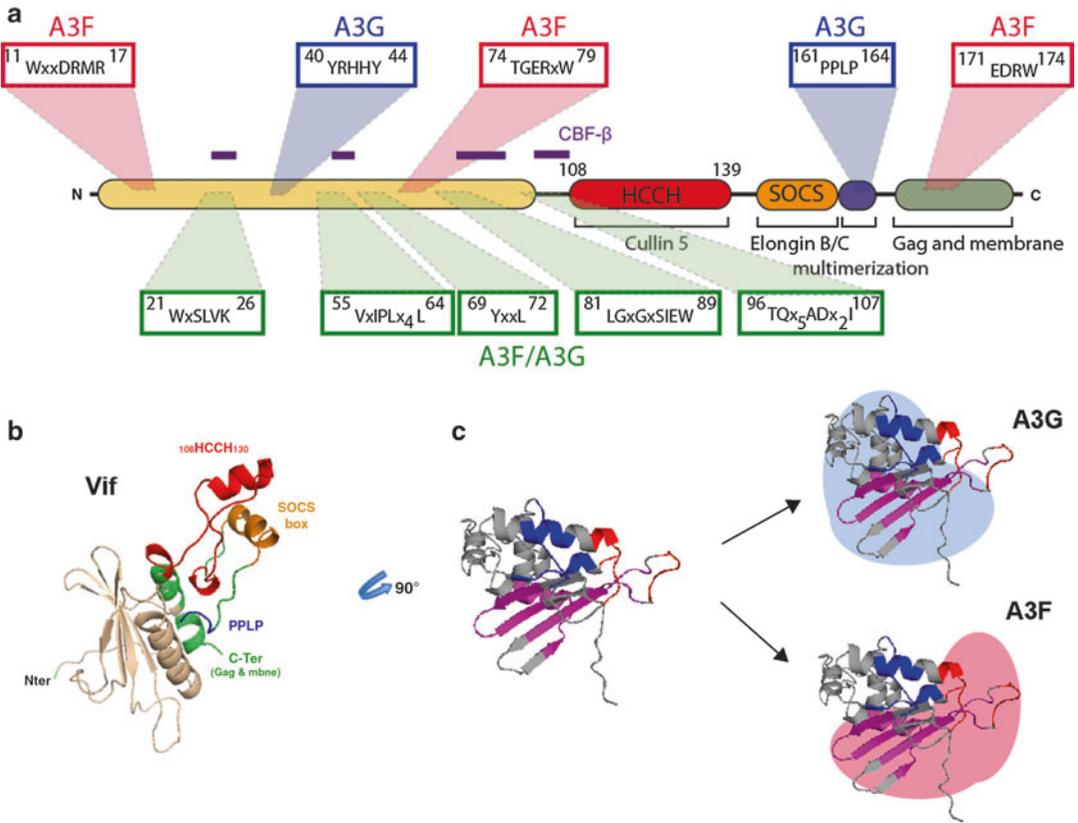
Functional Domains of Vif

Vif is a highly basic protein (pI = 10.7) composed of 192 amino acids (23 kDa). Alignments of lentiviral Vif sequences highlighted several conserved domains suggestive of a multifunctional protein.

- *The N-terminal domain*, enriched in tryptophan residues, is important for the binding of A3 proteins (Fig. 2a). Although A3F and A3G share common interacting motifs with Vif (²¹WKSLVK²⁶, ⁵⁵VxIPLx4L⁶⁴, ⁶⁹YWxL⁷², and ⁸⁵VSIEW⁸⁹), some domains are specific to A3G (⁴⁰YRHHY⁴⁴ and ¹⁶¹PPLP¹⁶⁴) and A3F (¹¹WQxDRMR¹⁷, ⁷⁴TGERxW⁷⁹, and

¹⁷¹EDRW¹⁷⁴). Other domains in the C-terminal domain have been described to be important for A3G and A3F binding, such as the ¹⁶¹PPLP¹⁶⁴ and ¹⁷¹EDRW¹⁷⁴ motifs, respectively (Fig. 2a, b). Such discontinuous interacting sites suggest that the correct folding of Vif is crucial to create the binding platform for A3 proteins. Moreover, Vif residues important for the interaction with A3H, A3C, and A3DE have been identified in the N-terminal region. The N-terminal region of Vif also interacts with the HIV-1 genomic RNA (residues 1–64 and 75–114) both in vitro and in infected cells. Finally, residues W₂₁, W₃₈, ⁸⁴GxSIEW⁸⁹, and ¹⁰²LADQI¹⁰⁷ are involved in the binding of CBF-β (core binding factor β).

- *The central region* of Vif constitutes a platform for the recruitment of an E3 ubiquitin ligase complex composed of Elongin B, Elongin C, CBF-β, Cullin 5, and Rbx2 proteins that targets A3 proteins for degradation by the proteasome. Residues 108–139 constitute a non-consensus HCCH zinc finger motif that binds zinc and Cullin 5; they are followed by a SOCS (suppressor of cytokine signaling) box motif (¹⁴⁴SLQYLA¹⁴⁹). This region promotes the interaction with the Elongin C/Elongin B (BC box) heterodimer through a hydrophobic platform on both proteins involving the SLQ residues and the A₁₄₉ residue of the SOCS box and α helices 3 and 4 of Elongin C. Very recently, the Cullin 5 box and the ¹⁶¹PPLP¹⁶⁴ motif have also been shown to participate to Vif-Elongin C/B binding.
- *The C-terminal region* of Vif starts with a putative Cullin 5 box (residues 159–173) that recruits the Cullin 5 with a lower efficiency than the HCCH zinc finger domain. This region covers the ¹⁶¹PPLP¹⁶⁴ motif involved in Vif multimerization. This PPLP motif is also involved in the binding with various partners such as A3G, Elongin B/Cullin 5, and the cellular kinase Hck. The last 25 residues of Vif are important for its interaction with Pr55^{Gag} and cytoplasmic membranes and interfere with the reverse transcriptase activity. In contrast to other regions of Vif, the C-terminal

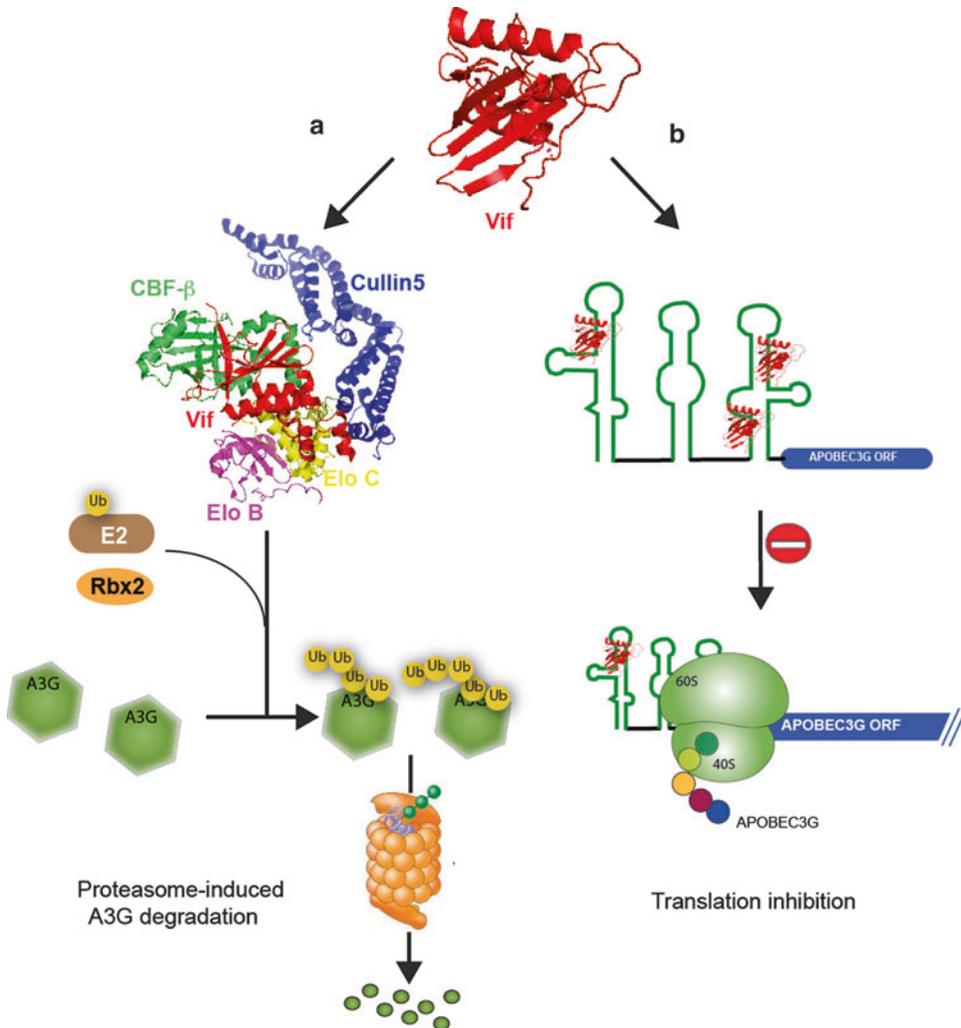


APOBEC3F/G and Vif: Action and Counteractions, Fig. 2 Vif: domains, structure, and interactions with A3F/G. (a) Schematic representation of the Vif functional domains together with the interaction map with A3G, A3F, and other Vif partners. (b) Structure of the Vif protein extracted from the structure of the Vif-E3 ubiquitin ligase complex solved by Guo et al. (2014) (PDB: 4N9F). Colors correspond to the domains described in (a). (c) Vif

structure with A3F (red) and A3G (blue) binding domains. The Vif structure has been rotated to highlight the specific (blue/red) or the common (magenta) interaction areas. The possible orientation of A3G (in light blue) and A3F (pink) proteins is depicted on the Vif structure. Figures were drawn with the PyMOL software (PyMOL Molecular Graphics System, Version 1.5.0, Schrodinger, LLC)

appears to be intrinsically disordered. This property is characteristic of proteins carrying RNA and/or protein chaperone activities: such proteins catalyze the folding of both the chaperone and the target molecules upon binding, reconstituting an active complex. Chaperone activities have been extensively described for several HIV-1 proteins such as NC and Tat and more recently for Vif (Sleiman et al. 2014). The Vif RNA chaperone activity may modulate viral activities such as the structural maturation of the HIV-1 genomic RNA and initiation of its reverse transcription to optimize viral infection and propagation.

Due to its intrinsic biochemical properties, the determination of the 3D structure of Vif has been a real challenge and a matter of intensive research. It is only very recently that the Huang Group successfully crystallized Vif in a pentameric complex (Fig. 3a) including the subunits of the E3 ubiquitin ligase complex: CBF-β, Elongin C/Elongin B, and Cullin 5 (Guo et al. 2014). The crystal structure of Vif has been solved at 3.3 Å resolution (PDB: 4N9F) and showed an elongated conical structure constituting two binding platforms involved in the recruitment of the E3 ubiquitin ligase complex and of A3 proteins (Fig. 2b). This is consistent with previous



APOBEC3F/G and Vif: Action and Counteractions, Fig. 3 Schematic representation of the mechanisms by which Vif reduces the A3G cellular level. (a) Vif binds to A3G and recruits an E3 ubiquitin ligase, leading to the polyubiquitination of A3G and its degradation by the 26S proteasome. (b) Vif impairs the translation of A3G

mRNA through an mRNA-binding mechanism, involving the highly structured 5' UTR of A3G mRNA. These different activities of Vif on translation and degradation not only deplete A3G from virus-producing cells but also prevent A3G from being incorporated into virions

mutational analyses showing that both Cullin 5 and Elongin C interact with the C-terminal domain of Vif, whereas A3F/G and CBF-β bind to the N-terminal domain. Interestingly, while the A3F/G binding motifs appear discontinuous in the linear Vif sequence (Fig. 2a), the Vif structure highlights how the different residues are organized in two different binding domains that overlap in the central part of Vif (Fig. 2c). Of note, the

extreme C-terminal part of Vif does not harbor any defined structures, consistent with an intrinsically disordered region and compatible with the RNA chaperone activity of this region.

Vif Hijacks an E3 Ubiquitin Ligase Complex to Degrade A3 Proteins (Vif/A3 Interactions)

Several mechanisms have been proposed to explain how Vif limits the antiviral activity of

A3F/G by preventing its encapsidation. The first and more documented mechanism so far is the polyubiquitination and proteasomal degradation of A3F/G by Vif (Marin et al. 2003; Sheehy et al. 2003). Proteasomal degradation results in a strong reduction of the intracellular levels of A3F/G. As described above, Vif is able to bind A3F/G through various domains and to recruit an E3 ubiquitin ligase complex, leading to the polyubiquitination and degradation of A3F/G. Vif therefore connects the E3 ubiquitin ligase complex (Elongin B/C, Cullin 5, CBF- β) to its A3G substrate (Fig. 3a). Then, an E2 ubiquitin-conjugating enzyme interacts with Rbx2 and allows the polyubiquitination (⁴⁸K-linked ubiquitins) of A3G that is targeted to the 26S proteasome for degradation. Recently, the transcription cofactor CBF- β has been shown to be an integral component of the ubiquitin ligase complex and required for the Vif-mediated degradation of A3G. Therefore, Vif binds the E3 ubiquitin ligase complex through at least 3 binding domains: the SOCS box allows the interaction with Elongin B/C, the HCCCH zinc finger mediates binding to Cullin 5, and, finally, the N-terminal domain binds to CBF- β .

The A3G lysine residues targeted by the E3 ubiquitin ligase complex are not clearly defined: if one study identified four critical lysines in the C-terminal region of A3G (K₂₉₇, K₃₀₁, K₃₀₃, and K₃₃₄) (Iwatani et al. 2009), others showed that A3G lysines are randomly susceptible to ubiquitination.

Degradation-Independent Inhibition of A3 Proteins

Translation Although the Vif-induced degradation of A3F and A3G by the 26S proteasome has been well studied, it is not the only mechanism by which Vif counteracts A3 proteins. Indeed, Vif decreases the steady-state levels of A3F/G in the presence of proteasome inhibitors in rabbit reticulocyte lysates and cell cultures, suggesting that A3G translation is affected by Vif (Mariani et al. 2003; Stopak et al. 2003). Recently, binding of Vif to the highly structured 5' untranslated region (5' UTR) of A3G mRNA has been shown to be responsible for the inhibition of A3G

translation by Vif (Mercenne et al. 2010; Fig. 3b). Although the exact mechanism is not understood yet, it is possible that Vif relocates A3G mRNA to specialized compartments such as P-Bodies or stress granules where translation is stopped. Indeed, Vif has been shown by immunofluorescence to co-localize with A3 proteins in P-Bodies (Wichroski et al. 2006).

Virion Encapsidation As discussed above, Vif can reduce the expression of A3F/G proteins through the proteasome/degradation pathway and by inhibiting its mRNA translation, ultimately leading to a strong reduction of A3F/G encapsidation into new budding particles. One estimate indicates that 1–2 to 3–11 A3G proteins are encapsidated per virion in the presence or absence of Vif, respectively (Xu et al. 2007). Interestingly, the use of an A3G C97A mutant suggested that degradation and packaging of A3G are independent properties of Vif (Opi et al. 2007). Indeed, although this A3G mutant is resistant to Vif-mediated degradation, less protein is found in virion in the presence of Vif. The molecular mechanism is still not understood but a competition between Vif and A3G toward their specific binding sites, such as the HIV-1 genomic RNA or Pr55^{Gag}, is possible (see also section “[The APOBEC3 Proteins: A Potent Army Against HIV](#)”).

Intravirion Deamination Despite the fact that a few A3G molecules (1–2) are packaged into virions in the presence of Vif, deamination activity per protein is less potent in these viral particles (Britan-Rosich et al. 2011). Several studies have shown that Vif is able to decrease the deaminase-specific activity of A3G and this effect requires Vif binding to the ssDNA that is the substrate of A3G (Feng et al. 2013). The Vif-mediated inhibition of A3G deaminase activity is the consequence of the reduction of A3G processivity. Indeed, A3G performs deaminations on ssDNA using both sliding and jumping mechanisms (see also section “[Deamination-Dependent Mechanisms](#)”). Interestingly, a study reported that Vif from two different HIV-1 isolates (HXB2 and IIB) does not have the same effect on A3G

deaminase activity (Feng et al. 2013). While Vif_{IHXB2} inhibits the jumping of A3G, Vif_{IIB} alters the sliding movements. Moreover, the intravirion deaminase activity of the A3G D128K mutant is unaffected, suggesting that inhibition of this activity requires the interaction between Vif and A3G.

Inhibition of APOBEC3G Degradation: A Therapeutic Perspective

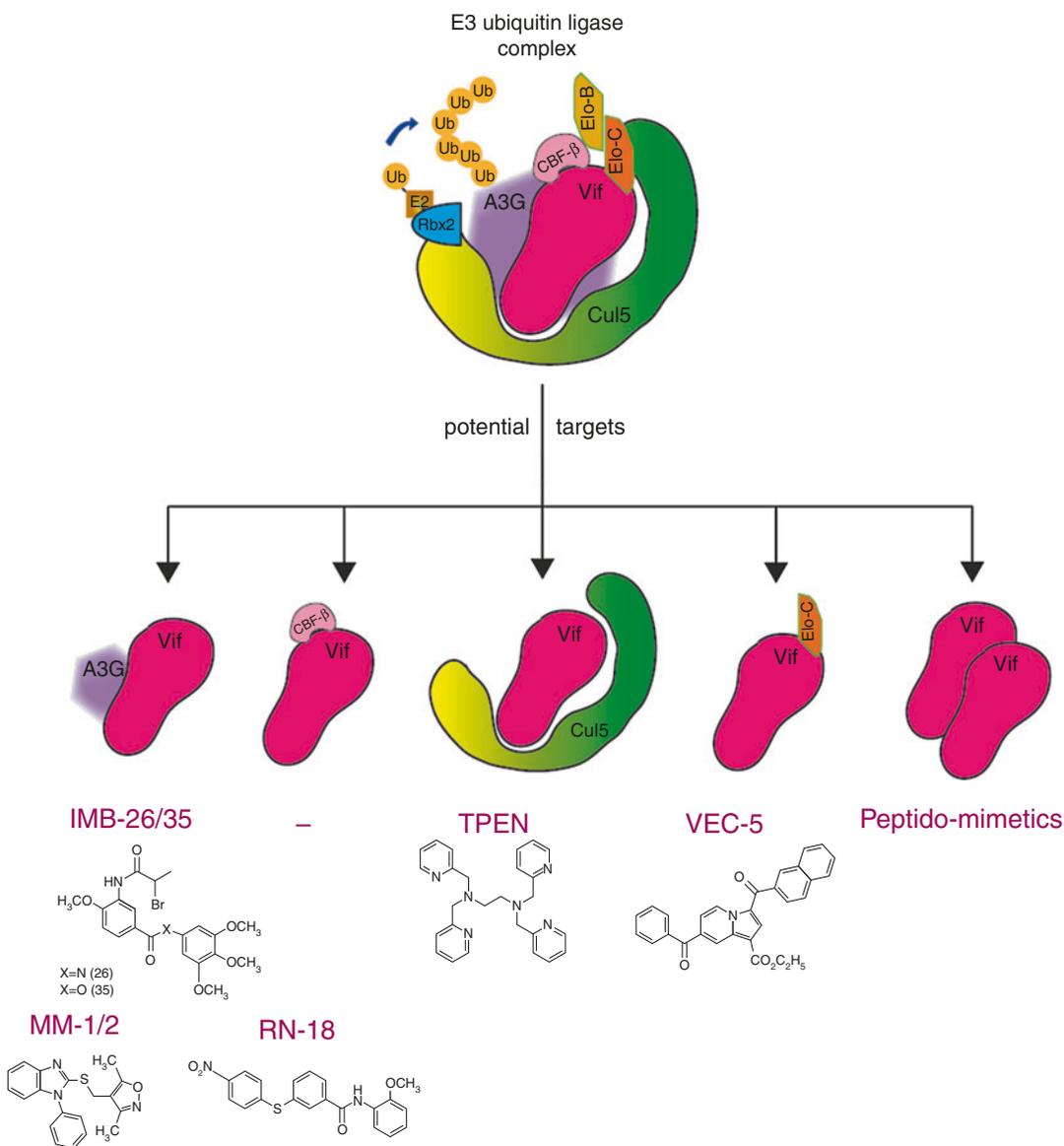
The relationship between Vif and members of the A3 family offers perspectives for developing novel therapeutics. High-throughput screening studies have identified several families of molecules that can restore the A3G intracellular concentration despite the presence of Vif. Some compounds are antagonists of Vif and restore the A3G enzymatic activity (and thus its antiviral property), whereas others block the A3G catalytic activity (Li et al. 2012) and are proposed to decrease the resistance to antivirals due to the reduction of viral quasispecies. There are at least five interfaces that can potentially be targeted to inhibit Vif functions: A3 proteins, CBF- β , Elongin C, Cullin 5, and its dimerization domain (Salter et al. 2014; Fig. 4).

Targeting the interface between Vif and A3 proteins is a real challenge. Indeed, Vif interacts with each of A3 proteins (A3D/F/G/H) through distinct and unique interfaces, and the development of a specific drug targeting each A3 protein is time consuming and thus unlikely. Moreover, another hurdle for the development of such inhibitors is the overlapping of binding sites for different A3 ligands: e.g., the Vif/A3G interface coincides with amino acids required for A3G encapsidation, oligomerization, and processivity (Huthoff et al. 2009). Nevertheless, different classes of compounds have been identified that potentially interfere either directly with the Vif/A3G interaction through binding to the N-terminus of A3G (IMB-26/35) (Cen et al. 2010) or indirectly by stabilizing A3G during the polyubiquitination steps (MM-1/2) (Matsui et al. 2014) or by decreasing Vif expression in the presence of A3F/G (RN-18) (Nathans et al. 2008; Fig. 4). Another strategy to restore the deaminase/antiviral activity would be to block the Vif/E3 ubiquitin ligase

complex and CBF- β interactions. For instance, a zinc chelator named TPEN (N,N,N',N'-tetrakis-(2-pyridylmethyl) ethylenediamine) has been shown to specifically inhibit the Vif-Cullin 5 interaction and to inhibit Vif functions (Xiao et al. 2007). The Vif-Elongin C interaction can also be affected by targeting the Vif SLQ motif with a small molecule (VEC-5) (Zuo et al. 2012). Interestingly, this compound does not only protect A3G but also A3C and A3F from Vif-induced proteasomal degradation, and it enhances A3G incorporation into the progeny virions. Finally, it may be possible to directly target Vif or CBF- β . In the case of Vif, its oligomerization domain provides an attractive target (Fig. 4). Although the molecular mechanism of Vif oligomerization is not clearly defined (Batisse et al. 2013), targeting the ¹⁶¹PPLP¹⁶⁴ motif with peptido-mimetics has been shown to block Vif-Vif interaction in vitro and lead to a decrease of virus replication in nonpermissive cells (Miller et al. 2007). The recent resolution of the Vif/E3 ubiquitin ligase complex structure (Vif/CBF- β /Cul5/EloC/EloB) (Guo et al. 2014) together with in silico screening of small molecules should enhance the number of lead compounds aimed at inhibiting the functions of Vif.

Conclusion

The innate immune system encodes several editing A3 proteins that are involved in the restriction of various retroviruses, viruses, and retrotransposons. Among these deaminases, A3F and A3G are the most potent against HIV-1 replication. These proteins need to be incorporated into new viral particles to induce DNA hypermutation. To prevent the antiretroviral activity of A3F/G, HIV-1 encodes Vif that targets A3F/G and reduces their intracellular concentrations by recruiting an E3 ubiquitin ligase complex to direct them to the proteasome, by inhibiting the A3F/G translation through binding to the 5' UTR of their mRNAs or by competing with A3F/G binding sites to avoid their incorporation into new viral particles. Vif also inhibits intravirion A3F/G



APOBEC3F/G and Vif: Action and Counteractions, Fig. 4 Potential therapeutic strategies and targets to inhibit Vif functions. Targeting one interaction between two partners can be sufficient to prevent the formation of the E3 ubiquitin ligase complex and to inhibit Vif activity. The Vif/A3G interaction can be targeted by IMB-26/35,

MM-1/2, and RN-18. The Vif/CBF-β interaction is a potential target site but no molecules have been identified yet. The Vif/Cul5 and the Vif/E1oC interactions can be inhibited by TPEN and VEC-5, respectively. Finally, the dimerization domain can be counteracted by peptido-mimetics

deamination. Thanks to the recent resolution of the structure of the Vif-E3 ubiquitin ligase complex, new potential therapeutic target sites may be discovered to inhibit Vif functions and restore

antiviral activities of A3 proteins. However, additional studies will be required to make sure the targeted interactions are not involved in essential cellular functions.

Cross-References

► Cellular Cofactors of HIV as Drug Targets

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Arp2/3

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Definition

Arp2/3 is a heptameric complex composed of the Arp complex proteins (ARPC) 1–5 and the actin-related proteins Arp2 and Arp3 (Higgs and Pollard 1999, 2001). The Arp2/3 complex is highly conserved among eukaryotes, is a key regulator of cytoskeletal actin dynamics, and plays significant roles in cell motility, filopodia formation, cell adhesion, endocytosis, T-cell activation, and metastasis. Additionally, the Arp2/3 complex is utilized by several pathogens, including vaccinia virus, *Listeria monocytogenes*, baculovirus, and enteropathogenic *Escherichia coli* (EPEC) for

intracellular motility or, in the case of EPEC, the creation of an actin pedestal (Haglund and Welch 2011). Recently, the Arp2/3 complex and its upstream signaling regulator, WAVE2, have also been implicated in HIV infection of primary CD4 T cells and macrophages (Spear et al. 2014).

Introduction

In cells, spontaneous actin nucleation is prevented by G-actin-binding proteins such as thymosin β 4. Additionally, the kinetic instability of actin oligomers that are fewer than 3 actin subunits long renders spontaneous actin nucleation unfavorable. The Arp2/3 complex serves as an actin nucleator by overcoming the kinetic barriers to nucleation in a highly regulated manner (Higgs and Pollard 1999). Following activation by nucleation-promoting factors (NPFs), the Arp2/3 complex binds to the side of an existing filament through Arp3 and ARPC2, with Arp2 and Arp3 in close juxtaposition (Higgs and Pollard 2001). The Arp2:Arp3 dimer functions as an F-actin nucleus, promoting de novo actin polymerization with the aid of the associated NPFs (Higgs and Pollard 2001). As a result, a new actin filament is created, deviating from the original filament, or “mother” filament, by an angle of approximately 70° (Higgs and Pollard 2001).

The Arp2/3 complex is highly regulated by NPFs, which serve the roles of signal integrators, activators, and facilitators. Known NPFs include N-WASP, WASP, WAVE 1–3, WASH, WHAMM, and JMY (Padrick and Rosen 2010). All NPFs share an Arp2/3-activating motif, called the WCA, at their C-terminus (Padrick and Rosen 2010). The WCA is composed of a WASP homology 2 (WH2) domain, connector domain, and acidic domain (Higgs and Pollard 1999; Padrick and Rosen 2010). The consensus mechanism of NPF activation involves the release of inhibitory contacts engaging the WCA, which is followed by WCA exposure. The exposed WH2 domain binds to G-actin, and the CA domain binds to and activates Arp2/3, priming the Arp2/3 complex for nucleation (Higgs and Pollard 2001; Takenawa and Suetsugu 2007).

Regulation of the Arp2/3 Complex by WASP/N-WASP and WAVE NPFs

WASP/N-WASP: Wiskott-Aldrich syndrome protein (WASP) was identified as mutated in many patients with WAS, characterized by defects in platelet and leukocyte development and function. WASP is expressed primarily in cells of hematopoietic origin, whereas the related neural WASP, or N-WASP, is expressed in most tissues (Takenawa and Suetsugu 2007). Structurally, WASP and N-WASP share an N-terminal EVH1 domain (Ena/VASP homology 1), a basic domain, a GTPase-binding domain (GBD), a proline-rich domain (PRD) that interacts with SH3-containing adaptor proteins, and a C-terminal WCA domain (Padrick and Rosen 2010). The isolated WCA domain of all NPFs is capable of activating Arp2/3 in vitro (Higgs and Pollard 2001). However, in vivo, both WASP and N-WASP are inhibited by intramolecular interactions between the GBD and WCA domains; however, upon activation, the WCA is exposed (Padrick and Rosen 2010). Activation of WASP/N-WASP is often cooperative, with full activation occurring when the GBD, basic domain, and PRD are engaged by their respective ligands (Padrick and Rosen 2010). The GBD engages a Rho-family GTPase, *cdc42*, in its active, GTP-bound form; the basic domain binds to PIP₂ and other negatively charged phospholipids; and the PRD binds to several known ligands including Grb2, Nck, amphiphysin, and many others (Padrick and Rosen 2010; Takenawa and Suetsugu 2007). However, the details of cooperative activation differ between N-WASP and WASP (Padrick and Rosen 2010). Tyrosine phosphorylation by Src-family kinases may potentiate activation (Padrick and Rosen 2010). The oligomerization of WASP/N-WASP through PRD ligands may also potentiate Arp2/3 activation (Padrick and Rosen 2010).

WAVE1–3: WAVE (WASP-family verprolin-homologous protein) 1, 2, and 3 are structurally similar to the WASP-type NPFs, including a basic domain, PRD, and C-terminal WCA. In contrast, the N-terminus of WAVE proteins contains a WAVE homology domain (WHD), which is responsible for recruiting components of the

WAVE regulatory complex (WRC), composed of HSPC300, Abi1, Nap1, and Sra-1 (Padrick and Rosen 2010) (Takenawa and Suetsugu 2007). The WRC, much like native WASP, is inactive and is activated by binding to the GTP-bound form of Rac, PIP₃, the PRB binding to SH3-domain containing proteins (especially IRSp53), and phosphorylation (Padrick and Rosen 2010; Takenawa and Suetsugu 2007). Rac, another Rho-family GTPase, in its GTP-bound form binds indirectly to WAVE via Sra-1 (Takenawa and Suetsugu 2007). IRSp53 may also promote WAVE activation in a manner dependent on WAVE oligomerization and binding of Rac:GTP (Takenawa and Suetsugu 2007). WAVE1 and WAVE 2 play a significant role in generating the protrusive actin dynamics during cell motility.

Roles of Arp2/3 in Cellular Cytoskeletal Dynamics

As a result of signal transduction events, upstream regulators of the various NPFs are activated, resulting in WCA exposure and Arp2/3 activation. In migrating cells, this often results in a protrusive structure called the lamellipodium at the leading edge of the cell (Le Clainche and Carlier 2008). In the lamellipodium, numerous Arp2/3 molecules are activated behind the leading edge membrane, producing a dendritic array of actin filaments, with polymerization occurring towards the direction of migration (Le Clainche and Carlier 2008). Typically, this is mediated downstream of Rac and WAVE activation (Padrick and Rosen 2010). This process of branching and polymerization helps drive the protrusive actin dynamics required for membrane protrusion (Le Clainche and Carlier 2008). Also during cell migration and at the leading edge, thin filamentous protrusions consisting of bundled actin filaments are also produced, and these are called filopodia (Le Clainche and Carlier 2008). These structures may be generated downstream of a *cdc42*-N-WASP pathway (Yang and Svitkina 2011). Although dendritic branching may be structurally inconsistent with the bundles of actin filaments seen in filopodia, it is believed that

Arp2/3-generated filaments converge to produce these structures, recruiting fascin, ENA/VASP, and formins as structural and actin-polymerization driving factors, respectively (Yang and Svitkina 2011). Both structures are often observable during cellular migration, a process that is critical to normal development and immunological function.

Additionally, Arp2/3 plays a critical role during phagocytosis and endocytosis. As above, Arp2/3-mediated nucleation and polymerization drives protrusive actin dynamics that shape the nascent endocytic structures and facilitate membrane scission along with a host of other factors (Takenawa and Suetsugu 2007). During endocytosis, WASP-family NPFs engage membrane-binding and membrane-deforming proteins via its PRD (Takenawa and Suetsugu 2007). Subsequently, Arp2/3 activation results in membrane scission and drives vesicle movement away from the plasma membrane (Takenawa and Suetsugu 2007). A similar, WASP-family NPF-driven mechanism is thought to be involved in phagocytosis, in which Arp2/3 promotes phagocytic cup closure and scission.

Arp2/3 is also indispensable in cell-cell and cell-matrix adhesion, wherein the actin cytoskeleton is attached directly to extracellular contacts (Ren et al. 2009). Cortactin is one of the pivotal regulators at these cellular adhesions, coordinating cellular signals from integrins, cadherins, Src-family kinases, and NPFs (Ren et al. 2009). Cortactin also has an N-terminal acidic (NTA) domain that acts as a weak activator of Arp2/3 (Higgs and Pollard 2001; Ren et al. 2009).

Role of Arp2/3 in HIV Infection

Arp2/3 has been implicated in HIV infection, though the exact mechanism remains unclear. In one study, HIV and SIV infection was inhibited by expression of the N-WASP WCA domain, expressed as GFP-WCA, which dysregulates and dislocates Arp2/3 from physiologically relevant conditions (Komano et al. 2004). This inhibition was not related to fusion, as determined by a cell-to-cell fusion assay in which the target cell

expressed CD4 and a T7 RNA polymerase-dependent reporter vector, and the donor cell expressed HIV Env and T7 RNA polymerase (Komano et al. 2004). Additionally, VSV-G pseudotyping of HIV-1 particles mitigated inhibition, suggesting that the route of entry confers dependency on Arp2/3 (Komano et al. 2004). Furthermore, HIV-1 inhibition could be mediated by the CA or acidic domain alone. Though no mechanism was elucidated in this study specifically, constitutive expression of GFP-VCA significantly delayed the kinetics of HIV-1 replication in H9 cell clones.

Another study corroborated that Arp2/3 is important in HIV infection; however, this study indicated that inhibition occurred during a post-hemifusion step, such as pore formation or expansion (Harmon et al. 2010). Additionally, siRNA knockdown of the upstream regulators of Arp2/3, WAVE2, IRSp53, Rac, Tiam-1, and Abl generates similar fusion defects, indicating a potential pathway of activation necessary for HIV entry and infection (Harmon et al. 2010). In addition, the Abl inhibitors imatinib, nilotinib, and dasatinib also blocked HIV entry as determined by fusion assay (Harmon et al. 2010). Molecules that induced positive membrane curvature, overcoming blocks to hemifusion, diminished the effects of siRNA knockdown of Tiam-1, Abl, Rac, IRSp53, WAVE2, and Arp3 (Harmon et al. 2010).

As the findings of these two studies are somewhat incompatible, perhaps due to differing methodologies and cell systems, the exact mechanism of how Arp2/3 is involved in HIV entry remains uncertain. Recently, the Arp2/3 complex and WAVE2 have been implicated in the nuclear migration of the viral core following entry (Spear et al. 2014). Stable shRNA knockdown of Arp3 inhibited viral infection of CD4 T cells without blocking viral entry and early DNA synthesis (Spear et al. 2014). However, the accumulation of 2-LTR circles, a correlative of viral nuclear migration, was inhibited (Spear et al. 2014). A small molecule inhibitor of Arp2/3, CK-548 (Nolen et al. 2009), was also found to inhibit viral nuclear migration: viral DNA synthesis was not inhibited by CK-548, while the 2-LTR circles were reduced (Spear et al. 2014). CK-548 was also found to

block HIV latent infection of resting CD4 T cells at dosages that did not inhibit T cell activation (Spear et al. 2014). It was demonstrated that during HIV infection of resting CD4 T cells, the virus actively promotes Arp2/3 activity through triggering WAVE2 phosphorylation on serine 351. This HIV-mediated WAVE2 activation was observed in X4 (CXCR4) virus infection of resting CD4 T cells and in R5 (CCR5) virus infection of primary macrophages (Spear et al. 2014). In the X4 virus infection of resting CD4 T cells, WAVE2 phosphorylation could be blocked by AMD3100, a CXCR4 antagonist, suggesting that the signal was transduced from HIV gp120 interaction with CXCR4 (Spear et al. 2014). However, treatment with pertussis toxin, which inhibits Gai signaling, only partially blocked WAVE2 phosphorylation at later time points (Spear et al. 2014), indicating that the WAVE2 phosphorylation induced by HIV gp120 is likely dependent on both Gai and Gaq (Spear et al. 2014). It was postulated that Arp2/3 could be anchored onto the viral pre-integration complex (PIC) through the viral core binding to actin filaments. This Arp2/3-mediated actin polymerization may drive the PIC towards the nucleus (Spear et al. 2014).

The HIV pathogenicity factor and accessory protein Nef has also been linked to Arp2/3. Expression of Nef in peripheral blood mononuclear cells and Jurkat T cells inhibited immunological synapse maturation and cell spreading induced by anti-CD3/CD28 antibody-coated cover slips, a model system that mimics T-cell activation (Haller et al. 2006). This effect was dependent on the Nef SH3-binding domain, as a well-characterized Nef mutant lacking this motif ameliorated defects in T-cell spreading and contact maturation (Haller et al. 2006). This effect was further linked to an overall reduction in active, phosphorylated N-WASP, including at TCR-stimulatory contacts (Haller et al. 2006). Direct inhibition of N-WASP utilizing wiskostatin mimicked these Nef-mediated effects, corroborating evidence that Nef-mediated defects in immunological synapse maturation and cell spreading are mediated by N-WASP dysregulation (Haller et al. 2006). PAK2, a serine/threonine kinase and a Nef interactor, was necessary for these

Nef-mediated effects, as observed in siRNA knockdown experiments (Haller et al. 2006).

Conclusion

The Arp2/3 complex is a critical regulator of actin dynamics that mediates F-actin nucleation and branching. This process is critical for lamellipodium formation, T-cell activation, certain types of endocytosis, and metastasis. Arp2/3 is dynamically regulated by nucleation-promoting factors (NPFs), including WASP/N-WASP, WAVE family proteins, WASH, WHAMM, and JMY. Known NPF activation schemes require signal convergence from phospholipids, GTPases, adaptor proteins, and kinases, which induce the exposure of the Arp2/3-activating WCA domain found at the C-terminus of the NPF. HIV-1 infection has recently been shown to require Arp2/3 and the upstream regulators such as WAVE2, for nuclear migration. HIV-1 Nef also interferes with N-WASP signaling, which may affect CD4 T-cell activation at the immunological synapse.

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Attachment/Binding

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Definition

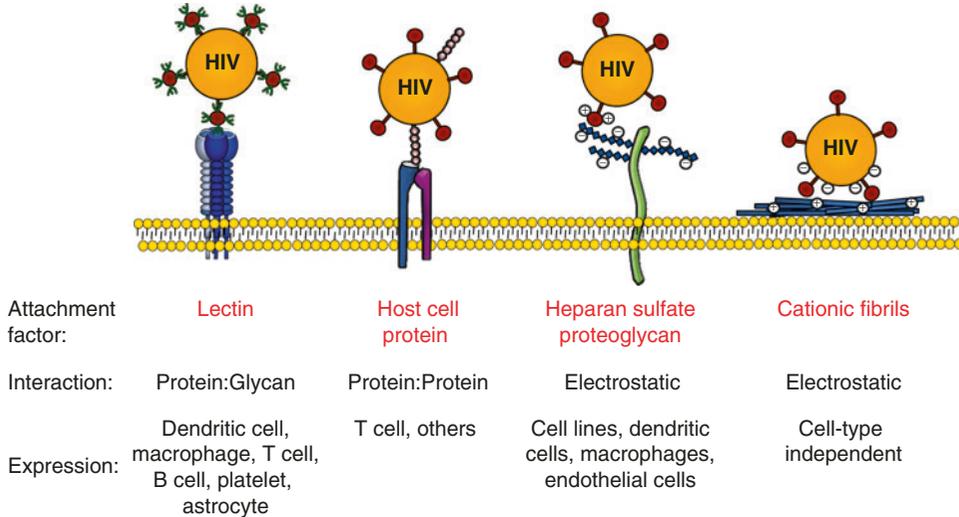
The envelope glycoprotein (Env) of the human immunodeficiency virus (HIV) mediates entry into target cells. Binding of Env to cellular attachment-promoting factors (attachment factors) is frequently unspecific and generally dispensable for entry. However, engagement of attachment factors can profoundly augment HIV infectivity and might impact viral tropism and spread. The lectin DC-SIGN is an archetypic attachment factor, which can facilitate capture of infectious HIV by dendritic cells and might impact HIV transmission and dissemination.

Introduction

The human immunodeficiency virus (HIV) uses its envelope protein (Env) as a key for entry into target cells, mainly CD4⁺ T cells and macrophages. Env is synthesized as a polyprotein in the constitutive secretory pathway of infected cells. An N-terminal signal sequence mediates import of the nascent protein into the endoplasmic reticulum where Env is extensively modified by high-mannose glycans (Scanlan et al. 2007). Upon transport of Env trimers into the Golgi apparatus, the high-mannose glycans are processed into complex glycans. However, processing is incomplete, in part due to the dense packaging of glycans, and about half of the glycans remain in the high-mannose or hybrid form (Scanlan et al. 2007). Env proteins traversing the *trans*-Golgi network are cleaved into the surface unit, gp120, and the transmembrane unit, gp41, by the proprotein convertase furin, and cleavage is essential for infectivity. The two subunits of Env remain non-covalently associated, and trimers of gp120:gp41 heterodimers are incorporated in the plasma membrane, the site of HIV ► budding, and ultimately in the viral envelope.

The physical separation of Env into a surface and a transmembrane unit mirrors the separate functions of these subunits: The surface unit gp120 binds to the receptor and coreceptor, while the transmembrane subunit drives ► fusion of the viral and the cellular membrane, which allows delivery of the viral genetic information into the host cell cytoplasm. Specifically, binding of gp120 to CD4 creates or exposes a coreceptor binding site, and coreceptor binding then activates gp41 for membrane fusion (“► Fusion” and “► CXCR4, Co-Receptors”). The multiple subsequent interactions between Env, CD4, and coreceptor are highly specific and attractive targets for antiviral intervention.

Attachment of HIV to the cell surface can be mediated by factors other than CD4. For instance, binding of Env glycans to cellular lectins or the interaction of charged surfaces on Env with host cell proteoglycans can facilitate attachment (Fig. 1). In addition, the cognate recognition of ligands on the cell surface by cellular receptors



Attachment/Binding, Fig. 1 HIV attachment factors discussed in the present review. DC-SIGN and other cellular lectins can bind glycans on the viral envelope protein (*Env*) and transmit infectious virus to target cells in *cis* (i.e., infection of the lectin-expressing cell) and in *trans* (i.e., infection of an adjacent cell). Several host cell proteins are incorporated into the viral envelope during budding of progeny virions from infected cells. The interaction of host cell-derived proteins with binding partners expressed on the

surface of viral target cells can augment HIV infection in *cis*. Electrostatic interactions between the positively charged V3 loop of *Env* and negatively charged heparan sulfates facilitate binding of HIV to heparan sulfate proteoglycans. Bound viruses are infectious for target cells in *cis* and in *trans*. SEVI and other cationic fibrils bind to negative charges presented on the HIV surface and concentrate virions onto target cells, thereby augmenting infection efficiency

incorporated into the viral envelope can promote attachment. Since attachment of HIV to the cell surface is a rate-limiting step in the entry cascade, engagement of attachment factors can increase infection in *cis* (infection of the attachment factor expressing cell) and might expand viral tropism. Moreover, binding of HIV to attachment factors on cells negative for CD4 and coreceptor can promote infection of adjacent susceptible cells (a process termed *trans*-infection), which might facilitate HIV dissemination (Fig. 2). Here, we will introduce selected HIV attachment factors, and we will discuss how binding to these factors might impact HIV spread and pathogenesis.

HIV Attachment Factors

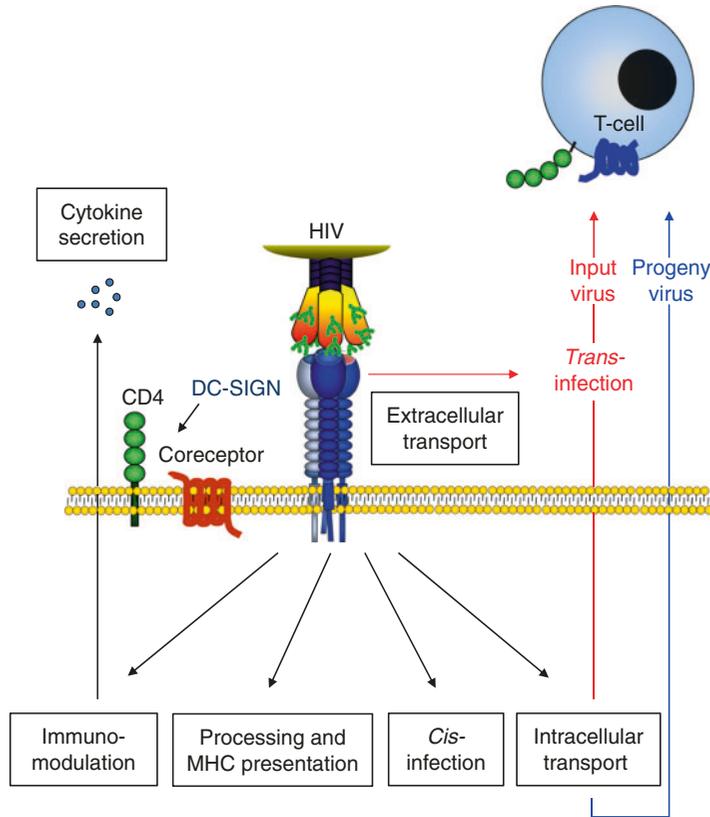
Lectins

DC-SIGN

The lectin *dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin*

(DC-SIGN, CD209) is a type II transmembrane protein consisting of an N-terminal cytoplasmic domain, a transmembrane domain, a stalk domain, and a calcium-dependent (C-type) lectin domain (Geijtenbeek et al. 2000; Fig. 2). The cytoplasmic tail contains LL and EEE motifs which mediate receptor internalization upon ligand binding and appropriate intracellular trafficking, respectively. The stalk and lectin domains are located on the cell surface and are intimately involved in ligand binding: The stalk region, which consists of 7.5 blocks of a 23-amino acid-comprising sequence, mediates DC-SIGN tetramerization which is essential for efficient recognition of multivalent ligands. In addition, the stalk region is responsible for adequate spatial orientation of the lectin domain, which binds to mannose- and fucose-containing glycans displayed on the surface of HIV and other viral and nonviral pathogens (Tsegaye and Pöhlmann 2010).

DC-SIGN is mainly expressed on dendritic cells (“► [Role of Dendritic Cells in HIV-2](#)



Attachment/Binding, Fig. 2 Multiple consequences of HIV binding to DC-SIGN. DC-SIGN on dendritic cells binds to glycans on the viral Env protein and modulates HIV infection via different mechanisms: capture of HIV by DC-SIGN can increase infection of the DC-SIGN-expressing cell (*cis*-infection), resulting in constant release of infectious particles. Alternatively, bound viruses can be

transmitted via the cell surface or upon intracellular trafficking to adjacent T cells (*trans*-infection). A substantial portion of the DC-SIGN-bound virions is endocytosed and processed for MHC presentation. Finally, binding of HIV glycans to DC-SIGN can induce intracellular signaling which modulates cytokine expression upon TLR stimulation (Figure adapted from (Tsegaye and Pöhlmann 2010))

Pathogenesis”) which are among the first cells exposed to sexually transmitted HIV and are believed to play an important role in HIV dissemination (“Modeling Early HIV-1 Infection and Dissemination” and “► HIV-1 Transmission; Cell-Types Associated with”). Specifically, DC-SIGN was detected on dendritic cells located in the submucosa of the vagina, rectum, and foreskin (Geijtenbeek et al. 2000). In addition, DC-SIGN is expressed by certain tissue macrophages, including placental Hofbauer cells, which might play a role in vertical HIV transmission (Tsegaye and Pöhlmann 2010; “► HIV-1 Transmission; Cell Types Associated with”). Finally, subsets of B cells and platelets express

DC-SIGN and were shown to capture infectious HIV via this lectin.

Dendritic cells are largely resistant to HIV infection but catalyze infection of adjacent T cells. This process involves the formation of so-called infectious synapses between virus-loaded dendritic cells and uninfected T cells, which provide ideal microenvironments for HIV transmission. The discoveries that (i) placental DC-SIGN binds to the HIV Env protein, (ii) DC-SIGN expression endows certain cell lines with the capacity to transmit HIV (Geijtenbeek et al. 2000), (iii) DC-SIGN can facilitate HIV uptake into dendritic cells and promotes the formation of infectious synapses (Blanchet

et al. 2011), and (iv) DC-SIGN contributes to the HIV transmission from dendritic cells to T cells (Geijtenbeek et al. 2000) suggest that DC-SIGN on dendritic cells might play a key role intra- and interindividual spread of HIV (Fig. 2). Thus, it was proposed that dendritic cells might take up sexually transmitted HIV at peripheral sites via DC-SIGN, migrate into lymphoid tissues, and transfer the virus to adjacent T cells, thereby facilitating viral dissemination (Trojan horse model; Geijtenbeek et al. 2000).

Important features of the Trojan horse model were challenged by subsequent studies (Tsegaye and Pöhlmann 2010): It was shown that dendritic cells can capture HIV in a lectin-independent fashion and that lectins other than DC-SIGN (e.g., mannose receptor and DCIR) can contribute to HIV interactions with these cells. In addition, capture of HIV by dendritic cells was shown to result either in productive infection, HIV transmission to T cells, or degradation of the virus for antigen presentation. Which pathway prevails seems to be determined, at least in part, by the nature of the glycans present on the HIV Env protein.

An additional layer of complexity in the HIV interactions with DC-SIGN and dendritic cells results from the ability of DC-SIGN to elicit intracellular signals, which promote HIV spread in several ways (Fig. 2). Thus, ligation of DC-SIGN on dendritic cells by an antibody or by HIV can trigger signaling via the nucleotide exchange factor LARG, Rho GTPases, and Raf-1. The DC-SIGN-dependent signaling can have multiple consequences: It forces the cells to maintain an immature state and reduces their ability to induce T cell proliferation upon treatment with LPS, a TLR4 ligand. In addition, signaling is required for the DC-SIGN-driven formation of infectious synapses and can trigger an increase in the production of IL-6, IL10, IL-12p35 and IL12p40 upon exposure of dendritic cells to TLR4 ligands. Finally, stimulation of DC-SIGN by binding to the viral Env protein and simultaneous activation of TLR8 by the HIV mRNA is required for the production of full-length HIV transcripts, due to Raf-1-dependent phosphorylation of NF κ B and recruitment of the

transcription factor pTEF-b (Gringhuis 2010). Thus, glycan-dependent binding of HIV to DC-SIGN modulates dendritic cell function and thereby facilitates viral spread. The HIV glycosylation is cell type dependent, and viruses produced in macrophages and T cells, the major viral target cells, exhibit a markedly different glycosylation pattern. Whether these differences impact the interaction with dendritic cells remains to be investigated.

Langerin

The C-type lectin langerin is expressed on Langerhans dendritic cells and, like DC-SIGN, binds to the HIV envelope protein. However, HIV capture by DC-SIGN and langerin may have differential consequences for infectivity: While HIV binding to DC-SIGN can increase viral infectivity, at least under some conditions, langerin was reported to endocytose bound virions into Birbeck granules, unique structures within Langerhans cells, where HIV is degraded. These observations were made with Langerhans cells isolated from human skin, underlining that langerin-dependent HIV degradation might be operative in vivo. Thus, Langerhans cells in the top layer of the anogenital mucosa were proposed to constitute a powerful defense against sexually transmitted HIV (de Jong and Geijtenbeek 2010). However, relatively high doses of HIV can override the antiviral effect of langerin, which can also be compromised by herpes simplex virus type 2 (“► Risk of HIV-1 Transmission; Coinfections Associated with Risk”) which downregulates langerin expression and competes with HIV for langerin binding. In addition, langerin expression does not inhibit HIV infection of Langerhans cells generated from CD34⁺ dendritic cells, and these cells can facilitate *trans*-infection of T cells upon activation. Within skin explants Langerhans cells are preferentially and productively infected by HIV and promote viral spread by catalyzing infection of adjacent T cells. Finally, HIV capture, migration, and *trans*-infection of T cells were shown for Langerhans cells within the foreskin and vaginal mucosa. While high viral inoculum and differential expression of langerin on Langerhans cells in different tissues might

account for some of these pro-viral effects, the role of langerin in HIV infection warrants further analysis.

DCIR

The finding that HIV transmission by dendritic cells can proceed in a DC-SIGN-independent fashion spurred efforts to identify the responsible factors. One such study showed that the C-type lectin DCIR (*dendritic cell immunoreceptor*), which is expressed on dendritic cells and many other antigen-presenting cells, contributes to dendritic cell-mediated HIV *trans*-infection of primary T cells and also augments *cis*-infection of dendritic cells (Lambert et al. 2008). However, a direct interaction between the Env protein of HIV and DCIR remains to be demonstrated.

The cytoplasmic tail of DCIR contains an immunoreceptor tyrosine-based inhibitory motif, which can repress cell activation, and mutation of this motif reduces the capacity of DCIR to augment *cis*-infection by HIV. In accord, inhibition of protein tyrosine phosphatases (SHP-1, SHP-2), tyrosine kinases (Src and Syk family members), serine and threonine kinases (PKC), and mitogen-activated protein kinases (ERK1/2 and p38) inhibits DCIR-mediated HIV *cis*-infection. Thus, as for DC-SIGN, HIV might signal via DCIR to generate an environment suitable for efficient viral replication. Interestingly, soluble factors released in the context of HIV infection induce DCIR expression on both infected and uninfected bystander T cells, and upregulation is associated with increased HIV *cis*- and *trans*-infection. Therefore, several cell types might augment HIV spread in a DCIR-dependent fashion in HIV-infected individuals.

Mannose Receptor

The mannose receptor (MR) contains a large extracellular domain consisting of a membrane-proximal cysteine-rich domain, a fibronectin type II domain, and eight carbohydrate recognition domains (CRD). The cysteine-rich domain can bind to sulfated glycans, while the CRDs 4–8 facilitate calcium-dependent binding to mannose-, fucose-, or *N*-acetylglucosamine-containing ligands, including HIV gp120.

The HIV capture activity of MR might play a role in vaginal transmission. Thus, MR⁺, DC-SIGN⁺ dendritic cells, and MR⁺ macrophages are located in the submucosa of the endocervix, while CD68⁺, DC-SIGN⁺, and MR⁺ cells are present in the lamina propria of the ectocervix. Expression of MR on vaginal epithelial cells was shown to facilitate gp120 binding to these cells, and a link between MR expression on vaginal epithelium and sexual acquisition of HIV infection was suggested. Binding of gp120 to MR on the vaginal epithelium induces expression of matrix metalloproteinases (MMP), and MR-induced expression of MMPs might increase susceptibility of vaginal cells to HIV infection due to degradation of components of the extracellular matrix. MR and heparan sulfate proteoglycans might also promote capture of infectious HIV by spermatozoa, and these cells can present infectious virus to target cells.

Interactions of HIV with MR might also impact viral spread after the establishment of primary infection. For instance, binding of HIV to MR on astrocytes facilitates infection of these cells, which are CD4 negative, and triggers signals which induce expression of MMPs. Moreover, MR on macrophages and dendritic cells can promote attachment of HIV to these cells and the bound viruses are infectious for adjacent T cells. However, presentation of infectious, MR-bound virus is transient since HIV is taken up into macrophages in an MR-dependent manner and uptake does not lead to establishment of productive infection (Trujillo et al. 2007). Thus, HIV binding to MR could augment and repress HIV spread, as reported for DC-SIGN. Which effect prevails might depend on complex parameters, including cell type, coexpression of CD4 and coreceptor, and HIV glycosylation. Finally, it is noteworthy that MR expression is downregulated in HIV-infected cells in a partially Nef-dependent fashion, which might compromise innate defenses and HIV capture by infected cells.

Galectin-1

Attachment of HIV to target cells can be modulated by both membrane-associated and soluble lectins. The dimeric lectin galectin-1, which is

secreted by activated T cells, macrophages, and several other cell types, binds to beta-galactoside-containing glycans present on gp120 and on the surface of HIV-infected cells (Sato et al. 2012). Binding of galectin-1 to the surface of cells can induce apoptosis and is increased in the context of HIV infection, which alters the glycosylation status of host cell factors (“► [Lymphocyte Apoptosis](#)”). In addition, binding of galectin-1 to gp120 can augment viral infectivity. This effect is due to simultaneous binding of galectin-1 to CD4, which cross-links virus and receptor and thereby boosts infectious entry (Sato et al. 2012). Morphine increases galectin-1 expression and macrophage infection, suggesting that this lectin might augment viral spread particularly in drug users.

Proteoglycans

Proteoglycans are proteins covalently linked to one or more glycosaminoglycans (GAG), unbranched polysaccharide chains built from disaccharide units. Five classes of GAGs are known: hyaluronan, chondroitin, dermatan, heparin/heparan, and keratan. Apart from hyaluronan, GAGs are sulfated and thus contain a negative charge, which is important for ligand binding. Proteoglycans are integral parts of the extracellular matrix but are also found within cells and at the cell surface. The electrostatic interactions between sulfated proteoglycans presented at the plasma membrane and positively charged structures on the virion surface can promote cellular attachment of non-enveloped and enveloped viruses. HIV engages heparan sulfate but not chondroitin sulfate for attachment to T cell lines and HeLa cells, which express high levels of proteoglycans. In contrast, expression of heparan sulfate proteoglycans (HSPG) on primary T cells is low, and HSPGs have little impact on HIV attachment to these cells, which is largely mediated by Env binding to CD4.

The role of HSPGs in HIV attachment is mainly determined by the net charge of the V3-loop. Thus, viruses with a high net charge of V3 due to the presence of basic amino acids will bind robustly to negatively charged sulfates within HSPGs, resulting in efficient attachment and entry via CD4 and coreceptor. In particular, Arg-298 in the V3 loop determines the interaction

with HSPGs by binding to 6-O sulfates, and this amino acid also governs Env engagement of CCR5 via recognition of sulfated tyrosines at the receptor N-terminus. In contrast, viruses with a low net charge in V3 seem to be repulsed by HSPGs resulting in decreased attachment and infectious entry. Indeed, the analysis of a larger panel of Env sequences revealed that the infectivity of most HIV isolates might be reduced by HSPGs (Zhang et al. 2002). The coreceptor binding site, which is only exposed/created upon gp120 binding to CD4, is a second determinant of HSPG interactions, although its physiologic importance is less clear. Finally, it is noteworthy that GAGs can also modulate HIV infectivity in an indirect fashion by interacting with the CCR5 ligand CCL5 (RANTES). Within a certain concentration range, CCL5 blocks HIV infection by binding to CCR5. However, at higher concentrations CCL5 can form oligomers which cross-link enveloped virions to cells by binding to GAGs present on the surfaces of virions and cells. In addition, CCL5 can bind to GAGs attached to CD44, which activates MAPK, and both the cross-linking of virions and the activation of the MAPK pathway augment HIV infection. In sum, HSPGs bind to HIV Env in a charge-dependent fashion and can modulate viral infectivity for cell lines but might have little impact on viral infection of T cells in patients (Fig. 1).

Macrophages can sustain high viral loads upon depletion of T cells and constitute important viral reservoirs. These cells express high levels of proteoglycans, which facilitate HIV attachment to the cell surface. Specifically, expression of betaglycans and syndecans was detected on macrophages and the latter were shown to capture infectious HIV and to augment viral replication in a syndecan transfected cell line expressing low amounts of CD4. Syndecan-1, syndecan-2, syndecan-3, and syndecan-4 capture HIV upon directed expression in cell lines in a heparan sulfate-dependent manner and capture activity extends to SIV (Gallay 2004). More importantly, endogenous expression of syndecan-2 and syndecan-4 endows human endothelial cells with the ability to capture and transmit HIV to T cells. The infectivity of bound virus is conserved over

days, and a comparison between syndecans and DC-SIGN, for which conservation of infectivity was initially reported, showed that the proteoglycans are slightly more adept than the lectin in maintaining HIV in an infectious form. Syndecan-3 is also expressed by dendritic cells, and inhibition analysis revealed that C-type lectins and syndecan-3 contribute to HIV capture by these cells, with blockade of both factors diminishing HIV attachment to background levels (Gallay 2004). Binding of HIV to syndecan-3 on dendritic cells is heparan sulfate-dependent, promotes infection in *cis* and in *trans*, and conserves viral infectivity, indicating that syndecan-3 plays an important role in HIV interactions with dendritic cells. In sum, syndecans might aid HIV transmission and spread by promoting viral attachment to macrophages, dendritic cells, and endothelial cells. Whether HIV binding to syndecans induces signaling events which increase the susceptibility of cells to *cis*-infection or augment their capacity to transmit the virus to adjacent cells remains to be established.

Host Cell Factors Incorporated into the Viral Envelope

The envelope of HIV is derived from the host cell and is acquired during budding of progeny particles from the plasma membrane. Approximately 8–10 Env trimers and a substantial number of membrane-associated host cell factors are incorporated into the envelope of budding particles, including HLA-DR, CD40, CD40L, and CD86. Some of the cellular factors inserted into the viral membrane serve as receptors or ligands for structures presented on the surface of HIV target cells. As a consequence, cellular factors inserted into the viral envelope can bind to their partner molecules on the cellular membrane, and these cognate interactions can promote attachment of HIV to host cells (Fig. 1). One of the best studied examples is the incorporation of cellular ICAM-1 into the HIV envelope and its interaction with LFA-1 on the surface of target cells (Pöhlmann and Tremblay 2007), which is in the focus of the present discussion.

The interaction of ICAM-1 and LFA-1 promotes cell-cell interactions, for instance, during

the formation of immunological synapses. HIV infection upregulates ICAM-1 expression, and ICAM-1 and LFA-1 produced in T cells are incorporated into budding particles. The incorporation of ICAM-1 does not depend on the presence of the viral envelope protein but is promoted by interactions between the cytoplasmic tail of ICAM-1 and the p55 Gag protein. Virion-associated ICAM-1 interacts with LFA-1 on target cells, and this interaction can increase HIV infectivity for peripheral blood mononuclear cells (PBMCs) and *ex vivo*-cultured lymphoid tissue. Cross-linking of the T cell receptor (TCR) transiently increases the affinity of LFA-1 for ICAM-1, resulting in augmented infectivity of ICAM-1-bearing HIV. In addition, TCR cross-linking induces the formation of LFA-1 clusters, which contain CD4 and CXCR4 and are bound by HIV particles. The formation of these clusters and augmentation of infectivity require the activities of ZAP-70, phospholipase C γ 1, and calpain. Calpain abrogates the association of LFA-1 with the actin cytoskeleton and thereby increases its lateral motility and thus its ability to form clusters within the plasma membrane.

The presence of ICAM-1 in the viral envelope and LFA-1 on target cells could impact control of viral spread by the humoral immune response and by therapeutic agents. Thus, virion-associated ICAM-1 increased the neutralization resistance of CCR5- and CXCR4-tropic viruses and reduced sensitivity towards the membrane fusion inhibitor T-20. It has been proposed that the resistance to T-20 might be due to accelerated membrane fusion which shortens the time during which the T-20 target structure is exposed. However, a recent study indicates that ICAM-1 binding to LFA-1 promotes viral attachment but not membrane fusion, and the mechanism underlying relative resistance against membrane fusion inhibitors and neutralizing antibodies merits further analysis.

The association between ICAM-1 on the viral surface and LFA-1 on the target cell surface might also account for important features of the viral cell tropism: HIV-1 is known to preferentially target the memory subset within the CD4⁺ T cell compartment, and virion incorporation of ICAM-1

notably augments HIV-1 infection of these cells (Tardif and Tremblay 2005). In contrast, the presence of ICAM-1 on the virion surface has relatively little effect on infection of naïve T cells. Finally, it is noteworthy that ICAM-1/LFA-1-mediated binding of HIV to receptor-negative cells can promote *trans*-infection of adjacent susceptible cells, although this process does not seem to contribute to HIV transmission by macrophage and dendritic cells.

Cellular attachment of HIV facilitated by ICAM-1/LFA-1 interactions is a potential target for therapeutic inhibition. Thus, the inhibitory effect of cholesterol depletion on HIV infection might in part be due to abrogated ICAM-1/LFA-1 interactions. More specifically, statins and the small molecule integrin inhibitor XVA143, which are known to block binding of LFA-1 to ICAM-1, were shown to inhibit HIV replication in T cells by inhibiting ICAM-1/LFA-1-dependent viral attachment to target cells. Blockade of the ICAM-1/LFA-1 interaction increased inhibition of HIV by T-20, indicating that combinatorial approaches targeting attachment and entry in parallel might be promising.

SEVI and Other Cationic Fibrils

The most frequent mode of HIV transmission is sexual intercourse, and the identification of cellular and viral factors which determine transmission efficiency is of high interest to the development of preventive strategies (Doncel et al. 2011). Screening of a semen-derived peptide library resulted in the identification of a fraction containing fragments of prostatic acidic phosphatase (PAP), which potently enhanced HIV and SIV infection (Münch et al. 2007; “► HIV-1 Transmission; Influence of Bodily Secretions”). The predominant peptide, PAP248-286, assembles into amyloid fibrils termed semen-derived enhancer of viral infection (SEVI), which bind to HIV and the cell and augment viral infectivity by facilitating viral attachment (Fig. 1). Augmentation of infectivity is independent of viral coreceptor tropism and virus-producer/target cell type and has also been observed for other retroviruses. Enveloped viruses outside the retrovirus family have not been examined so far. Mutagenic

analysis and zeta potential measurements revealed that the interaction of SEVI with HIV depends on the positive surface charges of the fibrils. Thus, the infection-enhancing activity of SEVI but not its capacity to form fibrils is abrogated upon substitution of lysines and arginines by alanines.

The formation of SEVI fibrils is promoted by anionic constituents of seminal plasma and the SEVI levels correlate with the ability of semen to augment HIV infectivity in cell culture. Thus, SEVI and semen components which facilitate formation of SEVI fibrils are potential targets for microbicides (“► HIV-1 Transmission Blocking Microbicides”). Initial studies showed that epigallocatechin-3-gallate, present within green tea, and theaflavin derivatives promote SEVI degradation and polyanionic compounds, including self-assembling peptides, interfere with SEVI's infection-enhancing capacity. However, antiviral approaches targeting SEVI need to take into account that semenogelin fragments present with semen can also assemble into fibrils and augment HIV infectivity, suggesting that HIV might hijack several fibril-forming peptides to ascertain infection of cells within the anogenital mucosa.

Conclusion

Several secreted and membrane-associated cellular proteins can bind HIV and promote viral attachment to target cells. The interaction of HIV with these attachment factors is dispensable for infectious entry but can profoundly augment viral infectivity. Engagement of attachment factors frequently depends on the charge and glycan composition of the viral Env protein. Alternatively, the interaction of host cell factors inserted into the viral envelope with their binding partners on the surface of target cells can promote HIV attachment. Attachment factors can augment viral infectivity by concentrating viral particles on the cell surface, thereby increasing the chance that Env proteins interact with CD4 and coreceptor. In addition, binding of Env to attachment factors can elicit intracellular signals which render cells more susceptible to infection or to transmission of the virus to adjacent cells. However, attachment

factor binding can also promote HIV degradation and parameters like target cell type and HIV glycosylation control whether infection is augmented or repressed. Inhibition of attachment factors in cell culture can markedly decrease viral infectivity, and expression of several attachment factors on important viral target cells suggests a role in viral spread in patients. The potential of attachment factor inhibitors to prevent viral transmission and dissemination therefore merits further analysis.

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Autophagy and HIV Infection

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Definition

Autophagy is a fundamental and highly regulated lysosomal degradation mechanism, dependent on specialized autophagy-related proteins (Atgs) (Mizushima et al. 2011). It can be classified into macroautophagy, microautophagy, and chaperone-mediated autophagy, but only

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macroautophagy will be described and hereafter referred to as autophagy since it is the major lysosomal route for the turnover of cytoplasmic constituents. It is characterized by the formation of membranes that engulf cytoplasmic material through the formation of autophagic vacuoles, called autophagosomes. These structures fuse with lysosomes to form autolysosomes where the sequestered material is digested by lysosomal hydrolases. Before this degradative step, autophagosomes can also fuse with endosomes to form amphisomes, making a direct connection between the endo-lysosomal and autophagic pathways. This process is highly dynamic, and constituents are continuously recycled through lysosomal transporters toward the cytosol. Autophagy plays essential physiological roles in survival, homeostasis, and development and is closely linked to several mechanisms of cell death such as apoptosis, pyroptosis, and necroptosis. It is also involved in the defense against invading intracellular pathogens and acts in both innate and adaptive immunities.

Introduction

Autophagy has an essential role in cell homeostasis and has been implicated in several pathologies including cancer, neurodegeneration, and

myopathies. It is a constitutive mechanism further induced by different stresses or stimuli that are dangerous for the cell. For example, autophagy is essential to provide energy and amino acids during starvation. It also prevents cell death or senescence due to accumulation of defective organelles, in particular damaged mitochondria, and large macromolecular aggregates.

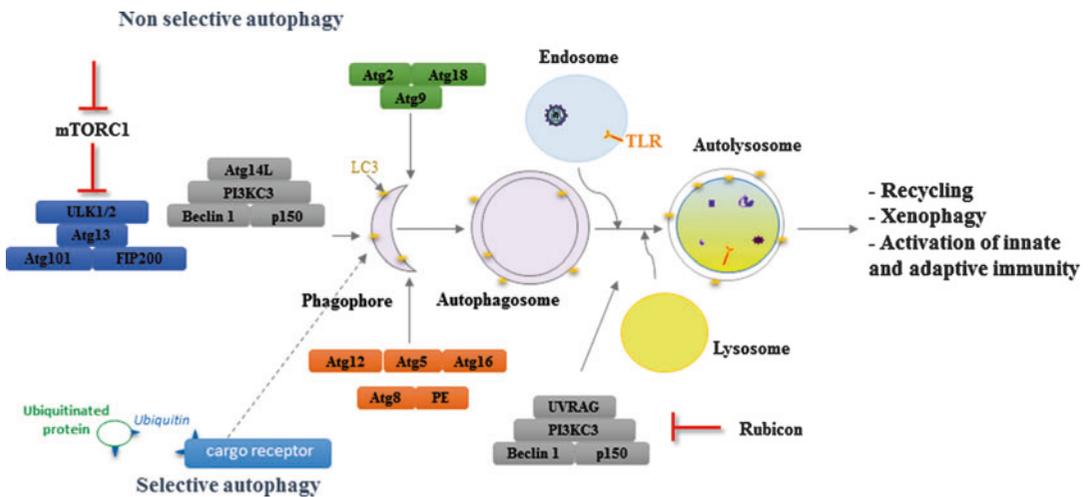
The global autophagy process is presented in Fig. 1.

It requires several specific protein complexes regulating different steps of the autophagy flux (for review (Ravikumar et al. 2010)).

The ULK1/ULK2 complex is involved in the initiation step of autophagy. The function of this multi-protein complex, composed of ULK1/ULK2, Atg13, Atg101, and FIP200, is tightly controlled by the mammalian target of rapamycin (mTOR) complex 1 (MTORC1), which represses autophagy flux initiation under nutrient-rich conditions.

The second complex is composed of class III phosphatidylinositol 3-kinase (PI3KC3), Beclin 1, p150, and Atg14L, which produces an autophagy-specific pool of phosphatidylinositol 3-phosphate (PI3P). Several additional proteins interacting with Beclin 1 ensure a timely and spatial regulation of PI3P formation during the autophagy process.

Then, two unique ubiquitin (Ub)-like conjugation systems drive the elongation and closure of



Autophagy and HIV Infection, Fig. 1 Autophagy pathway

the autophagic membrane. In the first conjugation system, Atg12 is conjugated to Atg5, in a reaction mediated by the Ub-activating enzyme (E1)-like Atg7 and the Ub-conjugating enzyme (E2)-like Atg10. No specific and exclusive Ub ligase (E3)-like has been discovered so far. Atg16L1 interacts with the Atg12–Atg5 conjugate to form a scaffold necessary for LC3 lipidation. The second conjugation system leads to the conjugation of proteins of the Atg8 family to the lipid phosphatidylethanolamine (PE) through a mechanism controlled by Atg7 and Atg3, another E2-like enzyme. The Atg12–Atg5 conjugate is an E3-like for the Atg8–PE conjugation reaction. In humans, the Atg8 family comprises three microtubule-associated protein 1 light chain 3 (LC3A, LC3B, and LC3C), one gamma-aminobutyrate receptor-associated protein (GABARAP), and three GABARAP-like proteins (GABARAPL1–3). However, only LC3B has been extensively studied and will be hereafter referred to as LC3. LC3 is synthesized as pro-LC3 and is very rapidly processed by the protease Atg4 to expose its C-terminal glycine. During autophagosome formation, a fraction of this cytosolic, soluble form of LC3, also called LC3-I, can be conjugated to PE to generate the lipid-conjugated form of LC3, or LC3-II, which becomes tightly associated with the autophagosomal membrane while the Atg5–Atg12 conjugate is removed from the neo-formed vesicles. Interestingly, LC3-II can also return to an unlipidated state via the proteolytic activity of Atg4, indicating, therefore, that this process could be reversible, although its functionality is not yet understood.

In turn, Beclin 1 recruits several proteins such as UVRAG and PI3KC3 to form a complex that plays a major role in the maturation step of autophagy. This complex is negatively controlled by the RUN domain-containing protein Rubicon. It is worth noting that Beclin 1 interacts with the anti-apoptotic protein Bcl2, as well as Bcl-XL, Bcl-w, and Mcl-1. Beclin 1 binding to Bcl2 represses autophagy when Bcl2 is localized in the endoplasmic reticulum. Upon stress, Beclin 1 dissociates from Bcl-2, allowing the activation of PI3KC3 and the subsequent stimulation of autophagy. This is one of the multiple links that exist between

autophagy and apoptosis. Although not yet fully investigated, other cross talks also exist between autophagy and other programmed cell death pathways (necroptosis, pyroptosis) and the Ub–proteasome system, the other main degradative pathway.

Autophagy was first described as a non-selective bulk degradation pathway. However, specific forms of autophagy can also selectively degrade cytoplasmic components such as organelles (e.g., mitochondria, peroxisomes, lipid droplets, ribosomes) and proteins that can also be under aggregate forms.

This function is supported by cargo receptors (e.g., p62/SQSTM1, NBR1, and NDP52), which bind to both “eat-me” signals, mainly molecules of Ub, and Atg8 family members present on the autophagosomal membrane. Binding to Atg8 family engages a specific motif, called an LC3-interacting region (LIR), with the general sequence W/F/YxxI/L/V preceded by acidic residues.

There is now a growing line of evidence that autophagy is also an essential pathway for host defense against viral infection. Autophagy acts at different stages of antiviral immunity, including the degradation of entire viruses or specific viral proteins by a process termed xenophagy, the activation of innate immune signaling by delivery of viral nucleic acids to endosomal Toll-like receptors (TLRs), and the activation of adaptive immunity by presentation of viral antigens to major histocompatibility complex (MHC) class I and MHC class II molecules. As a consequence, the viruses have evolved strategies to counteract or to exploit autophagy for their own profit.

HIV-1, as many other viruses, manipulates autophagy for its own replication. Its regulation depends on the cell type (CD4⁺ T cells, macrophages, or dendritic cells) and the status of the cells (i.e., infected or uninfected cells). This process is also at the center of the innate and adaptive immune responses against HIV-1.

Autophagy is mainly an anti-HIV mechanism, although several Atgs, including Atg7, Atg12, Atg16L, and GABARAPL2, are seemingly required for HIV-1 infection, as demonstrated through a functional genomic screen using

HeLa-derived TZM-bl cells (Brass et al. 2008). Several Atgs, in particular Atg5 and Atg16, are also needed for HIV-1 replication in CD4⁺ T cells (Eekels et al. 2012), suggesting that either HIV-1 needs the initiation step of autophagy or at least some of its components. It is worth noting that autophagy-related proteins can also function independently of the autophagic process, and this aspect has to be taken into consideration when trying to decipher the link between autophagy, autophagic proteins, and HIV-1 infection.

Strikingly, autophagy is also responsible for the apoptosis of bystander CD4⁺ T cells triggered by HIV-1 envelope.

Autophagy in CD4⁺ T Cells During HIV-1 Infection

CD4⁺ T lymphocytes represent the main target cell population for HIV-1 infection, and their progressive destruction is the hallmark of AIDS (Barré-Sinoussi et al. 1983). Depletion of this cell population is mainly due to apoptosis of bystander uninfected CD4⁺ T cells (“► [Lymphocyte Apoptosis](#)” and Finkel et al. (1995)) for which the viral envelope, composed of gp120 and gp41, plays an important role since it constitutes the primary interface between viruses and target cells. In particular, the gp41-mediated fusion process occurring during HIV-1 entry triggers activation of the intrinsic pathway of apoptosis, with activation of the caspases 9 and 3 (Roggero et al. 2001; Blanco et al. 2003; Garg and Blumenthal 2008). Recently, abortive HIV-1 infection, occurring in the vast majority of bystander quiescent CD4⁺ T cells, was shown to be responsible for an inflammatory form of cell death, called pyroptosis (“► [Pyroptosis and HIV Replication](#)”), characterized by the activation of caspase 1 and the release of inflammatory cytokines upon viral DNA sensing by the interferon- γ -inducible protein 16 (IFI16) (Doitsh et al. 2014; Monroe et al. 2014).

Importantly, autophagy is also activated in bystander CD4⁺ T cells after their contact with infected cells, through the gp41-dependent fusion of both membranes, and leads to their apoptosis (Espert et al. 2006; Denizot et al. 2008).

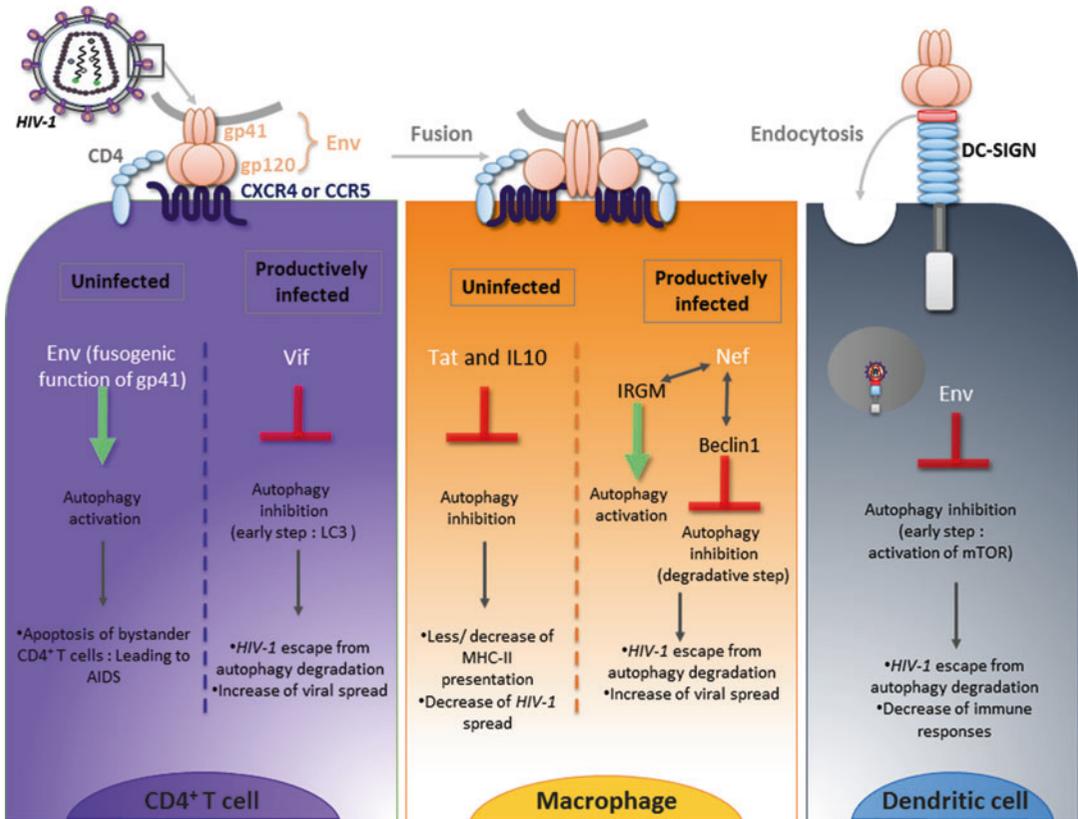
Autophagy contributes, therefore, to the depletion of CD4⁺ T cells during HIV-1 infection.

Autophagy is an anti-HIV-1 process by specifically degrading Tat, a viral protein essential for viral replication (Sagnier et al. 2015). As a consequence, autophagy is repressed in productively infected cells. Very recent data indicate that autophagy in CD4⁺ T cells could be indeed targeted by the viral protein Vif upon specific binding to the autophagy-related protein LC3 (Borel et al. 2015) (Fig. 2, left part).

Autophagy in Macrophages During HIV-1 Infection

In contrast to CD4⁺ T cells, bystander macrophages do not undergo autophagy after contact with HIV-1 envelope (Espert et al. 2009). This blockade is dependent on the activation of Src–Akt and Stat 3 by HIV-1 Tat and IL-10, both previously reported to inhibit autophagy (Van Grol et al. 2010). HIV-1 Tat was also shown to suppress IFN- γ -induced autophagy in primary macrophages (Li et al. 2011), suggesting that inhibition of autophagy could negatively impact on innate immune defenses of myeloid cells devoted in the killing of intracellular pathogens. Overall, this result correlated well with the physiopathology reported from HIV-1-infected patients. Indeed, macrophages do not undergo Env-mediated apoptosis and are seemingly not subjected to depletion during HIV-1 infection, at least compared to the level of CD4⁺ T cell depletion. These observations raise many relevant but still unsolved questions, including why the fusogenic function of gp41 induces autophagy only in CD4⁺ T cells and why Tat and IL-10 would not inhibit autophagy in bystander CD4⁺ T cells.

Conversely, in cells from the monocyte/macrophage lineage, autophagy is induced following a productive infection (Espert et al. 2009) and acts as an antiviral mechanism (Espert et al. 2009; Kyei et al. 2009). Surprisingly, two populations of autophagic cells are present, one highly autophagic and the other weakly autophagic, and viruses could be detected in the weakly



Autophagy and HIV Infection, Fig. 2 Relationship between autophagy and HIV-1 infection of its main target cells, namely, CD4⁺ T cells, macrophages, and dendritic cells

autophagic cells but not in the highly autophagic cells, suggesting that autophagy might still be controlled by HIV-1 in these cells to avoid degradation (Espert et al. 2009). Indeed, Nef regulates autophagy in these cells by two ways. First, Nef binds to the immunity-associated GTPase family M (IRGM), inducing autophagy (Gregoire et al. 2011), and second, Nef binds to Beclin 1, blocking the degradative step of autophagy (Kyei et al. 2009).

The dual interaction of HIV-1 with autophagy thus enhances viral yields by using the early stages while inhibiting the late stages of autophagy (Fig. 2, central part).

Apart from Nef, the antisense protein ASP, produced from antisense transcription of the HIV-1 genome, partially co-localizes with LC3. Preliminary data suggest that expression of ASP induces autophagy and increases viral replication

in the promonocytic U937 cell line (Torresilla et al. 2013).

Autophagy in Dendritic Cells During HIV-1 Infection

Although some dendritic cell (DC) subsets could differ in phenotype and functions, one of their hallmarks is their propensity to regulate innate and adaptive immunity.

DC (DCs) are known to regulate innate and adaptive immunity, and most of the subsets express a plethora of pathogen recognition receptors (PRR) (“Pathogen Recognition Receptors (General)”) facilitating their antigen sampling activity and contributing to their quickness in efficiently igniting adapted immune responses. Some DC subsets were also reported to be

involved in the early events of HIV transmission. Even if they are mostly refractory to productive HIV infection, these cells can readily internalize virions via different receptors expressed at the surface, like C-type (CD209/DC-SIGN) or I-type (CD169/SIGLEC-1) lectins, and transmit them toward CD4⁺ T cells.

Upon viral challenge, autophagy is rapidly inhibited in DCs through the activation of mTOR by HIV-1 envelope. This autophagy blockade impairs Toll-like receptor-mediated innate immune response while also strongly affecting antigen processing and MHC-II-mediated antigen presentation to CD4⁺ T cells (Blanchet et al. 2010) (Fig. 2, right part) potentially limiting anti-HIV immune responses.

Conclusion

Taken together, these results suggest a complex, cell-type-specific relationship between HIV-1 and the autophagic response and highlight the complexity of HIV-1 pathogenesis. Autophagy appears to play different roles in controlling HIV-1 replication, depending on the cell type. In turn, HIV-1 has evolved individualized strategies to manipulate autophagy in each cell type. Whatever the cells involved, autophagy is unambiguously involved in HIV-1 pathogenesis, and more investigation on its antiviral role should strengthen our understanding of the interplay between the virus and the host.

Further studies are needed to (i) decipher the mechanisms that are activated after gp41-dependent membrane fusion during the entry of the virus leading to autophagy, (ii) understand the link between autophagy and apoptosis or other cell death mechanisms in the bystander CD4⁺ T cells, (iii) determine the viral proteins responsible for the control of autophagy in the infected cells, and (iv) understand the connections between autophagy and viral trafficking through endocytic compartments linked to immune responses in DC.

Modulation of the autophagy flux might thus be a promising approach to improve immunomodulatory approaches to treat HIV-1 infection aimed

to increase immune responses in complementation to HAART.

Cross-References

- ▶ [Lymphocyte Apoptosis](#)
- ▶ [Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense](#)

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Bacterial Respiratory and Invasive Pneumococcal Infections and HIV

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usual pathogens, and in AIDS patients with neutropenia, infection with *Pseudomonas aeruginosa* can occur. In severely immunosuppressed patients, additional opportunistic infections (OI) with *Rhodococcus equi* and *Nocardia* spp. (both CDC category B OI diagnoses) occur (Feldman and Anderson 2013).

This entry will discuss factors predisposing HIV-infected patients to bacterial lower respiratory infections, the epidemiology of such infections, the major bacterial pathogens involved, and their pathogenesis, diagnosis, management, and prevention. *Streptococcus pneumoniae*, the most important pathogen in this group, for which a specific prevention strategy in the form of vaccination is available, will be discussed in depth.

Definition of the Problem

HIV-infected patients have a high risk of bacterial lower respiratory tract infections, even in the era of highly active antiretroviral therapy (HAART). Recurrent pneumonia (≥ 2 episodes/year) is both an indication for HIV testing and an acquired immunodeficiency (AIDS)-defining event. The clinical presentation of community-acquired pneumonia (CAP) is usually similar to that in HIV-uninfected patients, although the prognosis can be worse in the severely immunosuppressed. *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* are the

Introduction

Since the beginning of the HIV epidemic, the vulnerability of the respiratory system of HIV-infected patients to the development of severe infections caused by opportunistic pathogens has been clearly evident. Lower airway infections, specifically those caused by *Pneumocystis jirovecii*, *Mycobacterium tuberculosis*, and the pneumococcus, are the leading causes of death resulting from the severe immunosuppression, which accompanies advanced HIV infection. In the western world, CAP is now the most common pulmonary infection, while

tuberculosis is the most common infection in Africa. The CD4 cell count is inversely correlated with pneumonia risk and recurrent pneumonia (two or more episodes within a 1-year period) is an AIDS-defining CDC stage C condition. However, even in the HAART era and in patients with relatively normal CD4 cell counts, the CAP incidence remains elevated. Not only does the mortality remain high in HIV-infected persons with CAP, but the occurrence of CAP is also associated with a permanent decline in lung function and an increase in long-term mortality in these patients (Feldman and Anderson 2013).

Bacterial Pathogens Causing CAP

Excluding tuberculosis, the most common bacterial pathogens are *Streptococcus pneumoniae*, which will be discussed in detail separately, and *Haemophilus influenzae*, which accounts for 10–15% of bacterial pneumonias with known pathogens in HIV-infected patients. *H. influenzae* is more frequent in advanced immunosuppression and tends to present subacutely, with diffuse pulmonary infiltrates. *Staphylococcus aureus* ranks third in frequency (around 5% of CAP in HIV-infected persons). Pathogenesis includes either septic pulmonary embolization from central venous catheters or injection drug use-related right-sided endocarditis or superinfection after viral respiratory infection such as influenza. Colonization and/or infection with highly virulent Panton-Valentine leukocidin-producing community-acquired methicillin-resistant *S. aureus* (PVL-CA-MRSA) is a third possibility. The most frequent gram-negative pathogens are *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Moraxella catarrhalis* (the latter mostly in patients with chronic obstructive pulmonary disease). The atypical pathogens, *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae*, appear to be much less common in HIV-infected patients, as do infections with *Legionella* spp., although these occur slightly more frequently than in the general population (Feldman and Anderson 2013).

Epidemiology of CAP and Pneumococcal Disease in the HIV-Infected Persons

As indicated above, *S. pneumoniae* is the most common bacterial cause of CAP in HIV-infected persons, being implicated in 20% of cases overall, in 40% of cases in which a microbiological diagnosis is confirmed, and in 70% of cases that are associated with the occurrence of bacteremia (Feldman and Anderson 2013). Several studies have documented a substantially increased risk of bacteremia in HIV-infected patients with CAP, with rates of invasive pneumococcal disease (IPD) of up to 100-fold higher than among HIV-uninfected patients (Raju et al. 2012; Feldman and Anderson 2013, 2014a). In addition recurrent infections have been commonly noted. There has been some conflicting data as to whether the rates of CAP, and in particular invasive pneumococcal CAP, in HIV-infected persons have decreased considerably with the use of HAART and/or pneumococcal vaccination; nevertheless even if the rates of invasive pneumococcal disease (IPD) have decreased with the use of these measures, they still remain very high, being approximately 35-fold higher than in HIV-uninfected persons (Siemieniuk et al. 2011; Feldman and Anderson 2013). While rates of IPD declined within 3 years following vaccine with the 23-valent polysaccharide vaccine, 74% of IPD that occurred was due to serotypes contained in that vaccine (Siemieniuk et al. 2011). Part of this ongoing and increased risk appears to relate to the negative impact of cigarette smoking and injection drug use, with several studies showing the positive benefit of smoking cessation on rates of CAP (Siemieniuk et al. 2011; Feldman and Anderson 2013).

In the UK, the overall rates of IPD in HIV-infected persons have been documented to be 245/100 000 persons, which was 50 times higher than that of the general population; rates were 281/100 000 persons in patients not on HAART and 563/100 000 persons in cases with advanced immunosuppression (Raju et al. 2012). Since HIV infection is one of the most important risk factors for IPD, it is recommended that

patients presenting with pneumococcal bacteremia should be tested for HIV infection, if they have not been previously tested, irrespective of the presence or absence of other IPD risk factors (Feldman and Anderson 2014a). In the study from the UK described above, 2.4% of adults aged 15–44 years with IPD had undiagnosed HIV infection (Raju et al. 2012).

It is well recognized that in addition to smoking and alcohol use, various underlying medical conditions are associated with an increased risk of IPD (Kyaw et al. 2005). Investigations have been undertaken documenting the incidence rates of IPD in adult patients with specific medical conditions, comparing the rates of IPD in those adults with and without those medical conditions in the USA (Kyaw et al. 2005). In one study the overall incidence rates in healthy adults were found to be 8.8/100 000 persons, with rates, for example, in diabetes mellitus of 51.4/100 000 persons and in chronic lung disease of 62.9/100 000 persons. However, incidence rates in those with HIV/AIDS were 422.9/100 000. Overall, in patients with diabetes mellitus, chronic heart, and lung disease, there was a three- to sevenfold increased risk of IPD compared with healthy controls, whereas adults with immunocompromising conditions, including HIV/AIDS, had a 23–48-fold higher risk of IPD than healthy adults (Kyaw et al. 2005). Risk factors for IPD in HIV-infected persons in the HAART era are said to be similar to that of HIV-uninfected persons and include current smoking, alcoholism, underlying comorbid conditions, and prior hospitalization, as well as low CD4 cell counts in the former patients (Feldman and Anderson 2015).

HIV Infection and Increased Vulnerability to Pulmonary Infection

Suppression of Pulmonary Host Defenses

The major protective airway host defenses which are progressively and significantly compromised by HIV-mediated depletion of the CD4⁺ T lymphocyte population are as follows:

- Infection of a subtype of CD4⁺ T helper cell known as follicular cells (Tfh) (Ueno et al. 2015). These cells which abound in secondary lymphoid tissues, such as the highly organized network of mediastinal lymph nodes in the lungs, provide essential help to antibody-producing B lymphocytes. However, infection and functional dysregulation of Tfh cells by HIV result in defective production of mucosal IgA and IgG antibodies, severely compromising mucosal antibacterial host defenses.
- Infection and depletion of the CD4⁺ Th1 and Th17 T cell subsets resulting in decreased production of the monocyte/macrophage/neutrophil recruiting and activating cytokines IFN- γ and IL-17A, also contributing significantly to predisposition for the development of severe mucosal bacterial infection.

In addition, neutropenia is also a common manifestation of advanced HIV infection and a probable contributor to susceptibility for IPD, as well as for pneumonia due to *Pseudomonas aeruginosa*.

Pathogenesis of Pneumococcal Disease: From Colonization To Invasive Disease

Pneumococcal infections are broadly grouped into either invasive or noninvasive (mucosal) infections (Ludwig et al. 2012; Drijkoningen and Rohde 2014). The term invasive infections refers to infections in which the microorganism is found in normally sterile body sites, such as the blood, the cerebrospinal fluid (CSF), pleural and joint fluid, etc. Noninvasive infections, which can become invasive, include otitis media, sinusitis, and some patients with pneumonia.

As with the development of severe pneumococcal disease in the general population, nasopharyngeal colonization of the upper respiratory tract in HIV-infected subjects is a prerequisite for subsequent translocation of the pathogen to the lower respiratory tract and development of invasive disease (Steel et al. 2013; Huson et al. 2015).

Colonization necessitates evasion of the expulsive actions of the mucociliary escalator and is achieved via the complementary interactions of several pneumococcal virulence factors. These are (i) the highly electrostatically charged, anti-phagocytic, polysaccharide capsule, the primary virulence factor of the pneumococcus, which also prevents attachment of the pathogen to respiratory mucus and (ii) the pneumococcal cytotoxins, hydrogen peroxide, and pneumolysin, which damage the ciliated respiratory epithelium, interfering with ciliary beat frequency (Steel et al. 2013; Huson et al. 2015). Pneumolysin is the major protein virulence factor of the pneumococcus.

Evasion of the mucociliary escalator, in turn, facilitates interaction of the pathogen with the respiratory epithelium, a process which is dependent on a transient decrease in the thickness of the capsule, enabling exposure of an array of underlying cell wall-associated protein adhesins. Like pneumolysin, many of these are immunogenic and have been identified as being priority candidates for development of novel protein-based vaccines (Feldman and Anderson 2014b). These include pneumococcal surface adhesin A (PspA) and pneumococcal surface protein C (also known as CbpA and SpsA) which mediate binding of the pathogen to epithelial E-cadherin and the polymeric immunoglobulin (Ig) receptor, respectively. In addition, cell surface phosphorylcholine promotes attachment of the pneumococcus to the platelet-activating factor (PAF) receptor, which is also expressed on epithelial cells (Steel et al. 2013; Huson et al. 2015; Feldman and Anderson 2014b).

At this point, containment of the pathogen is dependent on the efficacy of innate airway host defenses. Notwithstanding the mucociliary escalator and various antimicrobial peptides/proteins present in epithelial lining fluid, the major cellular effectors include resident macrophages, dendritic cells, mast cells, and groups 1 and 3 innate lymphoid cells, as well as epithelial cells. Pattern recognition receptors (PRRs) expressed on/in these cells interact with various pathogen-associated molecular patterns (PAMPs), triggering a protective inflammatory response. Foremost

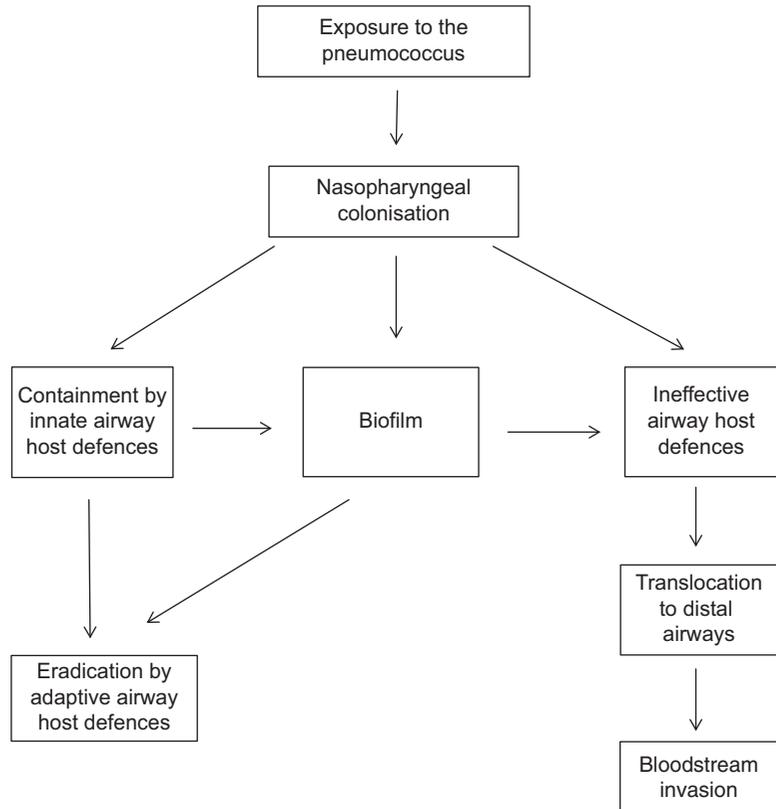
among the various families of PRRs are (i) the Toll-like receptors (TLRs); (ii) nucleotide-binding oligomerization domain (NOD) receptors, including the subfamily of inflammasomes; and (iii) various cytosolic microbial nucleic acid sensors. Activation of these various PRRs by pneumococcal constituents such as cell wall teichoic acid/peptidoglycan, pneumolysin, and DNA results in the generation of a series of pro-inflammatory cytokines/chemokines which act in concert to promote mobilization and recruitment of monocytes/macrophages and neutrophils. These include the cytokines interleukin (IL)-1 β , IL-6, IL-12, IL-17A, IL-18, interferon-gamma (IFN- γ), and tumor necrosis factor (TNF), as well as the monocyte/neutrophil chemokine, IL-8 (Huson et al. 2015). In addition, these innate immune mechanisms are also essential in shaping the more slowly occurring adaptive immune response to pneumococcal antigens.

Onset of the adaptive immune response is characterized by the production of specific IgA and IgG antibodies which target cell wall adhesins and the polysaccharide capsule, preventing adhesion to respiratory epithelium and promoting phagocytosis of the pathogen. In addition, pneumococcal protein antigens also activate cell-mediated responses involving CD4⁺ helper T lymphocytes, specifically those of the Th1 and Th17 subtypes, which as mentioned above, produce the cytokines IFN- γ and IL-17A, respectively. These cytokines enhance the antimicrobial activities of monocytes/macrophages and neutrophils (IFN- γ), while promoting the recruitment of these cells to sites of pneumococcal colonization (Huson et al. 2015). Eradication of the pneumococcus is dependent on these antibody- and cell-mediated adaptive immune mechanisms operating effectively and in unison.

Two scenarios now exist: these are either eradication of the pneumococcus or its coexistence as a seemingly passive, but nevertheless menacing, colonist. In the latter scenario, the pneumococcus, present in and/or on respiratory epithelial cells, may protect itself against airway host defenses by concealment in an extracellular matrix of polymeric materials, known as biofilm. The biofilm matrix consists predominantly of

Bacterial Respiratory and Invasive Pneumococcal Infections and HIV, Fig. 1

The events involved in the transition of the pneumococcus from nasopharyngeal colonization to lower airway and bloodstream invasion



polysaccharides, proteins, and nucleic acids. Encased in biofilm, which has been proposed to play a role in the pathogenesis of up to 80% of all microbial infections, the pneumococcus is relatively inaccessible to both antibiotics and host defenses. In this relatively dormant state, the pneumococcus can survive and reemerge as a dangerous invasive pathogen when airway host defenses are subdued either transiently or on a protracted basis. In this setting translocation of the pneumococcus to the distal airways causes pneumonia, as well as possible bloodstream invasion due to the cytotoxic effects of pneumolysin on the alveolar epithelial barrier (Huson et al. 2015).

The events involved in the transition of the pneumococcus from nasopharyngeal colonization to lower airway and bloodstream invasion are summarized in Fig. 1.

Taking into account the suppression of pulmonary host defenses described above, namely, the accumulation of dysfunctional T cells and

depletion of CD4 Th17 cells, it is easy to imagine how in HIV-infected patients the balance can be tipped from pneumococcal colonization toward pneumococcal disease and invasion. The fact that the extremes of age and a multitude of comorbidities predispose patients to pneumococcal disease regardless of their HIV status illustrates the paradigm of pneumococcal pathogenesis. The pneumococcus mostly behaves as a commensal in the nasopharynx. However, already weakened mucosal host defense mechanisms can allow it to become a pathogen causing both mucosal and invasive disease.

Pulmonary Microbiota in HIV-Infected Patients

The few studies performed in HIV-infected individuals have identified unique microbiota in the lower airway compared with HIV-negative people, such as an increase in *Tropheryma whipplei*

lung colonization (Gingo et al. 2013). However, a causal link between altered pulmonary microbiota and pulmonary function or susceptibility to infection cannot yet be made. Changes in oral, upper airway, and gut microbiota due to HIV infection per se or due to antibiotic prophylaxis (azithromycin, co-trimoxazole) may well turn out to affect pulmonary infection rates in HIV patients in the future (Clarke et al. 2010; Vujkovic-Cvijin et al. 2013; Brown et al. 2015).

Bacterial Lower Respiratory Tract Infections: Clinical Syndromes and Course

Community-Acquired Pneumonia

There has been conflicting data on whether HIV infection has any impact on the clinical presentation and outcome of CAP, and in particular pneumococcal CAP, with earlier studies suggesting that the mortality was no different when comparing HIV-infected and HIV-uninfected patients (Feldman and Anderson 2013). However, in one multicenter, international, prospective observational study of bacteremic pneumococcal pneumonia, HIV-infected cases were more likely to be of younger age, to be IV drug users, and to have a higher frequency of respiratory and systemic symptoms (rigors, pleuritic chest pain, cough, sputum production, hemoptysis, diarrhea, vomiting, and headache) than HIV-uninfected cases (Feldman et al. 2007). Furthermore, when cases were stratified according to age and severity of infection, the mortality in patients with severe CAP was higher in HIV-infected cases compared with HIV-uninfected cases, with a significant trend for increasing mortality associated with lower CD4 cell counts (Feldman et al. 2007; Feldman and Anderson 2013). Therefore some experts have suggested that both a low CD4 count (<200 cells/ μ L) and a high severity of illness score (PSI score IV and V) should be considered as potential reasons for admitting an HIV-infected patient with bacterial CAP to hospital (Feldman and Anderson 2013). Other investigators have documented that the overall mortality rate for hospitalized patients with IPD has hardly

changed between the years 1952 and 2001 and still remains in the region of approximately 12% (Ludwig et al. 2012).

Pathogens Causing Cavitating Bacterial Pneumonia

In addition to mycobacterial and fungal infections, cavitating lung disease may also be caused by a range of other bacteria, often presenting in the form of subacute or acute pneumonia. Among the abovementioned CAP etiologies, *S. pneumoniae* can cause lung abscesses, mostly with contiguous empyema, while *S. aureus*, either in the form of bloodstream infection with septic pulmonary infarctions or in the case of PVL-CAMRSA, can cause multiple lung cavities. Anaerobic lung abscesses occur more frequently in persons who use drugs as well as in patients with gingivitis/periodontitis. Rare reports have described cavitating pneumonia due to *Legionella* spp. or *Salmonella typhimurium* infection in HIV-infected patients.

Pseudomonas aeruginosa can present both as CAP or hospital-acquired pneumonia, and there are several case series describing subacute presentation with cavitating disease which tends to have a milder course than non-cavitating disease. Severely immunosuppressed patients with CD4 counts <50 cells/uL and accompanying neutropenia have the highest risk of such disease.

The gram-positive aerobic actinomycete *Nocardia* has a predilection for patients with defective cellular immunity and characteristically causes cavitating pneumonia. In HIV-infected patients, susceptibility is highest in those with CD4 counts below 200 cells/uL. Most likely as a result of co-trimoxazole prophylaxis in these patients, nocardiosis (traditionally susceptible to co-trimoxazole therapy) remains somewhat rare.

Rhodococcus equi, an aerobic, weakly acid-fast gram-positive rod, is another infrequent cause of subacute cavitating pneumonia in severely immunosuppressed HIV patients. Presentation typically includes fever, cough, pleuritic chest pain, and weight loss. Patients are often bacteremic and prognosis in the pre-HAART era was dismal (Shepp et al. 1994; Gallant and Ko 1996; Feldman and Anderson 2013).

Diagnostic Evaluation of Patients with Suspected Bacterial Pneumonia in HIV-Infected Patients

In HIV-infected patients with clinical suspicion of a bacterial lower respiratory tract infection and consistent findings on pulmonary imaging, the following diagnostic studies should be performed.

A sputum of good quality should be obtained, before initiation of antibiotic therapy, in those patients able to comply. When using sputum analysis, one has to bear in mind that only isolation of an obligate pathogen, such as *Legionella* spp. or *M. tuberculosis*, for example, can firmly establish an etiology. Isolation of organisms that can be both colonizing and pathogenic makes establishing an etiology much more difficult.

Since many pathogens causing bacterial pneumonia in HIV-infected persons can be associated with bacteremia, obtaining two sets of blood cultures is usually recommended, particularly in hospitalized patients (Feldman and Anderson 2013). Establishing a firm diagnosis using blood cultures is not only of importance to individual patient management but also for surveillance of pneumococcal disease. Many countries base their surveillance of antibiotic resistance and serotype distribution of *S. pneumoniae* exclusively on invasive pneumococcal isolates. In the face of the continuously evolving serotype distribution under vaccine pressure such serotype surveillance is crucial. If unusual pathogens are suspected, such as *Nocardia* spp., one can consider holding blood cultures for 2 weeks in order to try and detect this pathogen.

If resources permit, urinary antigen tests for *Legionella pneumophila* serotype 1 should be obtained, and despite some debate in the literature, the use of the BinaxNOW[®] pneumococcal urinary antigen test in patients who have not had potential pneumococcal disease in the previous 3 months is recommended. In adult patients with a compatible clinical picture, the test has sufficient specificity to allow for narrowing of the antibiotic spectrum to *S. pneumoniae* (Sordé et al. 2011). In the future more sensitive and serotype-specific pneumococcal urinary antigen tests may become routinely available, such as illustrated by a urine

antigen detection test specifically designed for vaccine efficacy studies (Bonten et al. 2015).

Clinicians often adhere to the principle of Occam's razor that advocates the least possible number of diagnoses for a given combination of symptoms. In HIV patients with advanced immunosuppression, the counterargument of Hickam's dictum "Patients can have as many diagnoses as they please" is often more appropriate. Therefore the question remains as to how much further routine diagnostic workup needs to be done in patients with suspected bacterial pneumonia. In certain clinical situations, the possibility of infection with pathogens other than bacteria may be suspected; in addition, patients with suspected CAP may not respond adequately to initial treatment, either clinically or radiologically. In both these situations, additional investigations need to be performed.

In areas with high rates of tuberculosis, any patient with a history of preceding constitutional symptoms (weight loss, night sweats), cavitating disease, hemoptysis, or failure to respond to CAP therapy should be suspected of having tuberculosis and investigated routinely using sputum examination by smear and culture and/or, if available, GeneXpert TB testing.

Isolator blood cultures can be performed for detection of dimorphic fungi, as well as sodium citrate blood cultures if atypical mycobacteria are suspected. Depending on local epidemiology, other fungal, urinary antigen tests may be indicated (e.g., *Cryptococcus* spp., *Histoplasma* spp.). In hospitalized patients clinicians may also recommend PCR-based testing (nasopharyngeal secretion or bronchoalveolar lavage (BAL)) for respiratory viruses to assist with clinical management, including initiation and/or stopping of appropriate isolation precautions.

If TB or other cavitating, nodular, tree-in-bud pattern causing pathogens, as well as *Pneumocystis jiroveci* pneumonia (PCP), cytomegalovirus (CMV), cryptococcosis, or other interstitial pattern pathogens, are a possibility, or in the seriously ill, and severely immunocompromised, or in patients with undiagnosed, unclear, or protracted disease, a BAL with or without biopsy and full pulmonary opportunistic workup are

recommended (infections with PCP, CMV, respiratory viruses, atypical bacteria including *Legionella* spp., *Nocardia* spp., mycobacterial infections, and infections with molds and dimorphic fungi need to be sought, and cytology/histology specimens need to be obtained to investigate for noninfective conditions). Pleural effusions not responding to therapy may necessitate diagnostic and therapeutic thoracentesis to exclude empyema, tuberculosis, or neoplastic disease.

Obtaining a CD4 cell count in patients with acute CAP may not be helpful since CD4 cell counts may be transiently depressed during acute illness without any prognostic value. However, in HIV patients admitted with known recent CD4 counts, the test may be of use in choosing the appropriate diagnostic approach as well as for prognosis (Feldman and Anderson 2013).

Approach to Therapy of Bacterial Pneumonia

The treatment of CAP in HIV-infected patients is considered to be very similar to that of HIV-uninfected patients. It should be directed at the most commonly expected bacterial pathogens and their local antibiotic susceptibility patterns and modified according to microbiological findings. Usual recommended empirical treatment is the use of either a beta-lactam/macrolide combination or fluoroquinolone monotherapy. If there is concern that the macrolide may be interacting with the patient's antiretroviral therapy, the use of newer fluoroquinolone monotherapy or beta-lactam plus doxycycline can be considered, or alternatively azithromycin could be used instead of clarithromycin [hiv-druginteractions.org]. If the patient has a prolonged QTc interval, doxycycline can replace both macrolide and fluoroquinolone for coverage of suspected *Legionella* spp., infection, pending urinary antigen testing.

After numerous meta-analyses and randomized trials, the matter of the best empirical CAP treatment, i.e., a beta-lactam/macrolide combination versus beta-lactam monotherapy versus fluoroquinolone monotherapy, is still not completely settled. However, a recent meta-analysis of

critically ill patients with CAP clearly showed benefit of macrolide containing regimens, such that the authors concluded that combination antibiotics with macrolides should be considered as first line therapy in severely ill cases with CAP (Sligl et al. 2014). In less severely ill cases, beta-lactam monotherapy seems to be non-inferior to the other approaches (Garin et al. 2014; Postma et al. 2015). There are, however, no randomized controlled trials on this issue specifically targeting HIV-infected patients.

However, since HIV patients are at risk of severe disease, we would recommend the use of combination therapy (such as beta-lactam/azithromycin and occasionally beta-lactam/doxycycline) or fluoroquinolone monotherapy for the treatment of most cases. Fluoroquinolones should not, however, be used routinely as empirical therapy in areas in which tuberculosis is a highly probable differential diagnosis for fear of masking the disease and/or for selecting quinolone resistance in tuberculosis.

For patients not responding to empiric therapy, targeting routine bacterial pathogens; implementation of additional investigations, such as those described above; and appropriate escalation of empirical therapy may need to be considered (Feldman and Anderson 2013).

Prevention

There is no antimicrobial prophylaxis directed specifically at classical bacterial respiratory infections. Co-trimoxazole prophylaxis, irrespective of CD4 cell count, may have some protective effects against common bacterial pathogens causing CAP (Church et al. 2015). Whereas prescribing co-trimoxazole prophylaxis to patients not in need of prophylaxis against *Pneumocystis jirovecii* (e.g., CD4 counts above 200 cells/ μ L) may be considered in developing countries, especially in sub-Saharan Africa, this strategy is not recommended in resource-rich western countries. In the HAART era, azithromycin prophylaxis against *Mycobacterium avium/intracellulare* has become less common, but if used it may have protected patients from CAP in the past.

Annual influenza vaccination is recommended in HIV-infected persons since respiratory viral infections are common and may be associated with more severe infection, as well as increasing the risk of bacterial superinfection. Pneumococcal vaccination is a complex issue and is dealt with below. Other important issues that need to be considered are the need for strategies related to smoking cessation and the management of alcohol and drug abuse (Feldman and Anderson 2013).

Immunization Strategies Against *Streptococcus pneumoniae*

As mentioned above, even in the face of virologically suppressive HAART and associated immune reconstitution, HIV-infected patients remain at considerably higher risk than their HIV-uninfected counterparts for the development of IPD, underscoring the necessity for effective immunization strategies in this setting (Feldman and Anderson 2014a). Two types of pneumococcal vaccine are currently available. These are (i) the pneumococcal polysaccharide vaccine 23 (PPV23) containing a mixture of capsular polysaccharides derived from 23 common serotypes which induce T cell-independent antibody responses and (ii) several pneumococcal conjugate vaccines (PCVs) with improved immunogenicity in which capsular polysaccharides representative of the most common disease-causing serotypes are coupled to a nontoxic protein carrier, usually the modified diphtheria toxoid, CRM197. These vaccines evoke T cell-dependent antibody responses and PCV13 is the current front-runner in this category (Feldman and Anderson 2014b).

Although effective against invasive pneumococcal disease in immunocompetent, healthy young, and older adults, the evidence in support of a protective effect of PPV23 in high-risk HIV-infected patients remains contentious (Feldman and Anderson 2014a).

In the case of PCVs, PCV7, and PCV9, fore-runners of PCV13 have demonstrated efficacy in protecting against recurrent pneumococcal infection in HIV-infected adults and against development of a first episode of invasive disease in

HIV-infected children, respectively (Feldman and Anderson 2014a). Emerging evidence from several countries such as England, France, Mexico, South Africa, and the USA has also documented the efficacy of PCV13 introduced into routine national immunization programs. Like its predecessors, PVC13 is significantly impacting on the rates of invasive pneumococcal disease caused by vaccine serotypes, not only in vaccinated children but also indirectly in adults, a phenomenon known as herd protection.

However, the efficacy of PCV13 in the context of protection against IPD in HIV-infected subjects remains to be established. Nonetheless, in 2012 the United States Advisory Committee on Immunization Practices recommended a routine immunization strategy for immunocompromised adults aged ≥ 19 years which is based on sequential administration of PCV13 and PPV23 (Feldman and Anderson 2014a). This “prime-boost” strategy involves primary immunization with PCV13 followed 8 weeks later by a single dose of PPV23 and is recommended for all vaccine-naive HIV-infected subjects, with the possible exception of those with circulating CD4⁺ T cell counts of $< 200/\mu\text{L}$. With respect to patients who fall into this latter category, it has been proposed that administration of PPV23 be delayed until the numbers of circulating CD4⁺ T cells recover to levels above the threshold of 200 cells/ μL following implementation of HAART (Feldman and Anderson 2014a). The future development of protein vaccines based on either unencapsulated intact cells or recombinant proteins holds promise (Feldman and Anderson 2014b).

Conclusion

Despite all the advances that have been made in the management of HIV-infected patients since the introduction of HAART, CAP, and in particular pneumococcal infections continue to be a major challenge, being associated with significant morbidity and mortality. Lifestyle factors, especially cigarette smoking, contribute substantially to the ongoing risk of CAP, and smoking cessation strategies should be incorporated into the overall

management of HIV-infected patients. It has been recommended that the antibiotic treatment of HIV-infected patients with CAP should be similar to that of HIV-uninfected cases, but no specific studies of antibiotic strategies have been undertaken in HIV-infected patients. The pneumococcal conjugate vaccines offer the potential for safe and effective prevention of the most common pneumococcal infections in HIV-infected persons.

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BAF (BANF1)

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Definition

Barrier-to-autointegration factor (BAF or BANF1) is a highly conserved 10-KDa protein in multicellular eukaryotes. It was identified as a factor that blocks ► [integration](#) of Moloney murine leukemia virus DNA into itself (autointegration) *in vitro*. BAF binds DNA non-specifically and, because it is a dimer, bridges together DNA molecules. BAF also binds the LEM domain, a domain that is shared among lamina-associated polypeptide 2, emerin, and MAN1. BAF is an essential cellular protein, and knockdown or knockout results in a defect in chromosome segregation during mitosis.

Introduction

A role for barrier-to-autointegration factor (BAF or BANF1) in retroviral DNA ► [integration](#) was first inferred from *in vitro* experiments with Moloney murine leukemia virus. After entry of a virion into the cytoplasm of a newly infected cell, ► [reverse transcription](#) occurs within the reverse transcription complex, a nucleoprotein complex

derived from the core of the infecting virion. The resulting linear double-stranded viral DNA forms part of a large nucleoprotein complex called the preintegration complex (PIC). PICs contain a number of viral proteins, including integrase which is the viral enzyme that carries out the key DNA cutting and joining steps of DNA integration. Cytoplasmic extracts of virus-infected cells contain PICs, and the viral DNA within PICs efficiently integrates into an exogenously added target DNA *in vitro* in the presence of a divalent metal ion (Brown et al. 1987). This reaction system provided the foundation for early biochemical studies of retroviral DNA integration.

The products of integration of viral DNA within PICs into a plasmid DNA target *in vitro* can be readily monitored by digesting the integration product with appropriate restriction enzymes, followed by gel electrophoresis and southern blotting. The major reaction products result from intermolecular integration of the viral DNA into the plasmid target DNA. However, a minor fraction of integration products result from intramolecular integration of the viral DNA into itself. Such autointegration products had previously been detected *in vivo*. Autointegration is a dead end on the reaction pathway and the locally high concentration of viral DNA necessitates mechanisms that prevent this fate. It was found that when PICs are treated with high-ionic strength and then separated from smaller macromolecules by gel filtration or sedimentation, the preference for intermolecular integration is abolished, and autointegration products predominate (Lee and Craigie 1994). The preference for intermolecular integration could be restored by incubation with an extract of uninfected cells suggesting that a cellular factor was responsible for blocking autointegration and promoting intermolecular integration. Fractionation of cellular extracts, using blocking of autointegration as a functional assay, resulted in the identification of a single polypeptide barrier-to-autointegration factor (BAF) (Lee and Craigie 1998). BAF is a previously unidentified 89 amino acid cellular protein that at about the same time was also identified as an interacting partner of the LEM domain (Furukawa 1999), a domain that is conserved among a

family of proteins associated with the nuclear lamina including lamina-associated polypeptide 2, emerin, and MAN1.

BAF Structure and DNA Binding

BAF is a dimer in solution, and the structure has been determined by both NMR and X-ray crystallography. A helix-hairpin-helix motif was proposed to bind DNA (Umland et al. 2000). Consistent with the sequence-independent binding of BAF to DNA, the helix-hairpin-helix motif binds DNA through contacts between DNA phosphates and amide groups in the protein backbone. This mechanism of DNA binding was confirmed by solving the crystal structure of the BAF dimer in complex with DNA (Fig. 1; Bradley et al. 2005). In addition to the helix-hairpin-helix motif, DNA binding is aided by the first alpha helix which forms hydrogen bonds with phosphates. There are no contacts involving bases, and the BAF dimer does not undergo any conformational change upon DNA binding; the RMS deviation between the free and DNA-bound dimer is 3.5 Å for all atoms.

Mechanism of DNA Compaction by BAF

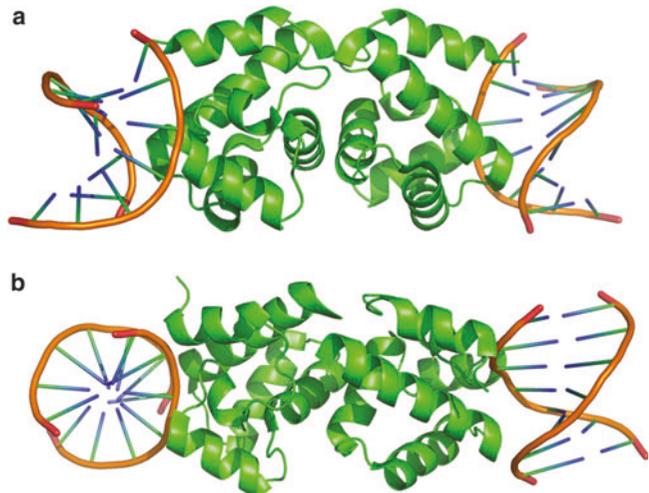
One DNA duplex binds to each monomer of the BAF dimer with the two DNAs forming an angle

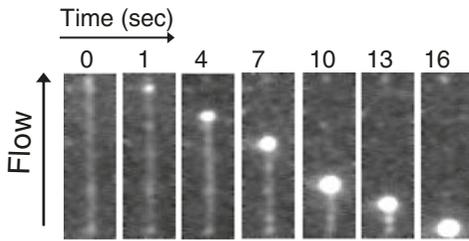
of close to 90 °C with one another (Fig. 1). Wrapping of a single DNA molecule around the BAF dimer to occupy both DNA-binding sites would be energetically unfavorable because of the required bending and the preponderance of negative charge on the surface of the BAF dimer between the two DNA-binding sites. The DNA-binding sites are therefore likely occupied by two difference of DNA molecules or sites on the same DNA molecule that are separated by a large enough distance that the DNA can form a loop between the two binding sites. At high DNA concentration intermolecular bridging will aggregate DNA. At a low DNA concentration, such as retroviral DNA within the cytoplasm of an infected cell, intramolecular bridging will result in compaction of the DNA molecule. It has been proposed that such compaction of retroviral DNA by BAF within the PIC makes it inaccessible as a target for integration and therefore blocks auto-integration (Lee and Craigie 1998).

The degree of compaction of viral DNA within the PIC correlates with protection against auto-integration. Salt-stripped PICs sediment more slowly in sucrose gradients. Treatment with purified BAF restores the protection against auto-integration and the faster sedimentation behavior in parallel. Other protein factors appear to not be required to compact the viral DNA within the PIC because incubation of BAF with plasmid DNA at DNA low concentration results in similar

BAF (BANF1),

Fig. 1 Ribbon representation of the BAF dimer in complex with DNA. BAF is shown *green* and the DNA helices in *orange*. The views in (a) and (b) are rotated about the horizontal axis (Note that the relative orientation of the two DNA molecules is close to orthogonal)





BAF (BANF1), Fig. 2 Compaction of DNA by BAF. A single 50-Kb DNA molecule was attached a flow cell and extended by buffer flow in the direction show. The DNA was labeled with the fluorescent dye YoYo1 and visualized by total internal reflection fluorescence microscopy. At time 0, the buffer was switched to buffer containing BAF. The DNA progressively condenses into a tight ball from the free end (*top*) toward the point of attachment (*bottom*) (Reproduced from Skoko et al. (2009))

compaction as judged by sucrose gradient sedimentation (Skoko et al. 2009).

DNA compaction by BAF can be directly visualized by total internal reflection fluorescence microscopy (TIRFM) (Skoko et al. 2009). Figure 2 depicts compaction of a 50-kb DNA molecule by BAF. The DNA is labeled with the fluorescent dye YOYO-1 and extended by buffer flow. At time zero BAF is introduced into the flow cell. The initially extended linear DNA rapidly condenses toward its attached point on the flow cell, and by about 15 s the DNA is condensed into a tight ball. The dissociation of BAF from DNA is surprisingly slow even in the presence of 1-M NaCl, with some BAF remaining bound even after 1 h of washing. The compacted structure likely presents a kinetic barrier to dissociation of BAF. Release of DNA from one of the two binding sites within the dimer does not result in immediate release of BAF because it remains anchored by the second binding site. Furthermore, the locally high DNA concentration favors rebinding at the first site before the second site dissociates. Thus the DNA-binding properties of BAF alone can account for its tight association with the PIC and the requirement for very high-ionic strength to release BAF and decondense the DNA. Autointegration may not require complete dissociation of all BAF from the PIC but rather sufficient dissociation to cause the viral DNA to adopt a more open structure.

Phosphorylation of BAF Abrogates DNA Binding and Protection from Autointegration

BAF exists in both unphosphorylated and phosphorylated forms. The major site of phosphorylation is Ser4 with a minor population phosphorylated at Thr2 and/or Thr3 (Bengtsson and Wilson 2006; Nichols et al. 2006). Phosphorylation of BAF abrogates DNA binding. The major kinase responsible for phosphorylation of BAF is vaccinia-related kinase 1 (VRK1) (Nichols et al. 2006). Treatment of MoMLV PICs with VRK1 and ATP abolishes intermolecular integration and promotes autointegration, consistent with a dominant role of BAF in blocking autointegration (Suzuki et al. 2010). The VRK1-treated PICs migrate more slowly in sucrose gradients consistent with a reduction in compaction resulting from a loss of BAF after phosphorylation. This change in sedimentation behavior resembles that resulting from treatment of PICs with high salt, which also causes a loss of BAF (Suzuki and Craigie 2002). It is noteworthy that treatment of HIV PICs with ATP promotes autointegration (Farnet and Haseltine 1991); this phenomenon may have resulted from phosphorylation of BAF by endogenous kinases present in the cell extract.

Role of BAF in POX Virus Replication

BAF has been implicated in host cell defense during poxviral infection (Wiebe and Traktman 2007; Ibrahim et al. 2011). Poxviruses, such as vaccinia, are DNA viruses that replicate exclusively in the cytoplasm. Vaccinia B1 kinase is an essential viral enzyme that is encapsidated within the virion. Mutations in B1 kinase cause a DNA replication defect that can be rescued by the cellular vaccinia-related kinase 1 (VRK1). The absence of known viral targets for the B1 kinase, and the fact that BAF is the major substrate for VRK1, led Traktman and colleagues to investigate whether the role of B1 kinase in poxvirus replication might involve phosphorylation of cytoplasmic BAF (Wiebe and Traktman 2007).

Several lines of evidence support this hypothesis. Firstly, phosphorylation of BAF increases with vaccinia virus infection. Secondly, in the absence of B1 kinase, BAF localizes to the sites of vaccinia virus DNA replication. Thirdly, increased BAF levels correlate with decreased vaccinia DNA synthesis. Finally, expression of a non-phosphorylatable mutant of BAF from the viral genome is lethal for vaccinia replication. The data support a model in which in the absence of phosphorylation cytoplasmic BAF compacts and sequesters the vaccinia DNA thereby blocking DNA replication. Thus the same mechanism that blocks autointegration of retroviral DNA can inhibit replication of poxviral DNA.

Function of BAF for the Host Cell

In this brief overview we focus what is known about the function of BAF for viral replication. However, BAF is an essential protein for the host cell, and this complicates *in vivo* functional studies because cells in which BAF is severely knocked down or knocked out are not viable. BAF plays a crucial role in chromatin segregation during mitosis and nuclear envelope assembly. Knockdown of BAF by RNAi in *C. elegans* results in a defect in chromatin segregation during mitosis (Zheng et al. 2000). In *Drosophila*, homozygous *BAF* mutants are lethal at the larval-pupal transition (Furukawa et al. 2003). Abnormalities in interphase nuclear structure were also observed, including clumping of chromatin and abnormal nuclear morphology. The molecular basis of these changes in chromatin organization is unclear, but studies with a temperature-sensitive BAF mutant in *C. elegans* show nuclear envelope defects are apparently independent of and before the defect in chromatin phenotype (Gorjanac et al. 2007).

BAF plays an important role in nuclear envelope formation. BAF interacts with the LEM domain of the nuclear envelope-associated proteins LAP2, emerin, and MAN1 (Furukawa 1999; Lee et al. 2001; Shumaker et al. 2001; Mansharamani and Wilson 2005). The structure of the BAF/LEM complex has been determined

by NMR (Cai et al. 2007). BAF has also been reported to interact with other cellular proteins including cone-rod homeobox (Crx) (Wang et al. 2002) and the C-terminal domain of MAN1 (Mansharamani and Wilson 2005), but a direct physical interaction is not supported by biophysical measurements (Huang et al. 2011).

Conclusion

The primary role of BAF for the host cell is in the assembly of the nuclear envelope and chromatin organization. Why is BAF also present in the cytoplasm where it serves no obvious cellular function? It has been proposed that cells employ the DNA compacting properties of BAF as a defense against DNA viruses and foreign DNA in general (Ibrahim et al. 2011). The same mechanism that blocks replication of DNA viruses can also compact the viral DNA within retroviral PICs making it inaccessible as a target for autointegration.

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Behavioral Aspects of HIV Mother-to-Child Transmission

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Definition

HIV mother-to-child transmission (MTCT) is defined as infection acquired by an infant from an HIV-positive mother during pregnancy, labor, delivery, or breastfeeding.

Introduction

Worldwide, an estimated 3.4 million children under the age of 15 years were living with HIV in 2011, of whom 330,000 acquired HIV that year; over 95% are believed to have acquired HIV by MTCT (UNAIDS 2012) (374, 359). Without intervention, 28–40% of infants born to HIV-positive mothers will acquire HIV, most during delivery; less frequently, intrauterine MTCT can occur. MTCT can also occur during breastfeeding, and over 10% of MTCT cases in breastfeeding populations may be due to this mode of transmission (Horvath et al. 2009). Prenatal interventions for prevention of MTCT (pMTCT) can decrease risk considerably. Multi-dose zidovudine and single-dose nevirapine regimens during pregnancy have been shown to decrease the risk of HIV

Robert Malow: deceased.

transmission to rates of 8–15% (384,414,381). Elective cesarean delivery can also reduce the risk of MTCT. The most effective method of reducing MTCT has been prenatal highly active antiretroviral therapy (ART), which has been able to reduce rates of HIV transmission to <1–2%, especially if administered for at least 3–6 months (Sturt et al. 2010). Exclusive artificial infant feeding eliminates postnatal MTCT due to breastfeeding. Cesarean delivery offers no additional protection if prenatal ART suppresses maternal viral loads to undetectable levels. Prenatal ART use has led to virtual elimination of MTCT in high-income countries and is currently the standard of care, making MTCT elimination theoretically achievable worldwide (US PEPFAR 2010).

Because ART provides health benefits to both the mother and the child, in 2010, the World Health Organization (WHO) introduced guidelines for moving beyond single-dose nevirapine for pMTCT, which offered no benefits to mothers, to lifelong ART for HIV-positive pregnant women if they needed it for their own health (381,414). Other options also recommended short-term ART to prevent MTCT if mothers did not need it, based on CD4 counts (WHO 2012). These recommendations were:

Option A: Initiating lifelong treatment as soon as possible after diagnosis if the CD4+ T-lymphocyte/mL³ (CD4) count is <350. If the CD4 count is ≥350, prenatal multi-dose zidovudine starting as early as 14 weeks' gestation, single-dose nevirapine and a dose of zidovudine and lamivudine during labor, and 7 days of zidovudine-lamivudine postpartum, with infant multi-dose nevirapine regimens of variable durations based on breastfeeding duration and maternal postpartum ART regimen.

Option B: Initiating lifelong ART including nevirapine or efavirenz as soon as possible after diagnosis if the CD4 count is <350; if the CD4 count is ≥350, starting ART containing efavirenz as early as 14 weeks' gestation, continuing through childbirth if not breastfeeding or until 1 week after breastfeeding cessation, with daily infant nevirapine or zidovudine regardless of feeding strategy (WHO 2012).

More recently, a third option (**Option B+**) was introduced which provides the same ART regimen (generally tenofovir-lamivudine or tenofovir-emtricitabine and efavirenz) to HIV-positive pregnant women beginning in prenatal care regardless of CD4 count and continues this therapy for life (WHO 2012). A single universal ART regimen has clinical and programmatic advantages: it treats HIV-positive women for their own health, prevents MTCT and streamlines, and strengthens pMTCT program performance through harmonization and linkages with ART standard programming for adults and adolescents, as seen in Option B (371).

Worldwide, the use of Option B+ is the most promising strategy for MTCT elimination (CDC 2013; Haas et al. 2016). However, because every HIV-positive pregnancy carries some MTCT risk, prevention measures have to be both accessible and sustainable. MTCT elimination is considered to depend on four key fundamentals or “prongs” (US PEPFAR 2010): (1) primary prevention of HIV infection in fertile women, including during pregnancy (Johnson et al. 2012) (312); (2) prevention of unwanted pregnancy in HIV-positive women (Reynolds et al. 2006); (3) prenatal and post-delivery maternal ART use for the mother's health and for MTCT prevention (WHO 2012) (373); and (4) effective integration of care, treatment, and support for HIV-positive women and their families (35) (Ackerman Gulaid and Kiragu 2012). The fulfillment of these key fundamentals requires the accessibility of and adherence to highly effective biomedical strategies, particularly ART regimens. Interpersonal and structural factors may influence the ability of HIV-positive women to initiate and adhere to ART during their pregnancy. Inequities and barriers to access, along with a lack of basic knowledge and information, may impede the uptake and sustainability of interventions to reduce MTCT in the most HIV-affected communities. Against this challenging backdrop, a “cascade” of prevention measures may identify HIV-positive women and their families at critical crossroads while providing strategies to negotiate these fragile prongs (Marcos et al. 2012), including prenatal counseling and education, specialized HIV care and ART,

perinatal testing and management, and infant feeding practices and family planning. The effectiveness of this cascade relies on a considerable degree on the robustness of the relationship between pregnant women and their providers (Barry et al. 2012). This section focuses on behavioral aspects of MTCT, including diagnosis and treatment of HIV infection in pregnant women, prevention or timely detection of maternal HIV infection acquired during pregnancy (Moodley et al. 2009), perinatal behaviors, and postpartum, post-delivery, and inter-pregnancy issues, including infant feeding, maternal contraception, and ART continuation.

pMTCT During Pregnancy and Delivery

Worldwide, HIV in women is most often acquired through unprotected sex with HIV-positive men, and many women are diagnosed with HIV during prenatal testing (UNAIDS 2011). Contact with providers who are able to administer or link women to HIV testing is thus a critical first step to diagnosing HIV infection and providing ART (WHO 2012). Evidence suggests that the vast majority of pregnant women accept universal HIV testing if it is routinely offered, either through opt-out or opt-in testing policies (Creek et al. 2009). Integration of HIV testing in prenatal and other maternal and child health programs has been particularly successful in enhancing acceptance of testing, if consent procedures are streamlined. The relatively high acceptability of routine, universal prenatal HIV testing to pregnant women and healthcare providers contrasts starkly with the low rates of acceptability for focused testing among women who considered “high risk” based on behavioral or other demographic characteristics that may stigmatize women, place providers in an uncomfortable position of explaining why a woman is considered “high risk,” lead to resistance to both offering and accepting testing, and therefore may miss the opportunity to test many women.

A significant barrier to women’s acceptance of prenatal testing, even when universally available and offered in a sensitive and respectful manner, is

the fear of loss of confidentiality, particularly the unauthorized disclosure of results to partners, in-laws, and employers. Fear that a positive test may result in loss of support or respect from acquaintances and friends and even violence, abandonment, and unemployment is an especially daunting barrier to the acceptance of testing, particularly in countries without robust, explicit legal protections for HIV-positive persons. For pregnant women, acceptability of universal HIV testing is greatest among women who are well informed regarding the preventability of MTCT and the availability at no cost of ART for both pMTCT and their own health, whose partners accept testing for themselves, and who know other women receiving ART for pMTCT (Creek et al. 2009). As such, the integration of “expert” mothers living with HIV in prenatal HIV testing programs (367), who can provide information regarding MTCT and offer support for ART initiation and continuation for those who test positive, is a promising strategy for pMTCT support and scale-up (Kim et al. 2012; Marcos et al. 2012)

In all settings, rapid tests are more acceptable than tests requiring the client to return days after for testing results (321). While drop-off and attrition are present at all levels of the cascade, the highest risk of loss to follow-up is arguably before an HIV-positive client receives her results. HIV-positive women are often overrepresented in the lowest income brackets; therefore, barriers to return for test results may include factors such as lack of transportation and/or child care, inability to take time off from work, and other obligations. For these reasons, rapid testing with results given within the same visit is the recommended standard. Currently available third- and fourth-generation rapid tests have extremely high specificity; the likelihood that a positive rapid test represents a false positive is extremely low, especially when the client can be retested with another rapid test using a different principle. As such, the HIV rapid test should be considered the highest priority, along with syphilis and anemia screening, in the first prenatal healthcare encounter.

The nature of the relationships between pregnant women and providers and women’s

satisfaction with their care are among the most significant predictors of maternal adherence to prenatal ART and, consequently, pMTCT effectiveness (Barry et al. 2012) (367). Strengthening the relationship between women and providers (Marcos et al. 2012) and addressing structural barriers faced by particularly marginalized and vulnerable populations are vital strategies to increasing prenatal ART adherence. The relationship between pregnant women and their providers depends upon the feeling that providers can be trusted, and women must feel that their disclosure decisions are respected. Additionally, women's disclosure of their HIV status to partners tends to be associated with increased ART and prenatal care attendance. However, immediately after HIV-positive pregnant women learn of their diagnosis, there are many priorities, such as ensuring housing stability and food and transportation access, which take priority over disclosure to the partner.

Many HIV-affected populations may have limited access to prenatal and delivery care (35, 325). In sub-Saharan Africa, while 71% of women have ≥ 1 prenatal care visit, only 44% have >4 visits; for delivery care, just 46% women deliver with assistance of a skilled attendant and 42% deliver in a hospital (UNICEF 2010). In other populations where prenatal care utilization for women may be higher, HIV-positive women may face significant barriers to prenatal care access, including lack of health insurance, lack of citizenship and/or documentation, or other marginalized statuses. In these circumstances, innovative strategies to offer prenatal testing outside of conventional prenatal care include mobile clinics, health fairs, and other approaches. Unconventional settings present challenges to effective linkage to care but may be an alternative strategy to at least provide HIV testing. Community engagement and support at multiple levels, such as assuring that the rights of HIV-positive persons are secured, have proven effective in ensuring the critical first steps: accepting testing and receiving results and accessing and adhering to ART (Ackerman Gulaid and Kiragu 2012) (321).

Special Circumstances

Acute HIV Infection During Pregnancy

An important but often overlooked component of pMTCT is the prevention of HIV infection during the time of pregnancy for HIV-negative women (Johnson et al. 2012). Because condom use tends to be higher among persons who desire contraception, pregnancy may be a particularly high-risk period for unprotected sex (312). Traditional taboos in some societies against sex during pregnancy, during the immediate postpartum period, and during lactation are rarely observed, especially if vulnerable women who depend for their livelihood on their partners feel that they may be replaced if they observe such abstinence periods. Acute HIV infection (AHI) during pregnancy, which is undetectable in early prenatal HIV testing, is extremely risky for MTCT, as AHI is generally asymptomatic or characterized by nonspecific symptoms, while viral loads reach extremely high levels during AHI. Encouraging pregnant women's partners to accept testing can help identify women at highest risk of AHI (those who are in stable discordant relationships with HIV-positive men [315,377]); partner testing also may increase women's acceptance of testing (Creek et al. 2009). Other strategies include encouraging providers to screen and treat genital conditions that may promote HIV transmission and encouraging pregnant women and their partners to adopt universal prenatal condom use. However, the most important behavioral measure to address acute HIV infection in pregnancy is motivating providers to facilitate third trimester or delivery retesting or rapid tests for newborns of women who test negative early in their pregnancy, regardless of maternal risk (Moodley et al. 2009). Women should be informed of the routine retesting policy during their initial prenatal test, as this may increase acceptability. Furthermore, both clients and providers need to understand that initiation of infant antiretroviral post-exposure prophylaxis (PEP) is very effective and of low risk for the newborn if started before 72 h postpartum. Infant PEP should be continued

according to Option B+ for 4–6 weeks or until after breastfeeding is discontinued, as in other perinatally exposed infants (WHO 2012) (384).

Serodiscordant Couples

Serodiscordant couples are couples in which one partner is HIV-positive and the other is HIV-negative; these couples are a small but critically important population in MTCT, particularly when the HIV-positive partner is the male (315). Artificial insemination with sperm separated from seminal fluids which could contain HIV (“sperm washing”) appears to be a safe and effective strategy (Semprini et al. 2013; Royal College of Obstetricians and Gynaecologists 2013). However, it requires considerable technological expertise and is financially unavailable to most couples worldwide who needed it. ART use by HIV-positive men in serodiscordant couples has emerged as an important alternative, offering health benefits to the HIV-positive partner even if they do not meet CD4 count criteria for ART initiation and reducing the risk of HIV transmission by over 95% to the HIV-negative partner and, thus, to a potential infant (Cohen et al. 2011; Royal College of Obstetrics and Gynaecologists 2013) (377). No assessment has been performed of the safety and effectiveness of “treatment as prevention” during the periconceptional period for prevention of HIV transmission to the female partner in serodiscordant couples desiring conception. However, it appears to be the most promising alternative and is tentatively being considered for couples where the HIV-positive male partner is adherent to ART, has an undetectable viral load and no sexually transmitted infections, and confines unprotected intercourse to HIV-negative female partner’s most fertile period (ideally at ovulation, confirmed by point-of-care testing). The use by the HIV-negative female partner of pre- and/or post-exposure prophylaxis (PrEP, PEP) with antiretrovirals to which the male patient’s strain is not resistant may be an additional protection (296,378,329,411). In these cases, both commonly used non-nucleoside reverse transcriptase inhibitors (NN-RTIs) are

relatively contraindicated, efavirenz (because of modest teratogenic potential) and nevirapine (because of the high risk of adverse reactions in multi-dose regimens for HIV-negative women). Therefore, PrEP and/or PEP regimens including only two N-RTIs or two N-RTIs with a boosted protease inhibitor are preferable. For cases in which the female partner is HIV-positive, conventional artificial insemination is safe and effective in protecting the male partner from HIV transmission during attempts to conceive, and pMTCT Option B+ is the most appropriate for the mother.

Single-dose nevirapine use should be confined to women whose HIV infection is diagnosed at onset of labor or at delivery, because it does offer a substantial decrease in MTCT risk (US PEPFAR 2010; WHO 2012), (414,381). Despite its apparent simplicity, adherence to this method can be quite low (in some settings, less than 70%) (Kuonza et al. 2010). In routine situations, the length of time between the HIV diagnosis (typically early in pregnancy) and the moment when a healthcare provider and the mother will be expected to act on the positive result can be over 6 months. During the critical time window (2–8 h pre-delivery for the maternal dose and less than 72 h postpartum for the infant dose) in which single-dose nevirapine doses must be administered, the mother may be the only person aware of her HIV status and may be overwhelmed requesting a single dose of an unfamiliar medication for pMTCT. Higher risk of non-adherence is associated with fewer prenatal visits, home deliveries, and multiparity, the latter, because of the higher likelihood of precipitous labor. Single-dose nevirapine use for pMTCT of maternal infections diagnosed before or during early pregnancy is clearly suboptimal, not only because of its inferior performance for pMCT but also because it makes other essential elements, including support for disclosure to partners and education to increase adherence to maternal treatment and infant nutrition and follow-up recommendations, very challenging. Conducting testing, receipt, and explanation of test results, safety and effectiveness of nevirapine, and subsequent steps in

pMTCT during a woman's labor in an open ward is also challenging (46). For these reasons, single-dose nevirapine should be considered a pMTCT strategy of last resort.

Post-Delivery and Infant Care

Infant Nutrition

Postnatal MTCT is generally related to breastfeeding. Exclusive artificial feeding can eliminate the risk of MTCT; however, the risks of malnutrition, diarrhea, respiratory infections, and other serious and often fatal complications of exclusive artificial and mixed feeding, along with infant formula's high cost, make this choice controversial (Creek et al. 2010; WHO 2012). In virtually all industrialized countries and in some low- and middle-income countries, provision of formula at no cost to mothers and capacity building for safe formula preparation have resulted in HIV-free infant survival with exclusive artificial feeding, often with lower infant mortality than in non-perinatally exposed breastfed infants (Ivers et al. 2011). However, the complexities implicit in exclusive artificial feeding, its stigmatization in some populations, and the lowered risk of breastfeeding-related MTCT and maternal and infant mortality when mothers adhere to post-delivery ART and administer PEP to their infants have made breastfeeding an appealing alternative (White et al. 2014).

Maternal adherence to postnatal MTCT prevention options may depend on the mother's receipt of accurate information regarding her options in a non-coercive and respectful way that supports her autonomy (WHO 2012). In the rushed, public setting of maternity wards, standard referral strategies to specialized services for perinatally exposed infants may fail to adequately inform the mother of the need for and location of these services, expose the mothers to stigmatization, and make reestablishing contact difficult—particularly if the infant's medical record is only initiated at the time of the initial infant visit at specialized services (Ciampa et al. 2012). Methods that have increased adherence rates by

over 50% include accompaniment of mothers by staff to visit the specialized infant services before their discharge from maternity wards; counseling in that private setting by maternity staff on infant care, feeding, PEP, and need for return; and infant medical record generation before discharge. Before discharge of perinatally exposed infants, maternity staff should identify and address concerns regarding housing, food insecurity, and transportation availability and ensure support in the mother's community, ideally by other mothers of perinatally exposed uninfected infants (367).

Family Planning

The roles of contraception and sterilization in pMTCT are extremely controversial. Coerced sterilization, often as a condition to providing prenatal or delivery services, such as cesarean delivery, has been documented worldwide and contributes to women's fears of HIV testing. Conversely, desired sterilization, as well as other elective surgical procedures, is often denied to women by healthcare providers worried about the risk of occupational HIV infection (360). Contraception is extremely cost-effective for pMTCT and has additional health benefits for women and children through spacing of pregnancies (Reynolds et al. 2006). Knowing one's positive HIV status is strongly associated with desire to limit childbearing through contraception and with increased condom use (Johnson et al. 2009). Fertility preferences are complicated in the diverse population of HIV-positive women, including mothers of HIV-positive children and those at highest risk (seronegative partners of HIV-positive men). Many women often desire to defer but not completely eliminate future childbearing options and prefer reversible rather than permanent contraception.

Despite its promise, reversible contraception use by HIV-positive women is extremely complicated. There are numerous interactions between the most widely used antiretrovirals, including efavirenz and nevirapine, and oral-combined hormonal contraceptives that considerably limit the use of oral contraceptives (US DHHS 2011). These interactions include lowering of blood

levels of estrogen components of combined hormonal contraceptives causing contraceptive failure when used concurrently with efavirenz; lowering of antiretroviral levels to subtherapeutic concentrations if used concurrently with various contraceptives; and elevations of antiretrovirals to toxic levels. These interactions may be inconsistent and difficult to control with dosing changes. Contraceptives that have virtually no interactions with antiretrovirals are less acceptable due to some of their other side effects; for example, long-acting progesterone-only injection contraceptives that need dosing only once every 3 months are especially attractive because of their limited adherence issues, high contraceptive effectiveness, non-interaction with antiretrovirals, absence of the cardiovascular risks, and non-contraceptive benefits, such as reduction or elimination of menstruation, with consequently lowered risk of anemia, a common problem in HIV-positive women (US DHHS 2011). However, many women do not understand that the irregular bleeding and spotting with injection contraceptives is short-lived and that long-term absence of menstruation related to prolonged use is not harmful and, in fact, is beneficial. Unfortunately, providers who could reassure potential users sometimes have misconceptions about the safety of amenorrhea similar to those of patients. Alternatively, the option of male sterilization among HIV-positive men is rarely discussed in the context of pMTCT, and male condom use is the only contraceptive option where the male partner's involvement is seriously considered.

Healthcare Providers

The knowledge, attitudes, and behaviors of healthcare providers are critical to successful pMTCT (360). They can provide information, counseling, testing, linkage to care, and treatment – factors which are critical to every level of the pMTCT cascade, including prevention of infection among female partners of HIV-positive men and unintended pregnancies in HIV-positive women. The strength and quality

of provider relationships with HIV-positive women are strongly associated with effective pMTCT (Barry et al. 2012). However, healthcare providers are often uninformed and lack confidence about best practices at critical decision points. Stressors such as exaggerated fears of occupationally acquiring HIV, misinformation about the risk of pMTCT (frequently including that all infants whose mothers have not received pMTCT interventions will acquire HIV, that pMTCT is only marginally effective, and that HIV infection is uniformly fatal in infants and their mothers), and overwhelming workloads and lack of psychological support serve to greatly reduce the potential for meaningful provider contributions. The inclusion of HIV-positive mothers as treatment partners for pregnant women in multidisciplinary teams can greatly improve quality of care for the patient as well as the experience of the provider (Ackerman Gulaid and Kiragu 2012) (367). Moreover, provider-created quality improvement strategies have been shown to be highly effective and among the most effective for promoting adherence and preventing loss to follow-up (Ciampa et al. 2012). Although less studied, the provision of support services, specifically for providers in pMTCT, may greatly increase their willingness to competently serve the women and infants in their care. These services include information regarding the low risk of occupationally acquired HIV from HIV-positive pregnant women, especially when they are receiving ART; on-site PEP for occupational exposure at no cost to the provider, provided confidentially and competently (329); and consistent access to the most important tools for pMTCT (384), including rapid tests, easily dispensed ART, and protective devices for procedures associated with occupational risk, particularly phlebotomy.

Conclusions

The elimination of HIV MTCT is potentially one of the most achievable HIV prevention goals; however, targeted strategies at all levels of the

prevention cascade must be introduced and maintained in diverse and challenging populations, including sexually active fertile women, their sex partners, and healthcare providers.

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Behavioral Aspects of HIV Treatment as Prevention

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Definitions

- **Combination prevention:** A term for an approach in HIV prevention that includes an array of behavioral, biomedical, and structural interventions, selected to best address the specific HIV prevention needs of a population.
- **Substitution prevention:** A term for an approach in HIV prevention that utilizes a singular ideal intervention, often pitting behavioral, biomedical, and structural interventions against one another.
- **Complementary prevention:** Similar to *combination prevention*; a term for an approach that appreciates the diverse context for HIV prevention efforts and synergistically involves prevention interventions from all domains to address clients' specific service needs.

Background

Certain behaviors and conditions such as unprotected anal or vaginal sex, having other sexually transmitted infections, sharing of contaminated needles or syringes, or receiving unsafe injections or blood transfusions put individuals at greater risk of contracting HIV (WHO 2012). Beyond these *proximal* risk factors, there are *distal* and *structural* factors such as homelessness or stigma,

which shape the general socio-environmental context that also drives HIV transmission. Additionally, policies and resources that influence the availability of HIV care and treatment are important factors in HIV transmission and risk. These structural, epidemiological, biological, and behavioral risk factors overlap and interact with one another, increasing risk for not only HIV and other sexually transmitted infections but also other conditions (e.g., mental illness, substance abuse) and social problems (e.g., homicide, violence, and crime) (Rotheram-Borus et al. 2009).

Due to the multilevel factors involved in HIV risk and transmission, optimally effective HIV prevention efforts will require a combination of behavioral, biomedical, and structural intervention strategies. The 2010 United States National HIV/AIDS Strategy endorses this “combination prevention” approach, emphasizing the need for clients to receive the best array of interventions to address their specific HIV prevention needs (The White House Office of National AIDS Policy 2010).

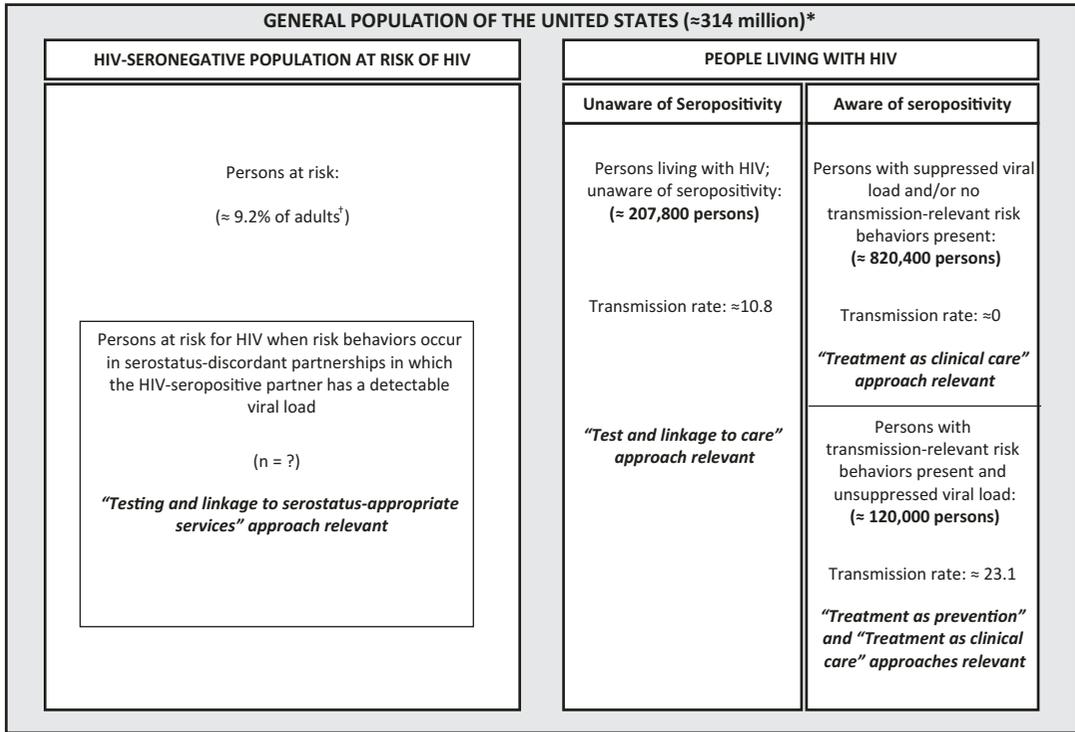
In addition to the established body of research on behavioral and structural interventions, emerging work in the field of biomedical HIV prevention strategies has received much attention in recent years. A recent NIH study, entitled HIV Prevention Trials Network (HPTN) 052, was touted as the “scientific breakthrough of the year 2011” by *Science* and showed that antiretroviral therapy for persons living with HIV (PLWH) can effectively reduce transmission in HIV serostatus discordant heterosexual partnerships (Cohen et al. 2011). The implications of this trial and of the growing body of work on Treatment as Prevention (TasP) on the broader landscape of HIV prevention have been profound and have led many to proclaim it as “The Beginning of the End of AIDS.” In a fact sheet addressing the state of the epidemic on World AIDS Day 2011, the Obama administration emphasized the significance of the recent breakthrough, but also highlighted the need for “combination prevention” efforts, acknowledging that not all types of intervention are appropriate for all populations and that it is important to consider strategic investment of funding in order to support the

interventions with the largest impact (White House Office of the Press Secretary 2011). In other words, though the recent biomedical breakthroughs provide a new class of prevention interventions, they should be considered in combination with existing intervention strategies rather than a direct substitution. Indeed, in subsequent discussions on the topic of TasP, the issue of HIV-related risk behavior has been nearly absent (Holtgrave et al. 2012; Small and Kerr 2011). The “combination prevention” discussion sometimes lapses into “substitution prevention” debates where one approach is pitted against the other (Holtgrave et al. 2012; Small and Kerr 2011).

While TasP is highly relevant for some subpopulations, the larger population of PLWH is far from homogenous in its risk of transmission and therefore in the optimal prevention intervention approach. Indeed as the scientific community and general public celebrate the NIH HPTN 052 study results and its potential impact on the fight against the epidemic, there is a need to emphasize a “complementary prevention” approach, choosing the best prevention interventions from all domains to address clients’ specific service needs (Holtgrave et al. 2012).

HIV Serostatus and Key Populations in the USA

Using a serostatus approach to consider the HIV epidemic in the USA (and as illustrated in Fig. 1), three key populations have been identified: (1) the general population, (2) HIV-seronegative persons at risk of infection, and (3) persons living with HIV (PLWH), which includes three subpopulations (persons unaware of seropositivity, persons aware and with suppressed viral load and/or engaged in no risk behavior that could result in transmission, and persons aware and engaged in unprotected serodiscordant risk behavior with unsuppressed viral load that could result in transmission) (Holtgrave et al. 2007, 2012). Due to the differences in serostatus, risk behavior, and transmission rates between these populations, interventions should be tailored to meet the unique needs of each group.



* Note: Figures are based on most recently available statistics described in the text.
[†] Percentage reflects federal estimates of risk behaviors among 15-44 year olds (Chandra et al., 2012).

Behavioral Aspects of HIV Treatment as Prevention, Fig. 1 Key U.S. populations defined by HIV serostatus and relevance of various treatment-related prevention approaches

The General Population

As of 2012, the US Census Bureau estimates that the US general population was nearly 314 million (US Census Bureau 2012). Although widespread education and awareness efforts are both necessary and valuable for the general population as a whole, specific behavioral and biomedical interventions, including TasP, are most relevant and cost-effective when directed at and custom-tailored for the specific subpopulations listed just above. The rationale for intervention tailoring is presented below for each of these groups, in turn.

HIV-Seronegative Persons at Risk of Infection

Federal estimates indicate that roughly 9.2% of seronegative adults between 15 and 44 years of age currently engage in some form of HIV-related risk behavior (Chandra et al. 2012). Within this group, there are an unspecified number of individuals who engage in unprotected transmission-

relevant risk behavior within a serodiscordant partnership where the HIV-positive partner has an unsuppressed viral load. This subpopulation represents the seronegative persons most at risk for acquiring the virus (this point is discussed further below).

People Living with HIV

The estimated number of PLWH in the USA as of 2009 includes both people aware of their serostatus (approximately 940,400 PLWH) and those unaware of their serostatus (approximately 207,800 PLWH) (CDC 2011; Hall et al. 2013). Among these subpopulations, there is a large discrepancy in the estimated transmission rates. While the estimated HIV transmission rate among PLWH who are aware of their serostatus is 3.0%, the transmission rate among PLWH who are unaware of their serostatus is an estimated 10.8% (due to the impact of behavioral changes upon learning one is

living with HIV and due to the impact of treatment on transmissibility). (Of course, the HIV transmission rate is the number of HIV transmissions to HIV-seronegative partners of 100 PLWH per year.) Due to this disparity, it is imperative that PLWH who are unaware of their serostatus should receive testing in order to improve linkage to HIV care for its clinical benefits and to limit the chance of HIV transmission. Although there are programmatic challenges in identifying undiagnosed PLWH (such as systematizing HIV testing in clinic settings and reaching PLWH who are entirely out of the healthcare system), awareness of serostatus is a major predictor of risk behavior and viral suppression and therefore of the potential risk of transmission (Hall et al. 2012, 2013).

The subpopulation of PLWH who are aware of their serostatus includes individuals who engage in unprotected risk behavior in serodiscordant partnerships with unsuppressed viral load (approximately 120,000 out of 940,400 persons) (CDC 2011; Holtgrave et al. 2012; Hall et al. 2013). Of the two subpopulations of PLWH who are aware of their serostatus, the vast majority (approximately 820,400 out of 940,400 persons) are not engaged in any risk behavior that may lead to transmission and/or has suppressed viral load, making the transmission rate for this subpopulation effectively 0% (Holtgrave et al. 2012; Hall et al. 2013). It has been estimated recently that approximately 120,000 diagnosed PLWH who are aware of their serostatus and who do engage in unprotected serodiscordant risk behavior in which the index partner has unsuppressed viral load account for about 27,800 HIV infections in the USA (the other roughly 22,400 HIV infections per year in the USA are transmitted from the approximately 207,800 undiagnosed PLWH) (Hall et al. 2013; Holtgrave et al. 2014). These key estimates are summarized in Figs. 1 and 2.

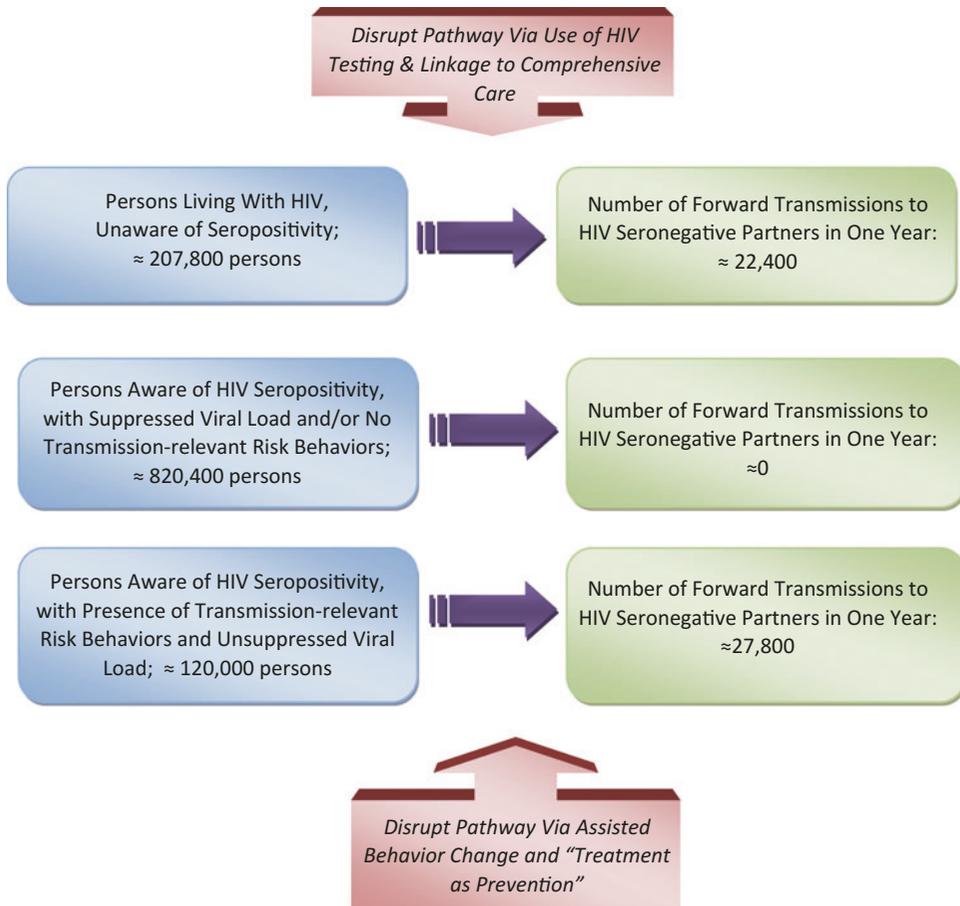
Further, as noted above, HIV-seronegative persons most at risk of infection are those who engage in unprotected risk behaviors with PLWH with unsuppressed viral load. Although this number of HIV-seronegative persons has not yet been specified in the literature, it could be approximated by (a) taking the number of

PLWH unaware of HIV seropositivity multiplied by the number of unprotected partnerships per unit time and then (b) adding to that product the number of PLWH who are diagnosed and engaged in unprotected risk behavior in serostatus discordant partnerships multiplied by the number of such partnerships per the same unit time.

Intervention Approaches by Subpopulation of PLWH

Table 1 displays the connection between key subpopulations of PLWH and the necessary array of client-centered HIV services. In summary (and also shown in Fig. 1), for PLWH, a “treatment as clinical care” strategy is always relevant as every PLWH should have unfettered access to high-quality, client-centered care (The White House Office of National AIDS Policy 2010).

For undiagnosed PLWH, a “treatment and linkage to care” strategy of HIV prevention is relevant since testing is the first step on a pathway to diagnoses, linkage to care, and ultimately suppression of viral load. For PLWH who are diagnosed and engaged in unprotected, serodiscordant risk behavior with unsuppressed virus present, there are two important pathways to avoid the transmission of the virus to seronegative partners: one pathway is behavioral risk reduction and the other is via TasP. (Of course, both HIV-related risk behavior and care access are influenced by broader social determinants as well.) Understanding the size of this particular subpopulation of PLWH provides insight into the impact that TasP strategies might have in the USA. For example, the impact of TasP on HIV incidence will be minimal if the next 10,000 persons entering care include very few PLWH who are engaged in transmission-relevant risk behavior. Conversely, the impact of TasP on incidence is potentially much greater if the next 10,000 persons entering care include many persons from this subpopulation. Again, this points to the need for truly synergistic combination HIV prevention approaches that meld together the best of behavioral and biomedical strategies (CDC 2009).



Behavioral Aspects of HIV Treatment as Prevention, Fig. 2 Number of forward HIV transmissions from subgroups of persons living with HIV, and potential points of prevention intervention

Further Key Behavioral Issues in HIV Care

Key steps in the continuum of care required for successful implementation TasP include HIV diagnosis, linkage and retention in care, prescription of antiretroviral therapy (ART), and ART adherence (CDC 2011). Persons retained in care can also benefit from counseling to reduce risk behaviors associated with HIV transmission. However, about one in five persons with HIV is unaware of their infection, less than half of persons with HIV are in regular care, and less than half of persons with HIV in care report receiving risk counseling in the past year (CDC 2011). Socioeconomic factors such as lack of health insurance, lower education, poverty,

unemployment, homelessness, lack transportation, feeling healthy, health literacy, and stigma are associated with lower retention in HIV care (Horstmann et al. 2010).

Mental health or substance abuse problems among PLWH also contribute to missed care visits and indicate greater need for health services. Structural factors that can enhance provider and patient participation and reduce stigma associated with HIV disease include policies for opt-out testing, HIV testing with routine blood draw from each patient entering the emergency department and inpatient facility, and reminders for appointments for care visits and risk counseling. A major factor in adherence to HIV care is a good provider-patient relationship, including effective communication, time to address individual needs, prompt

Behavioral Aspects of HIV Treatment as Prevention, Table 1 Key services needs of three subpopulations of persons living with HIV (PLWH)

Population	Testing	Linkage to care ^a	Care and treatment ^a	Behavioral prevention services	Mental health, reproductive health, housing, substance abuse treatment, sterile syringe and condom access, and other related services
PLWH, unaware	✓	✓	✓	✓	As client relevant
PLWH, aware; no risk behaviors	N/A	✓	✓	Support already existing behavior change	As client relevant
PLWH, aware; risk behaviors present	N/A	✓	✓	✓	As client relevant

“✓” indicates service is relevant for that subpopulation

“N/A” means not applicable

^a“Care” includes HIV specialty care and treatment, as well as comprehensive primary care, retention services, adherence support services, and ancillary support services such as transportation; linkage to care may include both initial and subsequent reengagement to care services

scheduling of appointments, and support through case managers (Aberg et al. 2009). In addition, care should be linguistically and culturally appropriate and address depression and substance abuse (Aberg et al. 2009). Effective interventions for linkage and retention in care must address barriers to accessing care. Some promising approaches using outreach to improve retention in care have been implemented (Bradford 2007; Horstmann et al. 2010); more research, however, is needed to develop optimal strategies and proven interventions for retention in care.

Conclusion

Scientific breakthroughs have clearly shown the importance of HIV treatment in terms of improving the morbidity and mortality of PLWH as well as on the disruption of HIV transmission. As a result, it is sometimes erroneously inferred that *only* HIV treatment is needed in HIV prevention. This is not the case, however, as an optimal combination of behavioral, social, and biomedical interventions creates a much stronger prevention package. It is only such complementary approach that has the potential to urgently and effectively address the HIV epidemic in the USA.

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Disclaimer The findings and conclusions in this study are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Behavioral Interventions for Adherence

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Definition

Management of HIV infection requires consistent medication use to keep viral load undetectable and prevent transmissions to sexual partners. While newer agents have made this process easier by creating simplified dosing, adherence still requires careful planning and behavioral management to ensure medications are taken correctly. To address this, psychosocial interventions have been developed to enhance adherence behaviors. Behavioral interventions for adherence have generally shown moderate success.

Behavioral Interventions for Adherence: Overview

Treating HIV to attain virological suppression is important both for long-term care of those living with HIV as well as minimizing the spread of HIV to others. Accordingly, interventions are needed for some individuals living with HIV/AIDS to maintain long-term adherence to antiretroviral therapy (ART). As a result, starting in the late 1990s when HIV went from a fatal diagnosis to a manageable chronic condition due to ART, medication regimens were quite complicated which spurred the creation of interventions with the goal of increasing or sustaining adherence to ART. These interventions have social-psychological health behavior models at their base and utilize various behavioral approaches such as problem-solving, cognitive behavioral therapy, or motivational interviewing to address barriers to adherence. The first set of meta-analyses suggest that the first major set of

adherence interventions were moderately successful in increasing or sustaining adherence (Amico et al. 2006; Simoni et al. 2006).

A central feature of most behavioral interventions for HIV medication adherence is that they have various skills-building techniques to address potential barriers to taking medication on time and as prescribed. Typical psychosocial theories for health behavior change are at the root of these interventions; strategies include attempting to change person-specific attitudes, norms, behavioral intentions, cognitions, and readiness to change. Other interventions have utilized therapy-based techniques including supportive psychotherapy, motivational interviewing, cognitive behavioral therapy, and problem-solving. Of the extant behavioral interventions for HIV medication adherence to date, most have fallen under one of the following three intervention categories: (1) individual education and behavioral skills building, (2) electronic reminder interventions, and (3) social support interventions. Each category is discussed briefly below with overviews of key trials and interventions.

Individual Education and Behavioral Skills Building

Education and behavioral skills building interventions are typically short term and performed in-person. These interventions emphasize basic medication management skills and problem-solving techniques that specifically address adherence barriers as opposed to problem-solving therapy, which teaches the general problem-solving skills. Consistent with the spirit of motivational interviewing, most of these effective interventions are conducted through a collaborative lens with both patient and counselor working together to achieve adherence goals. Interventions range in intensity from low to high (Amico et al. 2006) and from single-session to weekly sessions delivered intermittently over 12 months (Simoni et al. 2006). Approaches to treatment include psychoeducation about HIV and the importance of adherence to prevent viral mutation. However, for health behavior change interventions and

adherence to HIV medication interventions, education alone is necessary but not sufficient. As such, the inclusion of cognitive behavioral therapy and problem solving to address barriers to medication adherence is an important part of these interventions. These interventions often incorporate reminder systems or schedule planning to complement education and problem solving. In addition, the inclusion of cognitive-behavioral principles helps patients focus on changing thoughts and behaviors around taking medication. Most interventions also emphasize patient-centered care and utilize the therapeutic alliance to increase likelihood of adherence (Simoni et al. 2008). As an example, the LifeSteps intervention (Safren et al. 1999), one of the first published intervention trials of adherence counseling (Safren et al. 2001), coaches patients through a series of “steps” that address various barriers to adherence including attending medical visits, storing medication, and remembering to take medication. Another example of a similar approach utilizes “managed problem solving” that follows a 5-step process to identify barriers to adherence, brainstorm solutions, select the best strategy, monitor implementation of that solution, and evaluate whether that strategy was helpful for overcoming the barrier (Gross et al. 2013). These interventions specifically target psychosocial barriers to adherence compared to memory alone.

Electronic Reminder Interventions

One set of interventions has focused on enhancing adherence by utilizing technology, such as pagers or text messages, to set reminders to take medication. Electronic reminders through pagers or text messages are one intervention option that is both low-cost and feasible for large numbers of patients. These interventions may also be particularly useful, potentially as a complement to other interventions, for individuals with impaired memory possibly due to HIV associated neurocognitive disorders (HAND) or comorbidities including depression, dementia, and substance use (Andrade et al. 2005). In electronic reminder studies, patients typically receive automated alerts on

a personal pager or cell phone (e.g., “Take the 2 blue pills now” or “Eat lunch now before your 2 p.m. pill”) (Safren et al. 2003; Simoni et al. 2006). Interventions have also used pagers to deliver other forms of messages and encouragement including education about HIV and medication assessments to keep participants engaged (Simoni et al. 2009). More recently, two-way text messaging has been employed to try to increase adherence (Harris et al. 2010; Lewis et al. 2013) requiring a reply to the text to confirm that the medication has been taken. Despite directly addressing the most frequently given reason for lapses in adherence such as forgetting to take the medicine, studies employing electronic reminders have shown moderate change during the intervention period with mixed outcomes in long-term maintenance (Simoni et al. 2006, 2008).

Social Support Interventions

Social support consistently predicts positive health outcomes in a variety of illnesses. As a result, some HIV medication adherence interventions are largely based on social support and supportive therapies. These interventions utilize principles from the theory of reasoned action and planned behavior (Fishbein and Ajzen 1975) by emphasizing the role of social norms and principles of social learning theory (Bandura 1986), which posits that watching an individual perform a behavior can increase one’s own self-efficacy and likelihood of compliance with the desired behavior. Peer-led groups also offer an opportunity for observational learning and modeling, thus incorporating principles from social learning theory. In peer-led groups, HIV+ group facilitators can share their own experiences with participants, thereby modeling medication adherence strategies. Couples counseling for medication adherence have also shown to be effective (Remien et al. 2005) as support from a significant other may create an environment in which an individual can begin to value themselves enough to change. Overall, social support interventions have utilized a combination of informational and affirmational therapy (Simoni et al. 2006) as well as

emphasized the role that both functional and emotional support can provide in helping adherence. Topics covered include addressing barriers to adherence as well as how to engage and accept support from others. These interventions have been conducted face-to-face over a series of weeks and sometimes included additional support provided in the form of follow-up phone calls (Simoni et al. 2008, 2009). It is unclear, however, as to whether all forms of social support confer better adherence.

Limitations of Adherence Only Interventions

Overall, interventions that generally address the basic social-psychological barriers to adherence have had moderate results. While they improve adherence, additional interventions may be needed for individuals with the more severe adherence problems. Psychosocial comorbidities have been found as moderators of intervention effectiveness. Mental health and substance use can interfere with traditional, basic, short-term counseling approaches to increase adherence; these comorbidities or life conditions might diminish the benefit from brief or basic adherence interventions. As such, researchers started to address comorbidities within adherence treatments to try to further improve on already existing adherence therapy. The remaining portion of the chapter is dedicated to exploring interventions that have addressed comorbidities as a means of improving medication adherence in people living with HIV/AIDS (PLWHA).

Syndemics

From a theoretical perspective, one could see how mental health, substance use, and associated psychosocial problems could interfere with the ability of adherence counseling to make behavior changes. For example, with clinical depression, symptoms such as sadness, lack of interest, and loss of sleep can affect basic social-cognitive variables related to behavior change such as one’s

self-efficacy, one's ability to acquire new information, one's motivation for change, the ability to engage in new behavioral skills, as well as one's attitudes, norms, or beliefs. In meta-analyses, depression is associated with worse adherence in HIV (Gonzalez et al. 2011; Uthman et al. 2014). Additionally, traumatic stress, as in the case of posttraumatic stress disorder (PTSD), may make it difficult to benefit from adherence counseling via symptoms such as disassociation, sleep problems, concentration problems, or others due to a past or ongoing traumatic event. Symptoms of PTSD can also decrease medication adherence (Vranceanu et al. 2008). Similarly, substance use can interfere with normal decision-making processes. Acute intoxication can certainly affect the ability to carry out intended behavioral changes, and processes such as cravings or activities to obtain substances may interfere with problem-solving to keep medical appointments, refill prescriptions, or take medicines as prescribed. While acute substance use is associated with poor adherence, individuals with a substance use history appear to benefit from ART as much as others, and substance use disorders should not be a reason to withhold ART medications (Binford et al. 2012).

Furthermore, many of the psychosocial problems that occur in the context of HIV are interrelated; these problems are referred to as syndemics. A syndemic is defined as two or more conditions that interact synergistically to increase the burden of disease in a given population (Singer 1994, 1996). Some examples of these conditions are: alcohol or substance use/abuse, posttraumatic stress disorder, mood disorders, childhood sexual abuse, intimate partner violence, psychotic disorders, anxiety spectrum disorders, etc. One study has found additive effects of syndemics on ART adherence in individuals living with HIV (Blashill et al. 2015).

Depression

Despite the prevalence of syndemics and their adverse impact on medication adherence, there are few interventions that address both adherence

and a mental health or substance use comorbidity. One such intervention that addresses adherence and depression, which is prevalent in PLWHA, is cognitive-behavioral therapy for adherence and depression (CBT-AD). CBT-AD has emerging support among different populations, including HIV-seropositive men and women with depression receiving treatment for HIV in New England (Safren et al. 2009), PLWHA with depression and a history of injection drug use (Safren et al. 2012), HIV-seropositive Latinos on the US-Mexico border (Simoni et al. 2013), and a pilot study in South Africa with nurse interventionists (Andersen et al. 2015).

CBT-AD is a manualized, modular intervention (Safren et al. 2008a, b) and integrates psychosocial adherence counseling and treatment of depression in each session. CBT-AD consists of traditional CBT skills along with other skills to treat depression and improve adherence. In the context of HIV, the first module, Life-Steps, is a single session designed to increase motivation, improve adherence-related behavior, identify barriers, and solve problems related to ART adherence. Additional modules build off of this by reviewing barriers and developing techniques to overcome those barriers that emerge or are not resolved in the first session. The second module is psychoeducational and aims to introduce the patient to CBT for depression and how depression can affect adherence and self-care. The next modules consist of skills trainings for depression integrated with additional adherence counseling and include behavioral activation (examining and increasing activities that involve pleasure or mastery), cognitive restructuring (learning how to think more adaptively in difficult situations or situations that lead to depressed mood), problem solving, relaxation training, and relapse prevention. Throughout all sessions, CBT-AD simultaneously addresses depression and ART adherence as they relate to each other. Prospective trials in HIV that address depression alone but not adherence (e.g., psychopharmacological interventions, collaborative care) seem to result in improved depressive symptoms but have not improved HIV medication adherence (Tsai et al. 2013;

Pence et al. 2015). Therefore, when working with patients who have mental health comorbidities such as depression, it is important to evaluate and treat the mental health problem while concurrently addressing adherence in the context of the comorbidity.

Substance Use

Similarly, in the case of substance use, effective interventions address both adherence and the substance use comorbidity; the intervention combines motivational interviewing (MI) and cognitive-behavioral skills training (Parsons et al. 2007). This intervention, called Project PLUS, has been studied among HIV-seropositive adult men and women in New York City who met criteria for hazardous drinking. This intervention decreased the number of drinks per drinking day and improved adherence; these effects were sustained at the 6-month follow-up. Participants who received this intervention also had improved biologic outcomes (i.e., increased CD4 cell count; decreased viral load) at the 3-month follow-up compared to participants in the control condition, but not at the 6-month follow-up.

Project Plus consists of eight sessions and allows counselors to deliver information and skills training tailored to each individual's needs and motivation to change. The first two sessions are psychoeducational and motivational and are designed to increase motivation to change, encourage greater responsibility for one's adherence and alcohol use, and create behavior change plans. In the third session, a functional analysis of the patient's drinking and nonadherence behaviors is performed, which allows the counselor to select four skills-based modules, two to improve adherence, and two to decrease alcohol use. These modules are then covered in sessions four through seven, each of which include information/didactics from the counselor, self-assessment, skills-building activities, practice, and home-assignments (Parsons et al. 2007). The last session consists of relapse prevention.

Conclusions

General behavioral interventions for adherence demonstrate moderate effects that may be further enhanced by addressing co-occurring psychosocial problems, such as depression, trauma, and substance use, which might hinder the benefits of adherence interventions. Emerging studies that address both adherence and co-occurring comorbidities or problems have positive adherence outcomes for specific high-risk groups. Delivery of these interventions has varied greatly; however, most combine some form of either individual or group in-person sessions as well as an instrumental reminder (e.g., worksheets, pill diary, pager, cell phone) of adherence. In addition to needing more interventions that address adherence in patients with the greatest need and psychosocial comorbidities, a relatively unstudied area is how to best implement these interventions and disseminate them into settings where they would have a larger impact on long-term medication adherence behaviors.

Cross-References

- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Behavioral Science Highlights of Evidence and Research](#)
- ▶ [Comorbidity: Opioids](#)
- ▶ [Initial Antiretroviral Regimens](#)
- ▶ [Medication Adherence and HIV-Associated Neurocognitive Disorders \(HAND\)](#)
- ▶ [Retention in Care Interventions](#)

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societal challenges often faced in low-resource settings. Contextual factors influence human behavior and must therefore be addressed when fighting the disease (Myer et al. 2004). This includes addressing the micro- (individual), mesa (familial/social, community), macro- (government policy), and global (broad environment) levels.

Social Determinants of Population Health

The social determinants of health are the circumstances in which people are born, grow up, live, work, and age, as well as the systems put in place to deal with illness (WHO website 2014). These circumstances are in turn shaped by a wider set of forces: economic stability, education, politics, social and community context, health and healthcare, and neighborhood environment. Among these lie contributing factors that affect the reality of HIV/AIDS prevention, care, and treatment and overall health behaviors:

- Economic stability:
 - Poverty
 - Employment status/ income
 - Access to employment and migration
 - Housing stability/ access
- Education:
 - Lack of money for secondary school fees
 - High school graduation rates
 - School policies supporting public health
 - School environment
 - Enrollment in higher education
- Social and community context:
 - Family structure
 - Social cohesion
 - Religion
 - Stigma, discrimination, and equity
 - Civic participation
 - Incarceration/ institutionalization
- Health and healthcare:
 - Access to primary and HIV/AIDS health services
 - Access to preventive care and community-based health promotion

Behavioral Science Highlights of Evidence and Research

Ann F. Green

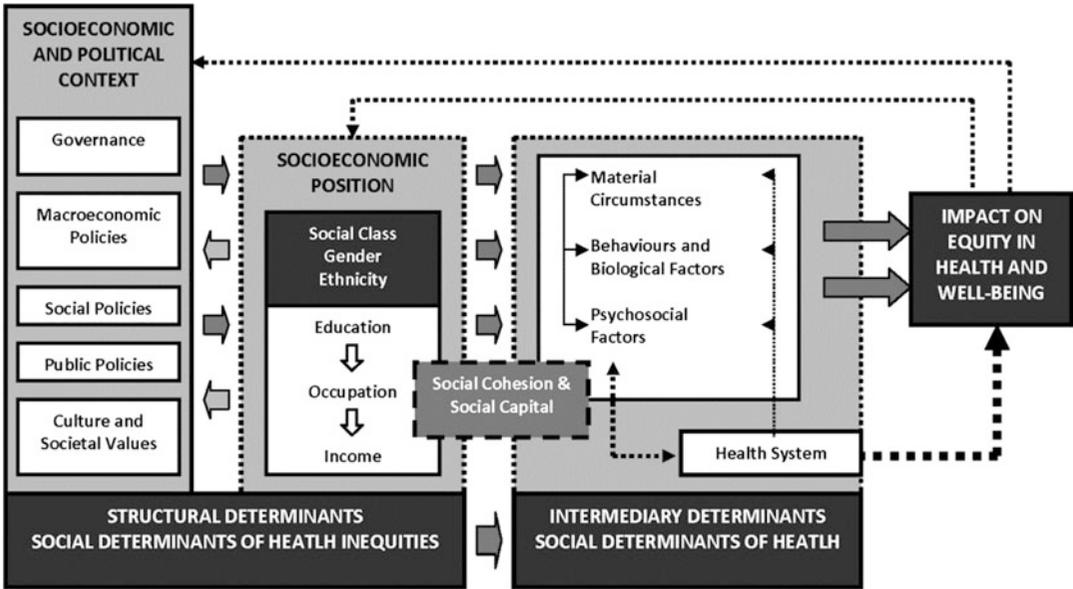
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Definition

Human behavior relates to HIV/AIDS risk as a sexually, pre-/postnatally, and blood-acquired virus that has been investigated since the recognition of the disease in 1981. The facets of sexual risk factors, mother-to-child transmission, drug injection and blood-borne risk, challenges in HIV prevention, medication adherence, and overall societal impact of HIV/AIDS have been characterized through thousands of observational and experimental studies. Existing scientific evidence details how aspects of poverty, political unrest, social exclusion, gender inequality, human rights violations, stigma, and other societal challenges influence human behavior.

Characteristics

The presence of HIV/AIDS in a community further exacerbates the effects of existing



Behavioral Science Highlights of Evidence and Research, Fig. 1 Conceptual framework for social determinants of health (developed by Solar and Irwin 2010 of WHO Commission on Social Determinants of Health)

- Health technology/equipment
- Neighborhood and built environment:
 - Housing quality
 - Violence and crime
 - Environmental conditions
 - Access to healthy foods

(Adapted from the HealthyPeople.gov website 2014).

The WHO has developed a conceptual framework for considering the interdigitation of social determinants of health (Fig. 1).

Sociodemographic Factors

Health inequities involve a lack of equality in health between groups of people within and between countries. Depending on the circumstances of economic and political stability in a region, these inequalities can arise at varying rates within and between societies. Social and economic conditions and their effects on a person's lives determine individual risk of illness and the actions required to prevent someone from becoming ill or treat illness when it occurs.

The following are examples of health inequities between countries:

- The infant mortality rate (the risk of a baby dying between birth and 1 year of age) is 2 per 1000 live births in Iceland and over 120 per 1000 live births in Mozambique.
- The lifetime risk of maternal death during or shortly after pregnancy is only 1 in 17,400 in Sweden, but it is 1 in 8 in Afghanistan.

The following are examples of health inequities within countries:

- In Bolivia, babies born to women with no education have infant mortality >100 per 1000 live births, while the infant mortality rate of babies born to mothers with at least secondary education is <40 per 1000.
- Life expectancy at birth among Indigenous Australians is substantially lower (59.4 for males and 64.8 for females) than that of non-Indigenous Australians (76.6 and 82.0, respectively).

(Adapted from the WHO website 2014).

Social Gradient

A correlation known as the social gradient exists globally whereby the world's poorest of the poor are believed to experience the worst health outcomes. A statistical measurement known as the Gini coefficient configures an inequality score ranging from zero (0), reflecting highest economic parity/equality in a region, to one (1), representing extreme inequality. From an HIV/AIDS perspective, many countries with the highest Gini coefficients are among those with the highest prevalence rates for HIV/AIDS. Efforts have been made to create a multi-dimensional poverty index that factors in a variety of indicators in an effort to measure the nuances of relative poverty and agency (Victor et al. 2014).

Women

Females comprise just over half of the estimated 36.7 million adults living with HIV in 2015. Evidence shows that the position of women and their role in a society is directly related to the health outcomes of children in that society (WHO Progress Report, 2016). Inequalities that may exist between men and women include access to education, income level, individual agency, and respect or hierarchy, which can manifest as gender-based violence and/or stigma. Despite these potential inequalities within a society, women tend to outweigh men in terms of health clinic attendance and have been proven to make more effective use of financial benefits or other patient rewards. Considering these factors, interventions often place emphasis on the female audience.

Many women bear the burden of juggling the role of both patient and caregiver for family members. Pregnancy and motherhood in women living with HIV has concentrated primarily on fetus health and the prevention of mother-to-child transmission. Currently, access to appropriate reproductive health services is less than optimal in most developing countries (Loutfy et al. 2013).

Youth

Roughly half of new HIV infections worldwide occur among young people aged 15–24 years; thus, behavior change effort geared toward this population group will be essential in fighting the pandemic. Research evidence illustrates how social and cultural factors influence the sexual behavior of youth. Seven key concepts or themes have emerged from the literature: young people assess potential sexual partners as either “clean” or “unclean”; sexual partners have an important influence on overall behavior; condoms can be stigmatizing and associated with lack of trust; gender stereotypes are a critical piece in determining social expectations and behavior; there are penalties and rewards for sex from a societal perspective; there is importance in reputations and social displays of sexual activity or inactivity; and social expectations can hamper communication about sex (Marston and King 2006). In addition, as HIV-positive adolescents mature into this age group, issues surrounding the disclosure of disease status become a critical area to navigate and address.

Male Engagement

One emerging area of focus for HIV-related interventions is the engagement of males. This includes advocacy for increased involvement in their partner's healthcare as well as education and awareness related to gender-based violence. Along these lines, evidence suggests that incorporation of male partners into prenatal care with expectant mothers improves health outcomes related to the prevention of mother-to-child transmission of HIV (Dunlap et al. 2014).

HIV Testing Coverage

In areas of highest HIV prevalence, such as sub-Saharan Africa, provision of healthcare and, specifically, HIV testing coverage and subsequent enrollment into care and treatment can be a challenge due to the rural areas of incidence. To help alleviate some of the barriers faced in resource-

limited settings, a multipronged approach is often taken toward HIV counseling and testing, incorporating voluntary counseling and testing centers and mobile campaigns in rural communities as well as provider-initiated HIV testing within health centers. Innovative strategies continue to be explored that make access to testing easier, including home visits for testing, kits for individuals to test themselves at home and education of traditional healers (Audet et al. 2014). Regardless of testing method, evidence has shown a need to tailor the testing approach/campaign toward the local cultural context.

HIV Knowledge

HIV knowledge within a population can have a strong impact on health outcomes related to HIV/AIDS. In addition to clinical outcomes, HIV knowledge and awareness can improve community support and reduce AIDS-related stigma. An HIV knowledge scale assessing a variety of key topics, such as modes and methods of transmission, has been validated in resource-limited settings and proven that low literacy and numeracy scores are related to low HIV knowledge scores (Ciampa et al. 2012). The validated scale is used to measure program and intervention effectiveness by taking pre- and post-measurements.

Clinics: Their Availability, Policies, and Effectiveness

Universal Antiretroviral Therapy (ART) Access The Alma Ata Declaration of 1978 declared the importance of primary healthcare for all citizens and set a goal of “health for all.” Decades later, in 2000, the United Nations issued a set of Millennium Development Goals (MDG), which included universal access to antiretroviral therapy for patients living with HIV by 2010 (Maddison and Schlech 2010). While this particular MDG was not met due to barriers in available

workforce, medication supply, and overall lack of resources, the push to make ART universally accessible continues. Research and interventions explore clinic capacity and accessibility as well as the politics and logistics surrounding HIV/AIDS prevention and support.

ART Expansion In many rural areas of resource-limited regions around the globe, expansion of ART services has been prioritized. Logistics of such expansion include human resource availability and capacity, drug supply chain structure, and capabilities to monitor and evaluate operations.

Task Shifting Due to the limited number of human resources for health in some areas, task shifting of responsibilities to a lower level cadre of health personnel has been incorporated into the care model for HIV/AIDS. This involves shared or trained responsibility for certain tasks related to HIV/AIDS care and treatment, such as the initiation of patients onto antiretroviral therapy being transferred from doctors to nurses. Evidence shows that under such circumstances, nurse provision of care and treatment is non-inferior to that of physicians (Fairall et al. 2012).

Option B+ A strategy referred to as option B+ offers lifelong antiretroviral treatment for all HIV-positive pregnant women and has been adopted by regions around the globe. Benefits of option B+ implementation include operational simplification, maternal health benefits, protection in future pregnancies, and reduction of sexual transmission. Implementation efforts report that the approach comes with challenges related to human and infrastructural resource availability, necessary training, strength in supply chain, monitoring, and evaluation. Behavioral issues loom large as women of childbearing age have no tradition of lifelong chemotherapy for a hidden disease that may not have even made them ill yet.

Mobile Clinics Mobile units outfitted for clinic purposes have been utilized in a variety of ways to offset challenges in HIV/AIDS care and treatment. These mobile clinics have been

incorporated for HIV counseling and testing and ART distribution as well as for provision of other health services such as family planning and cervical cancer screening. Models for operation include mobile clinics that constantly rotate among rural communities as well as those that compliment a resource-constrained facility, working alongside the existing structure to serve as mentors to the team while present.

Stigma and Discrimination

Research has shown that HIV/AIDS-related stigma can present itself in various forms such as community/familial ostracism, violence, loss of civil rights, and humiliation. Often this stigma is manifested in conjunction with other forms of stigma related to other at-risk populations (commercial sex workers, men who have sex with men (MSM), intravenous drug users (IDU), etc.). Evidence has revealed that perceived stigma can result in delayed initiation to HIV testing and/or care and treatment (Mukolo et al. 2013).

To combat such stigma, interventions have centered on community awareness and understanding of the modes of HIV transmission, treatment options, and dispelling existing myths about HIV/AIDS. While it is widely accepted that stigma is an integral factor that must be addressed in the fight against the disease, measuring the effectiveness of stigma-related interventions has proven challenging. Literature emphasizes the importance of incorporating a validated stigma scale when evaluating stigma-related interventions (Sengupta et al. 2011).

Discrimination against marginalized populations includes human rights violations such as homicide; physical and sexual violence from law enforcement, clients, and intimate partners; unlawful arrest and detention; discrimination when accessing health services; and forced HIV testing. Research stresses the importance of rights-based initiatives in the HIV response, which can prove challenging in low-resource settings rife with inequalities (Decker et al. 2015).

Health Communication

An increased awareness of the need for strong health communication strategies has influenced the engagement of communication experts to assist in program design for HIV/AIDS interventions. When biomedical and health communication approaches are strategically combined in evidence-based methods, interventions can have a stronger impact. Examples of the biomedical approaches to HIV control include treatment as prevention, voluntary medical male circumcision, preexposure prophylaxis, sterile needle exchange, opiate substitution therapy, and prevention of mother-to-child transmission (Vermund et al. 2014).

Outreach Programs for Enrollment and Retention

Without effective health communication, the success of biomedical interventions is limited. Often in the context of HIV/AIDS, it becomes difficult to retain all patients in care and/or treatment, and community level outreach is necessary. Evidence shows that barriers for patients include lack of transport, competing priorities such as employment, stigma, fear, and related costs of care and treatment. In an effort to combat these challenges, governments and nongovernmental and community-based organizations have identified various outreach interventions such as behavior change communication, community adherence groups, patient case finding, home visits, companion programs, patient reminders, and numerous other models being piloted.

Behavior Change Communication Mass media interventions are often incorporated into HIV/AIDS prevention to promote awareness or behavior change among an intended population through channels that reach a broad audience. These channels include radio, television, video, print, internet websites, and online social media, often taking the form of event announcements, dramas or plays, songs, interviews, music videos, instructional

films, brochures, billboards, posters, interactive websites, emails, text messages, or mobile phone applications. Research continues to explore audience response to such forms of communication as it relates to HIV/AIDS health behavior.

Community Adherence Groups Due to the strain of patient transport to health facilities on a monthly basis, it became necessary to explore options to limit the required visits related to HIV/AIDS treatment. A study led by *Médicins sans Frontières* in Tete Province of Mozambique explored a new model for distribution of antiretroviral treatment through self-forming groups of patients. Under this Community ART Group, patients who were stable on ART for 6 months were invited to form groups whereby members had four key responsibilities: facilitate monthly ART distribution to other group members, provide adherence and social support, monitor outcomes, and ensure that through the rotating clinic visits each group member undergoes a clinical consultation at least once every 6 months. Mortality and retention in care outcomes through this intervention were highly satisfactory, and Mozambique, as well as several other countries, has subsequently rolled out this mutual community support model nationally (Decroo et al. 2011).

Patient Case Finding/Home Visits Health facility monitoring and evaluation systems can produce lists of patients not attending scheduled visits or medication pickup, otherwise known as defaulting on treatment. Research continues to explore the range in time period for defining a patient as “lost to follow-up” from the health facility perspective, as this varies across countries and regions, and the optimal point in this range for seeking out these individuals (Shepherd et al. 2013). Teams of volunteers and/or health facility staff are frequently recruited to conduct community searches for patients lost to follow-up and encourage individuals to return to care. Often these cadres of community health workers conduct visits to patient homes to monitor status and offer support. In some regions, this community level access is utilized to provide health services at home such as testing, counseling, or medication provision.

Patient Reminders In some regions of the world, healthcare providers have explored the use of mobile phone technology and emails to remind patients of upcoming visits, missed appointments, schedule for taking medications, and messages for overall healthy living.

Microfinance/Conditional Cash Transfers In resource-limited settings, a lack of consistent income is often seen to be a primary limiting factor affecting patient health outcomes. For this reason, some community outreach efforts include the establishment of microfinance opportunities, income-generation projects among community groups, or conditional cash transfers, all in an attempt to alleviate the constraints of poverty on health. Evidence has shown that a comprehensive, tailored adherence intervention coupled with economic support is both feasible and effective among certain patients in some low-resource settings (Muñoz et al. 2011).

Conclusion

Behavioral risk is at the heart of the HIV/AIDS epidemic but is nested within gender-power imbalance, socioeconomic and cultural contexts, and stigma and discrimination. The powerful matrix complicates HIV prevention, diagnosis, care, and treatment.

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Blood and Blood Product Donors and Recipients in China, Epidemiology of HIV/AIDS

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Risk of HIV in Blood Recipients, and Rarely, in Blood Donors

While recipients of HIV-contaminated blood or blood products are well known to be at risk of HIV acquisition, China experienced an unexpected epidemic of HIV in blood/plasma donors in the early 1990s. To describe this event, we first present two definitions.

- Blood and blood products include (1) fresh whole blood or blood components, such as red blood cells (RBC), platelets, clinical fresh frozen plasma, cryoprecipitate, and cryodepleted plasma and (2) plasma-derivatives and recombinant products, such as albumin, immunoglobulins, and clotting factors.
- Blood transfusion is the process of receiving blood or blood products into one's circulation intravenously and is used for various medical

conditions to replace blood loss or deficiencies in certain blood components.

The use of blood and blood products can be lifesaving. Hence, blood and blood products are a vital resource derived from voluntary or paid donors, followed by processing and/or manufacture by public sector or commercial laboratories. The primary risk to blood recipients is the transmission of blood-borne diseases, including HIV and other viruses, bacteria, prions, and parasites. On rare occasions, donors may also have the risk of contracting these diseases through unsterile blood or plasma-collecting equipment or improper procedures such as the return of RBC to donors in ways that can mix them between patients or otherwise handle reinfusion in an unsterile fashion.

We review the practices and policy of blood donation and collection and the risk of HIV transmission to blood donors and recipients in China, including the historical event of HIV spread among paid plasma donors in rural provinces in central China during the early 1990s.

Blood Transfusion and Donation in China

Blood Shortages

Shortages of blood and blood products for medical use have been a chronic problem worldwide, particularly in low- and middle-income countries. Statistics from the World Health Organization (WHO) in 2010 showed that 50% of the all blood donations are collected in low- and middle-income countries, which are the home to 84% of the world's population. In the world's most populous country, China, blood and blood product shortages have become even more severe since its economy started to grow in the early 1980s and the demand for blood outstripped supply due to increased job- and transportation-related injuries, an aging population, and a rapidly rising epidemic of chronic diseases.

In general, people in China are reluctant to donate blood, as it is believed that losing blood is equivalent to losing one's bodily vigor or "Yuan

Qi," and therefore, the loss of blood is harmful to health. Only about 0.9% of the Chinese population donates blood in a given year. However, according to the WHO estimate, at least 1–3% of the population should donate blood to meet a nation's basic demand for clinical blood. In addition, more than 90% of whole blood donations collected in China are only 200 ml in volume, in contrast with the Western tradition of 400–500 ml per donation (Shan et al. 2002). Endemic hepatitis also limits voluntary blood donation from a large proportion of the general population, as blood is discarded when screened if found to be positive for hepatitis B surface antigen (HBsAg) or hepatitis C antibody (anti-HCV) (Qian et al. 2005).

Blood Collection and Donation

The policies and system for blood collection and donation in China have evolved through three major phases since China adopted a market economy in the early 1980s (Shi et al. 2014).

The first phase (Phase I) was from the early 1980s to the middle 1990s. The supply of blood and blood products relied primarily on paid or commercial donation, and the recruitment efforts for voluntary blood donation were minimal. For example, only 11% of blood for clinical use nationwide was collected from volunteer donors in 1996. Poorer residents, especially from rural areas, were motivated by monetary reimbursement in exchange for the donation. Most hospitals had a relatively stable team of donors who could sell blood to the hospitals when blood was needed for a surgery or other types of medical care. From 1991–1995, a large-scale collection of plasma using unsafe practices caused the spread of HIV, hepatitis, and other blood-borne diseases among many farmers who were blood donors in the rural areas of Henan Province and surrounding provinces in Central China (Shan et al. 2002; Qian et al. 2005, 2006).

The second phase (Phase II) began in 1998 when China enacted the "Law of Blood Donation." Large-scale HIV and hepatitis outbreaks among paid blood and plasma donors in the early 1990s (discussed below) led the central government of China to increase regulations on the safety of blood and blood products. The new

blood donation law became effective nationwide, banning commercial blood collection, encouraging voluntary donation, and requiring HIV screening for all blood donations. The commercial blood stations were closed, and the screening of blood collection was more strictly enforced. However, voluntary donation was still insufficient to meet national needs during this phase. In order to fill the gap of blood supply from the commercial sources in Phase I, blood collection relied on near-compulsory, employer-organized donation. State-run work units were assigned donation quotas, and employees were required to donate with minimal compensation (Shi et al. 2014). Recruitment efforts for blood donors were mainly targeted at public sector employees, college students, and military personnel. Although routine serologic screening of donors was implemented in employer-organized donation, employer-organized donors are not considered as the safest source of blood due to the wide use of employer incentives and coercion in some cases (Shi et al. 2014).

The third phase (Phase III) started in 2004 when the Chinese Ministry of Health mandated employer-organized donations to be discontinued within 3–5 years. Since then, China's blood donation system has transitioned to voluntary, non-remunerated donation. During this phase, blood centers have been actively engaged in donor mobilization and recruitment targeted to the general population. By 2008, most cities had already achieved the goal of meeting their regional blood needs with volunteer donations.

HIV Outbreak Among Paid Plasma Donors in the Early 1990s

From the early 1980s to the middle 1990s, China's blood donation system was largely driven by commercial interests, coinciding with the profound social and economic transition from a planned economy to a market economy. Though attempts were made in the 1980s to move to a voluntary system, these efforts were mostly unsuccessful due to reluctance in giving blood among Chinese people. In the early 1990s, China restricted the import of blood products due to concern about bringing in HIV from foreign countries, while

the demand for blood and blood products for advanced medical treatments had become ever bigger due to the rapid economic expansion and a growing population in China. Collection of blood, and especially blood plasma, became a lucrative business for blood traders or "bloodheads," who were businessmen or local government employees. These blood traders set up thousands of official and unofficial plasmapheresis stations in counties, townships, and villages in Henan Province and its neighboring provinces in Central China. In Henan Province, even local health officials promoted plasma collection activities through the "Plasma Economy" campaign, as selling blood and plasma was an easy way for poor farmers to augment their income (Shan et al. 2002; Wu et al. 2001). The average monthly income from farming was \$9–12 (USD) at that time, while a donor would receive 50 Chinese yuan (approximate \$6 (USD)) for each donation of 400 mL plasma and 200 yuan (approximate \$25 (USD)) for each donation of whole blood (Wu et al. 2001). Plasma donation was much more popular than whole blood donation because of commercial demand for plasma and a greater willingness of donors. A donor might make more money from selling plasma than selling whole blood, as a donor could sell plasma more frequently when anemia was reduced with re-infusion of pooled, same blood type RBC. According to the national regulation for plasma and blood donations, the shortest interval allowed between each donation was 15 days for plasma and 3 months for whole blood. However, this regulation was not adhered to by many stations. In addition, donors might use fake identification to sell plasma more frequently in different stations.

The blood/plasma collection practices in these commercial plasmapheresis stations generally had low health and safety standards and lacked proper sterilization procedures. Needles, blood bags, and other equipment in contact with blood were often recycled and reused. A physical examination and testing for HBsAg and anti-HCV were required for each donation according to national guidelines, but they were often omitted. HIV screening had also been required by law in large cities since

1993, but it was not performed at any of the local plasma collection stations, both for the purpose of reducing operational costs and because they believed they did not have an HIV problem.

The typical plasma collection procedures were: peripheral blood was drawn from several donors at the same time; donations with the same blood type were mixed together in the same centrifuge and the plasma was extracted; and the remaining blood constituents, such as RBC, were re-infused with normal saline solution to the donors in order to reduce anemia. In this fashion, even a single HIV-infected person who donated blood plasma could result in the infection of dozens of others, either through direct infection by mixed RBC that were reinfused to many or via contamination of equipment. Such RBC reinfusion procedures without screening tests, along with the reuse of needles and unsterilized equipment, became a very efficient way to spread HIV and other blood-borne diseases among local donors.

HIV infections among plasma donors were initially reported by local physicians, and the central government then sent a team to investigate and recognize the severity of HIV epidemic. The estimated number of total HIV infections among paid plasma donors from the government source was around 55,000 (Lu et al. 2006), while some AIDS activists estimate the number to be much higher.

From about 1995, the spread of HIV among commercial plasma donors began to be widely recognized. The central Chinese government took strong legal and police action to prevent further spread of HIV infection by closing commercial plasma collection stations and issuing new regulations for blood/plasma donation. By 1996, it was believed that all of the illegal, unregulated stations were closed.

Several lessons are learned from this public health tragedy. Failure to follow the regulations on blood and plasma donation was the major reason for the outbreak of HIV and other blood-borne diseases among donors. Pooling blood and reinfusing RBC to multiple donors is a prohibited plasma collection practice that can pass on any number of dangerous pathogens, yet this was common in these commercial stations. Equipment

was not sterilized. Testing for hepatitis B and C was not performed to save time and cost or donors avoided screening by bribing blood station staff. Donors had no knowledge of safe blood donation practices and had a low awareness of HIV risk. A decade prior to this HIV outbreak, the spread of hepatitis through unscreened blood collection had been reported as early as 1985 (Meng 1990). Unfortunately, this evidence did not become an alert among local health officials and bloodheads for avoiding unhygienic plasma collection and HIV outbreak in the early 1990s.

HIV Prevalence Among Donors and Secondary Transmission in Former Donating Communities

Since the Chinese government banned all commercial blood/plasma collection practices, HIV transmission through plasma collection has been under control in rural regions. However, secondary transmission from infected former donors to their spouses and children continued to be a public health concern. HIV cases among spouses and children had been reported by the mass media and were confirmed by the national HIV reporting and surveillance data. Until the early 2000s, very few community-based studies in the former plasma collection villages were conducted to evaluate HIV prevalence among former donors and the residents in these villages and assess the risk of secondary transmission.

A retrospective study was conducted during 2005–2009 among 420 HIV-infected former commercial plasma donors or recipients of contaminated blood, whose spouses denied any high-risk behaviors. The median time of potential HIV transmission between these spouses was 11.2 years, the spousal transmission rate of HIV was two per 100 person-years (2%), and rates were slightly higher for female-to-male (1.8%) than for male-to-female (2.1%) (Yang et al. 2010).

A census was conducted in 2004 among all villagers aged 18 to 64 years in four former plasma-donating villages in Shanxi Province that bordered Henan Province. Of 3,062 participants, 29.5% ($n = 904$) had a history of selling plasma or blood, 12.9% reported more than one lifetime sexual partner, and 6.4% had ever had

extramarital sex. HIV infection was detected in 1.3% ($n = 40$) villagers, including 4.1% ($n = 37$) detections among former plasma/blood donors and 0.1% ($n = 3$) detections among non-donors (Qian et al. 2006). Illicit drug use was rare in this rural population of farm families. The three infections detected in non-plasma donors were likely due to sexual transmission, though other routes such as blood transfusion or other blood contacts could not be excluded. Another census conducted in 2005 among villagers aged 25–55 years in 40 villages in Anhui Province, bordered with Henan Province, suggested a higher likelihood of sexual transmission; HIV prevalence was 10.8% among all villagers and 4.8% among non-donors (Ji et al. 2006).

A survey conducted in 2005 among villagers aged 2–79 years in Henan Province found that 36.3% (466/1285) villagers ever sold plasma/blood, and 15.3% (197/1285) were infected with HIV. In 197 HIV cases, 2.0% ($n = 4$) were regarded as sexual transmission from an infected plasma donor, 3.6% ($n = 7$) through mother-to-child transmission (MTCT) with the mothers having been former plasma donors, 0.5% ($n = 1$) through receipt of an unscreened transfusion, and 0.5% ($n = 1$) through nosocomial infection (Zhang et al. 2006).

HIV prevalence among donors and community members might be underestimated, as these surveys were conducted about a decade after the outbreak of the disease in the early 1990s. Some HIV-infected former donors may have died. In these censuses, all villagers' names were obtained from household registration and missing villagers could be identified; thus the likelihood of choosing not to participate due to stigma and concern about discrimination was small. The risk of secondary sexual transmission between discordant couples seemed to be at a low or moderate level. One possible reason is that it was common that both couples sold plasma or blood and as a result might both be infected via the unsafe donation procedures.

Current Blood Donation and HIV Risk

Following a few years of transition from employer-organized donation, China's blood

donation system changed to voluntary donation in the early 2010s. In 2011, the country's total whole blood collection volume was 4,164 tons (or 12.3 million whole blood donations), and 99% was from voluntary non-remunerated donors (Li et al. 2012). There were 452 blood establishments nationwide, including 32 provincial blood centers, 321 regional blood centers/stations, and 99 county blood stations. Blood centers/stations are the only authorized establishments to collect blood from voluntary donors, supply blood to hospitals for civilian clinical use, and charge fees from hospitals for covering the costs of blood screening and storage. However, some hospitals are still short of blood supply, particularly those in rural areas. A significant proportion of the blood supply is still dependent on family/replacement donors.

Although the safety of blood sources has been greatly improved in China, blood transfusion remains an ongoing risk factor for the spread of blood-borne infections. A survey among 4,366,283 donations collected from 2000 to 2010 in four Chinese regional blood centers in Guangzhou, Nanjing, Shenyang, and Yancheng found that the overall prevalence of HIV was 0.08%, HBsAg 0.86%, anti-HCV 0.51%, and syphilis (two rounds of enzyme immunoassay) 0.47% (Li et al. 2012). A meta-analytic review of 87 studies that reported HIV infection rates in voluntary blood donors from 2000–2009 showed that the pooled prevalence was 13.2/100,000 (range 0.74–126/100,000) and increased from 5.62/100,000 in 2000 to 28.9/100,000 in 2009 (Hong et al. 2012), which coincided with the rising HIV epidemics in both the general population and high-risk groups in China. HIV infections from blood transfusions were also reported by mass media. In comparison, global data in 2014 from the WHO showed the median prevalence of HIV infection in blood donations of only 0.002% (interquartile range: 0.0004–0.02%) in high-income countries, 0.12% (0.03–0.2%) in middle-income countries, and 0.85% (0.48–2.0%) in low-income countries (WHO 2014).

There are several major challenges for achieving a safe and adequate blood supply in China, including cultural and social barriers to blood

donation, lack of high-quality donor service and care, conservative selection criteria which eliminates many otherwise eligible donors, lack of a national/regional blood distribution mechanism for coordinating cross-region blood sharing, and lack of a national/regional blood donation information system for following up with blood donors to encourage repeat donation. According to the Law of Blood Donation, the central health department of China is responsible for enacting national standards and policies, while the public health bureaus at provincial, city, and county levels manage the day-to-day operations and planning for blood donation and mobilize and recruit blood donors by partnership with local blood centers/stations.

To mitigate the chronic blood shortage and reduce the risk of blood-transmissible diseases, both national policies and local operations may need to be modified. The following four strategies may be taken:

- (1) Reducing demand for blood and blood products that is not indicated clinically. Unnecessary blood transfusions and injections are too common in many low- and middle-income countries. Blood demand can be curbed through application of newer surgical methods, such as laparoscopic techniques that involve tiny incisions and little blood loss. Additionally, hospitals may implement more sophisticated blood management programs to reduce blood demand, such as using medications to reduce bleeding during surgery.
- (2) Increasing blood collection. Current donors are predominantly soldiers and college students who are living in urban areas. The voluntary blood drive campaigns should reach rural residents, as well as a larger proportion of urban residents. These campaigns may use psychological theory and evidence-based approaches to utilize positive factors such as altruism, support from family members, and role modeling by government leaders or movie stars. They may also minimize the influence of negative factors such as fear of losing blood, suspicion about blood centers' profiting from selling blood from volunteers to hospitals, and short-term donor deferral (e.g., sore throat). Lack of mobile vans limits blood drive campaigns, particularly for rural regions where blood shortage is more severe. The criteria for voluntary donation could also be changed. The upper limit of eligible age increased from 55 to 60 years in 2012, which is still lower than the limit of 65 or 69 years in other Eastern Asian countries or regions such as Japan, South Korea, and Taiwan. Currently, about 70% of donors are young adults aged 18–35 years, and older adults should be encouraged to donate blood. There is also a need to implement strategies to retain donors and increase collection from repeat donors who regularly donate blood.
- (3) Improving blood use. A national/regional coordinating mechanism should be established to improve distribution and sharing of blood and blood products across regions. A real-time communication for blood collection and supplies across blood centers/stations should be available so that surplus blood can be redistributed to the needy places in a timely manner.
- (4) Ensuring blood safety. All blood donations should be screened following the national guidelines. Health care providers should also ensure a safe transfusion practice and have a quality systems check throughout the blood transfusion process.

Conclusions

The unhygienic plasma collection practices in the early 1990s caused hundreds of thousands of HIV infections among rural residents in the poorer regions of Henan and its surrounding provinces in Central China. This led to the transition of a Chinese blood donation system from commercial donation from the 1980s to the middle 1990s, to employer-organized donation during 1998 and 2004, and to voluntary donation since 2004. Now, almost all blood supply comes from voluntary donors. However, more efforts are needed to

increase voluntary donation, to meet blood demand and to ensure the safety of blood transfusion. Furthermore, the Chinese experience provides cautionary guidance to other nations that struggle to implement universally safe blood donation and blood banking programs.

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Budding

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Definition

Budding is the process of lipid envelopment of viral particles by the cell membrane during exit from infected cells, a strategy that allows the virus to bypass the need for cell lysis to release newly formed virions and spread infection. As HIV-1 assembly progresses at the plasma membrane, viral capsids form on the inner leaflet of the cell surface and become gradually surrounded by the plasma membrane. In late steps of budding, the fully assembled immature capsids are completely surrounded by the membrane but remain connected to the cell by a membranous stalk. The ultimate separation of nascent virions from their progenitor cells involves host proteins of the endosomal sorting complex required for transport (ESCRT) pathway, a cell membrane fission machinery that will place the final cut and release the newly made virions.

Introduction

HIV-1 budding requires nascent virions to traverse the plasma membrane and acquire their envelope, a process that begins with the enfoldment of virus particles with the cell membrane and ends with the separation of progeny virions from cells to spread infection. Budding was originally thought to occur spontaneously driven by the outward egress of an “assembling” viral particle at the plasma membrane. In 1991, the removal of a short domain at the end of Gag, the viral structural protein responsible for virus particle formation, brought virus production to a halt and led to a remarkable accumulation of fully formed virus particles at the plasma membrane

(Gottlinger et al. 1991). These particles' failure to sever away from the cell surface revealed that specific regions in the Gag protein mediate virus release from the cell and identified budding as a distinct step in HIV-1 production. Further experiments led to the identification of short motifs responsible for mediating virus budding from the cell. Owing to their role in post-assembly events, these sequences were named late or L domains. Newly identified L domains became the focus of intense efforts to identify cellular factors involved and decipher mechanisms of function. A decade ago, members of the endosomal sorting complex required for transport (ESCRT) pathway, the host cell machinery required for membrane fission, were found to bind HIV-1 L domain sequences (Verplank et al. 2001; Garrus et al. 2001). These seminal findings provided key information on HIV-1 biology and signaled that release of an enveloped virus may require extensive assistance from the cell. Identification of these proteins was just the "tip of the iceberg" and subsequent work revealed that the cell membrane fission machinery is constituted of a network of multiprotein complexes that is evolutionarily conserved from Archaea to humans. An overview of HIV-1 budding mechanisms, host factors involved, and standing questions in the field are outlined below.

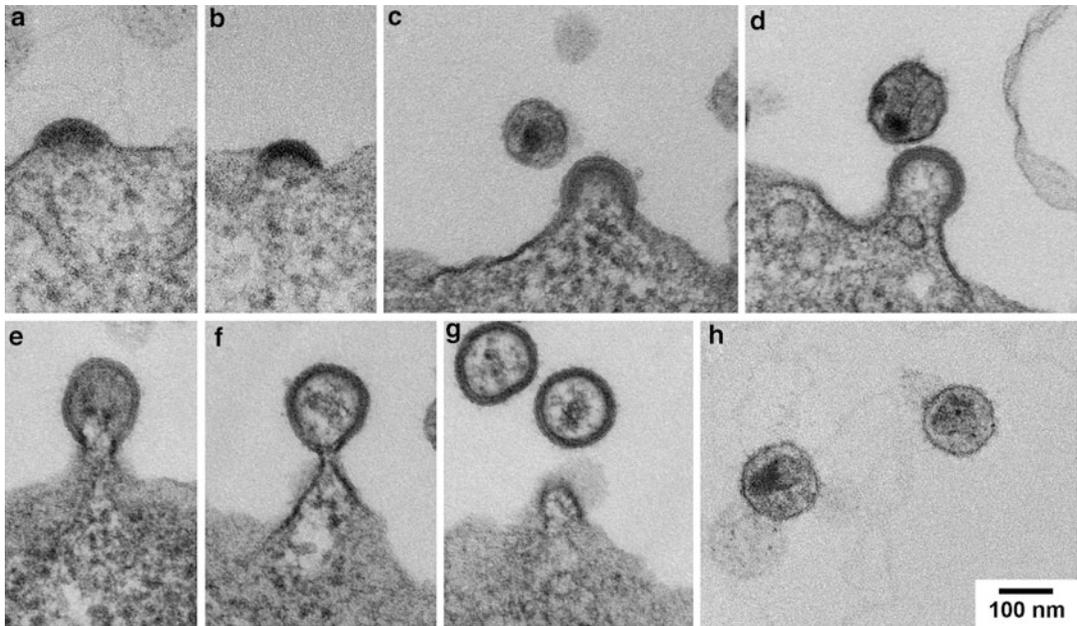
HIV-1 Particle Morphogenesis

HIV-1 must cross the plasma membrane in late steps of virus egress from cells to acquire its envelope. The major component that orchestrates virus particle assembly (► [Virus Assembly](#)), budding, and exit is the structural polyprotein Gag (Sundquist and Krausslich 2012). Gag is sufficient for the formation of noninfectious virus-like particles (VLPs).

Gag carries three domains: matrix (MA), capsid (CA), and the nucleocapsid (NC). The N-terminal end carries the MA domain, which anchors Gag molecules at the plasma membrane. Such interactions require a bipartite signal composed of a co-translationally acquired myristic

acid and a cluster of positively charged residues believed to engage in interactions with the negatively charged phospholipids at the membrane. MA is also responsible for the incorporation of the viral envelope glycoprotein in nascent virions. CA is endowed with Gag-Gag multimerization function responsible for the formation of large Gag complexes and assembly of nascent virus particles. NC serves primarily as a platform for the incorporation of HIV-1 genomic RNA to the assembling viral particle. HIV-1 harbors a fourth domain called p6, which is located in the C-terminal end of Gag. Not required for assembly, p6 mediates virus separation from infected cells, since its disruption leads to the accumulation of virus budding arrest on the cell surface. This domain is found in other primate lentiviruses where it appears to play similar roles.

Upon synthesis of Gag and Gag-Pol – a product of infrequent frameshifts that generate a Gag polyprotein in fusion to the *pol* gene, which encodes for the viral enzymes protease, reverse transcriptase (► [Reverse Transcription](#)), and integrase (► [Integration](#)) – viral assembly is believed to begin in the cytoplasm. These small assemblages are however invisible by electron microscopy and can only be isolated from cellular extracts using biochemical assays. Further multimerization of Gag on the inner leaflet of the plasma membrane leads to the appearance of a crescent shape electron-dense structure (Fig. 1a, b). Upon recruitment of additional Gag molecules, the latter evolves into a sphere-shaped particle, which becomes enveloped by the cellular membrane by budding outward away from the cytoplasm (Fig. 1c, d). Budding particles remain attached to the surface with elongated membranous stalks until complete viral release (Fig. 1e–g). Soon after or during separation from the cell, the Gag proteins that are part of the spherical particles are cleaved by the viral protease, inducing them to collapse into a central conical core (Virus Structure) in the newly released virions (Fig. 1h). This process is called maturation (► [Maturation](#)) and is central to the production of infectious virions.



Budding, Fig. 1 Steps of HIV-1 budding. Electron micrographs showing HIV-1 budding from human T cells in a chronological order. (a, b) The first visible budding structures are characterized by an electron-dense layer of Gag proteins that start to accumulate beneath the plasma membrane. (c, d) Upon addition and extensive multimerization of new Gag molecules, HIV-1 assembles its immature spherical shell that induces membrane curvature

and becomes surrounded by the plasma membrane. (e, f, g) In late stages of budding, thinning of the membrane connection between the nascent virion and the cell will ultimately lead to membrane fission that releases the new particle. (h) Soon after release, Gag is processed by the viral protease during a maturation step that leads to the formation of a central conical core, a hallmark of infectious HIV-1 virions

Late Domains and Their Interacting Host Cell Proteins

Soon after the identification of p6 as the region of HIV-1 Gag that drives virus separation from cells, virus exit determinants were further mapped to short motifs in p6 that are conserved among divergent retroviruses. As they function in late steps of particle morphogenesis, these sequences were named late or L domain (Wills et al. 1994). In HIV-1 Gag, a proline-rich motif carrying the PTAP or PSAP sequence (named PTAP hereafter) was the first to be described and mutational analysis revealed its important role in virus separation from cells (Huang et al. 1995). The PTAP motif is highly conserved in primate lentiviruses and is also found in viral proteins of other non-related enveloped viruses such as Ebola. Interestingly, the

equine infectious anemia virus (EIAV) lentivirus harbors a p9 domain in the same location as p6 but does not contain a PTAP motif. Instead, virus budding is supported by a LYPXnL-type L domain. Further mutational analysis in the p6 domain unveiled the existence of an equivalent L domain (LYPXnL) in this region of HIV-1 Gag, which functions alongside the PTAP motif to drive a secondary budding pathway (Strack et al. 2003). It became apparent that, in contrast to PTAP, the LYPXnL consensus motif exhibits greater sequence divergence that made its identification difficult. Three different types have been characterized: the type 1 LYPDL in EIAV, the type 2 LYPLASL in HIV-1, and a more divergent type 3 PYKEVTEDLLHLNSLF only found in some simian immunodeficiency viruses. Nearly all primate lentiviruses carry at least one of the LYPXnL

motifs, suggesting that its presence confers an advantage to the virus. A third type of L domain, not found in HIV-1 Gag, has been identified in the Rous sarcoma virus (RSV). Its core sequence was subsequently mapped using mutational studies to the proline-rich minimal motif PPXY (Wills et al. 1994). Regardless of their sequence, disruption of L domain integrity halted virus release and led to the accumulation of budding particles, emphasizing their important role in mediating virus detachment from cells.

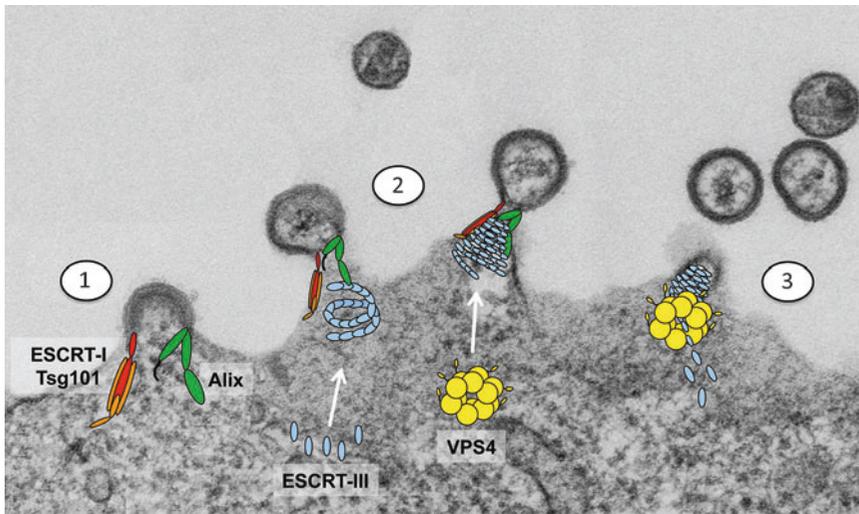
Virion release can also be restricted in certain cell types for HIV-1 mutants lacking the accessory gene *vpu*. This is due to the activity of tetherin/BST-2, a host cell factor believed to inhibit virus release by physically tethering virions to producer cells (Neil et al. 2008). Tetherin-mediated block of virus production is distinct from that resulting from interference with L domains. Whereas in the latter case, particles remain attached to the cell surface with elongated membranous stalks (indicating failure to detach) and appear immature, virions trapped by Tetherin/BST-2 exhibit a mature phenotype indistinguishable from released virions and remain tied to the cell surface and to each other with a thin proteinous tether further distinguishing this phenotype from deletion or disruption of L domain activity.

Clues that L domain motifs are docking site for host cell factors came from the observation that they can functionally replace one another in heterologous contexts. Indeed, RSV PPXY and HIV PTAP L domain motifs were shown to be functionally interchangeable (Parent et al. 1995). Groundbreaking studies led to the identification of the PTAP interacting factor as the tumor suppressor gene 101 or Tsg101 (Carter 2002). The LYPXnL L domain was found to bind Alix (Strack et al. 2003), whereas the RSV PPPY L domain interacts with members of the cell Nedd4 ubiquitin ligase family (Kikonyogo et al. 2001). Further seminal studies linked L domain-binding proteins to the ESCRT pathway, a set of cellular protein complexes involved in membrane fission. Tsg101 was later identified as an integral component of the ESCRT pathway, whereas Alix and Nedd4 are ESCRT-associated proteins that provide two additional entry points in the

pathway. Efforts to understand the role of these cellular factors in facilitating virus exit produced one of the best studied examples of how human viruses hijack host proteins to produce progeny virus and spread infection.

The ESCRT Pathway

The discovery of the interaction between the PTAP L domain and Tsg101 revealed that HIV-1 utilizes host cell proteins from the ESCRT pathway to catalyze scission of the newly made virions from the plasma membrane of the producer cell. The ESCRT pathway comprises four sets of multi-protein complexes (called ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III) in addition to the VPS4 complex and associated proteins, like Alix. ESCRT proteins were first identified in yeast where they are involved in the formation of late endosomal structures called the multivesicular bodies (MVBs), an organelle that sorts protein cargoes into the vacuole or lysosome for degradation (Henne et al. 2011). Cargoes destined for degradation are tagged with ubiquitin (see below) and as such recognized by the ESCRT-0 complex that initiates the sequential recruitment of the other ESCRT complexes on the endosomal membrane. Assembly of ESCRT-I and ESCRT-II complexes leads to the invagination of the cargoes into a vesicle that buds inside the endosome or vacuole following the action of the ESCRT-III complex. The latter catalyzes the membrane fission reaction required to release the vesicle into the endosome lumen (Hurley and Hanson 2010). The VPS4 ATPase complex comes last to disassemble the polymerized ESCRT-III proteins and recycle them for the next round of cargo sorting. Budding of HIV-1 at the plasma membrane of human cells is topologically equivalent to the biogenesis of intraluminal vesicles in MVBs as both exhibit invagination of a cellular membrane away from the cytoplasm and require the participation of a membrane fission machinery (ESCRT) acting from the cytoplasmic face of the budding neck to drive the membrane fission that frees vesicle (or the HIV-1 particle) in the luminal (or extracellular) compartment. In mammalian



Budding, Fig. 2 Schematic model of how proteins of the ESCRT pathway might drive HIV-1 budding. (1) The L domain-interacting Tsg101/ESCRT-I (in red and orange) and Alix (in green) factors are recruited in early stages of budding during the assembly of the immature viral particle. (2) When assembly is complete, ESCRT-I and Alix are believed to serve as a platform for the recruitment and the formation of the ESCRT-III filaments (in light blue). The

ESCRT-III CHMP2 and CHMP4 proteins assemble in a membrane-bound helical structure within the budding neck, driving its constriction. (3) The combined action of the ESCRT-III and VPS4 complexes (in yellow) triggers membrane fission and release of the nascent virion. VPS4 recycles the ESCRT-III proteins back as monomers that can be used in the next budding event

cells, proteins of the ESCRT pathway have an expanded repertoire of functions: they catalyze membrane fission in vesicle formation, abscission step that separates two daughter cells at the end of mitosis (Caballe and Martin-Serrano 2011), sealing of the nuclear envelope (Olmos et al. 2015; Vietri et al. 2015); assembly of nuclear pore complex (Webster et al. 2014) Viral budding from nuclear envelope (Lee et al. 2012); wound repair (Jimenez et al. 2014) exosome secretion (Baietti et al. 2012), and autophagy, a conserved mechanism for degradation of cytoplasmic components (Rusten and Stenmark 2009).

ESCRT-I

HIV-1 Gag recruits components of the ESCRT pathway primarily via the PTAP L domain, a binding site for the ESCRT-I subunit Tsg101 (Fig. 2). Interestingly, the ESCRT-0 component HRS also uses a PTAP (or PSAP) motif to recruit ESCRT-I during MVB biogenesis. HIV-1 Gag protein has evolved to mimic this cellular

interaction to hijack ESCRT-I from its normal cellular function in the ESCRT pathway. Consequently, the ESCRT-0 complex is not required for HIV-1 budding. Mammalian ESCRT-I is a heterotetrameric complex composed of a single copy of Tsg101, VPS28, VPS37, and MVB12 proteins assembled in a 1:1:1:1 stoichiometry. There is only one version of Tsg101 and VPS28 proteins in humans, but four isoforms of VPS37 (VPS37A–D) and three MVB12-like subunits (MVB12A–B and UBAP1) are present, giving rise to 12 possible ESCRT-I complexes. It is likely that the VPS37 and MVB12 isoforms confer specificities to ESCRT-I complexes in both interaction and function. HIV-1 has been shown to utilize VPS37B or C and any of the MVB12 isoforms. Structural studies have revealed that the ESCRT-I complex adopts an asymmetric conformation made of a headpiece attached to an extended rigid stalk with two flexible tethers on each side of the stalk. These two flexible regions are, on one side, the N-terminal UEV domain of Tsg101 that binds the PTAP L domain and, on the other, the C-terminal domain of VPS28 that interacts with

the downstream ESCRT-II, in MVB biogenesis, and possibly ESCRT-III complexes, at least in yeast (Votteler and Sundquist 2013). The requirement for ESCRT-I in HIV-1 budding first became apparent following the identification of Tsg101 as a binding partner of the PTAP L domain. Subsequent studies using RNAi knockdown experiments confirmed that depletion of cellular Tsg101 was highly detrimental to HIV-1 budding, leading to an accumulation of budding particles unable to detach from the cell plasma membrane. The same budding defects were observed upon mutation of the PTAP L domain in Gag or overexpression of the PTAP-binding N-terminal domain of Tsg101 that competes out the recruitment of the whole ESCRT-I complex (Carter 2002; Freed 2015).

ESCRT-II

The human ESCRT-II complex is a Y-shaped heterotetramer that consists of EAP30, EAP45, and two copies of EAP20. In yeast, where it has been extensively characterized, ESCRT-II is essential in linking ESCRT-I to ESCRT-III and nucleates the polymerization of the membrane-severing ESCRT-III proteins during multivesicular bodies biogenesis. In mammalian cells, knockdown studies revealed that ESCRT-II might not bear the same pivotal role than its yeast counterpart since it was shown that sorting of some endosomal cargoes could proceed independently of ESCRT-II. Similarly, HIV-1 budding was found to be insensitive to ESCRT-II depletion, raising the possibility that ESCRT-III recruitment could occur independently of ESCRT-II in humans, at least in some cases. The latter is also true in cytokinesis where a role for ESCRT-II in the separation of the two daughter cells has not been reported. Instead, the ESCRT-associated protein Alix (see below) is believed to recruit and nucleate the ESCRT-III complex through a direct interaction with CHMP4, a component of ESCRT-III. Interestingly, Alix is also recruited by HIV-1 Gag to sites of viral budding by interacting with the LYPXnL L domain. However, mutating this L domain or depleting Alix has only a mild effect

on HIV-1 budding in model cells, suggesting that HIV-1 Gag possesses yet another way (or multiple redundant ways) of recruiting ESCRT-III in the absence of ESCRT-II or Alix.

ESCRT-III

Unlike the other ESCRT complexes, ESCRT-III is not present as a stable complex in the cell but, rather, is recruited by the upstream complexes late in the pathway and assembles at sites where its membrane fission activity is required (Fig. 2). Humans encode 12 different ESCRT-III proteins, also known as the charged multivesicular proteins (CHMP), which are CHMP1A–B, CHMP2A–B, CHMP3, CHMP4A–C, CHMP5, CHMP6, CHMP7, and IST1. Among them, CHMP2A–B, CHMP3, CHMP4A–C, and CHMP6 form the core component of the membrane fission machinery. ESCRT-III core proteins are very similar in size and structure with an N-terminal basic and a C-terminal acidic domain. In the cytoplasm, CHMP proteins adopt an inhibited monomeric soluble form where the N-terminal domain folds over the C-terminal end. Upon activation following recruitment to budding sites, ESCRT-III proteins associate with membrane and polymerize extensively into helical filaments (Cashikar et al. 2014). In MVB biogenesis, CHMP6 is believed to act as a “nucleator” of ESCRT-III assembly owing to its membrane-binding ability. In turn, it recruits CHMP4B, the main component of the ESCRT-III filament. CHMP4 filaments are controlled by CHMP3 and capped by one of the CHMP2 isoforms that contained a binding site for the VPS4 ATPase that comes at the end of the pathway to disassemble the ESCRT-III polymers. Based on a study where depletion of individual ESCRT-III subunits was systematically analyzed, this model does not seem to hold true for HIV-1 budding. Instead, HIV-1 was found to rely on a surprisingly small subset of ESCRT-III proteins, with only CHMP2 and CHMP4 isoforms playing key functional roles in viral release. This could be explained by a direct interaction between CHMP2A and CHMP4B that is unique to humans. Although probably not essential,

CHMP3 was shown *in vitro* to synergize the assembly of CHMP2A on helical CHMP4 filaments. One of the major unanswered questions remains how CHMP4 is recruited to sites of viral budding, given that its known binding partners, CHMP6 and Alix, are not essential for HIV release. How ESCRT-III drives constriction of membrane at the budding neck is not fully understood either but an attractive computational model was put forward based on electron tomography imaging of *in vitro* assembly of ESCRT-III polymers. This model proposed that ESCRT-III proteins would assemble as a hemispherical dome-like structure within the neck of the membrane bud (Fabrikant et al. 2009). The dome formation, accompanied by the membrane attachment to the dome surface, would generate sufficient energy to drive the narrowing of the membrane neck up to the point where the two membranes are close enough for spontaneous fission to occur. This model is in agreement with the large body of evidence gathered from both *in vitro* and *in vivo* studies and is likely to be conserved in all ESCRT-mediated membrane fission events such as MVB biogenesis and the abscission step that separates two daughter cells at the end of mitosis (Guizetti and Gerlich 2012).

VPS4

The first evidence for a role of ESCRT-III in HIV-1 release originated from the finding that a dominant-negative VPS4 mutant, lacking ATPase activity, could block HIV-1 budding by preventing the nascent viral particle to pinch off. The VPS4 complex is the last component in the ESCRT pathway and is endowed of enzymatic activity required for the disassembly of the ESCRT-III lattice (Fig. 2). It consists of the VPS4 AAA-type ATPase and its activator LIP5. VPS4 exists as an inactive dimer in the cytoplasm and assembles into a high-molecular-weight complex upon activation and recruitment by ESCRT-III complexes on cellular membranes. Two isoforms, VPS4A and VPS4B, exist in humans and, according to knockdown studies, are believed to perform essential but redundant functions in

MVBs and HIV-1 budding. VPS4 proteins have a modular structure with a single ATPase cassette and an N-terminal substrate recognition region known as microtubule-interacting and trafficking (MIT) domain. The MIT domain of VPS4 proteins has been found to engage ESCRT-III proteins such as CHMP2 and CHMP6 in direct protein-protein interaction by recognizing a MIT-interacting motif (MIM) at the C-terminal of the CHMP protein. Structural studies and homology with other AAA-type ATPases suggest that the activated VPS4 is a mechanoenzyme complex made of the a double ring of hexamers with the flexible MIT domains projecting away to pull out ESCRT-III subunits from assembled lattice and release monomers of CHMP proteins through the central pore of the complex upon hydrolysis of ATP. This model suggests that removal of individual CHMP proteins could potentially constrict the ESCRT-III helical filaments and participates in generating the energy required for membrane fission. Whether VPS4 has an active role in membrane fission or is strictly limited to recycling remains an open question. Recent live imaging studies looking at HIV-1 assembly sites by TIRF microscopy indicate that VPS4 is recruited prior to particle release, therefore supporting a more active role for VPS4 in membrane fission events (Baumgartel et al. 2011; Jouvenet et al. 2011).

Alix

Alix is an ESCRT-associated protein that binds the LYPXnL L domain motif in the HIV-1 Gag p6 region and drives an auxiliary pathway of viral release (Fig. 2). In EIAV, a lentivirus that contains the type 1 LYPDL motif, Alix is sufficient to drive viral release in the absence of any other L domain. This can be explained by the fact that the EIAV L domain exhibits much higher affinity for Alix than the LYPXnL motif of HIV-1. HIV-1 release depends primarily on the PTAP L domain and its ability to recruit the cellular protein Tsg101. Mutations in the PTAP motif, which prevent Tsg101 recruitment to Gag, profoundly inhibit virus budding. In this context, however, increased

levels of cellular Alix can efficiently restore HIV-1 release and infectivity, revealing that Alix also functions in HIV-1 budding and can drive virus release independently of the PTAP L domain, provided Gag harbors an intact LYPXnL L domain motif.

Structural studies revealed that Alix comprises three distinct domains: an N-terminal Bro1 domain, a LYPXnL-binding coiled-coil V domain, and a C-terminal proline-rich domain (PRD). The Bro1 domain has a boomerang/banana-shaped structure and contains two main hydrophobic patches relevant for Alix function in HIV-1 release. The first patch or hydrophobic interface is localized in the concave face of the Bro1 domain and binds CHMP4 isoforms, connecting Gag directly to ESCRT-III isoforms (Zhai et al. 2007, *Nature Structural and Molecular Biology*). Mutations of residues involved in the Bro1-CHMP4 interaction prevent Alix to function in viral release, indicating that Alix is able to link Gag to the ESCRT-III complex and promote HIV-1 budding. A second hydrophobic surface is centered at the phenylalanine residue 105 (Phe105) at the tip of a long flexible loop protruding from the convex side of the Bro1 domain. Disruption of residues within this loop severely inhibited the ability of Alix to stimulate HIV-1 release, without affecting its ability to interact with Gag or its natural partner CHMP4 (Sette et al. 2011). The exposed nature of the Phe105 loop suggests that it could function as an acceptor site for a cellular cofactor of Alix that remains to be identified and characterized.

The central domain of Alix folds into a V-shaped structure that binds the LYPXnL L domain in HIV-1 p6 and other viral Gag proteins through a hydrophobic pocket located on one of its arms. Recently, the same V domain pocket was shown to interact with a cellular LYPXnL motif belonging to syntenin, a protein involved in exosome biogenesis (Baietti et al. 2012). In addition, the V domain is involved in Alix-Alix homodimerization, a property that appears to be required for Alix activation and function in virus release. Indeed, in its active dimeric form, Alix is believed to recruit and nucleate CHMP4 polymers during membrane budding processes. Recently, the Alix

V domain was also found to interact with K63-linked poly-ubiquitin chains. Mutation of the ubiquitin-binding motifs in the V domain impaired Alix ability to function in retroviral release, suggesting that Alix interaction with ubiquitinated proteins is critical for function. The nature of these ubiquitinated proteins and how they function with Alix remain to be elucidated, however.

The unstructured C-terminal PRD domain is essential for Alix activity in virus release. The current model suggests that Alix is present in the cytoplasm in an autoinhibited monomeric form with the PRD region folding back toward the upstream domains. In this closed conformation, the PRD prevents Alix interaction with the LYPXnL L domain. At the budding neck, the displacement of PRD is believed to trigger the activation and dimerization of Alix and subsequent binding to the L domain and possibly CHMP4. In addition to the LYPXnL-V domain interaction, a second point of contact between Alix and Gag was recently characterized involving the p6-adjacent nucleocapsid (NC) region of Gag and the Bro1 domain of Alix. Interestingly, Alix Bro1 domain alone was found to rescue budding defects of HIV-1 mutant lacking L domains, suggesting that Bro1 can act as a minimal functional unit of Alix that links Gag to the ESCRT pathway via interactions with NC (Dussupt et al. 2009). Findings that residues on both sides of the Alix Bro1-NC interface are positively charged, suggested that RNA is involved in this interaction, although molecular mechanisms involved are yet to be elucidated. Mutations that prevent Bro1-NC binding affect Alix ability to promote viral release, revealing its functional relevance. These studies suggested a model in which Alix engages in dual interactions with Gag via both NC and the adjacent p6 domain. Further studies are warranted to decipher the functional significance of the newly identified interactions.

Nedd4-Like Ubiquitin Ligase

Some retroviruses, including RSV, Moloney murine leukemia virus (MoMLV), and the human T-cell leukemia virus, utilize the PPXY-

type L domain to acquire an envelope and leave the cell. PPXY functions by recruiting members of the Nedd4-like ubiquitin ligase family and linking Gag proteins to the host cell ESCRT pathway. Nedd4-like family of ubiquitin ligases has a common structural organization with an N-terminal C2 domain involved in membrane binding, multiple WW domains for substrate recognition, and a C-terminal HECT domain with an intrinsic catalytic activity. The catalytic activity of the HECT domain is essential for their functions in the sorting of cargo proteins in endosomal/lysosomal compartments as well as in virus budding, suggesting that ubiquitin conjugation is central for function in these processes. Despite the absence of a PPXY L domain in its Gag protein, HIV-1 appears to be sensitive to the effect of Nedd4-like ubiquitin ligases. One of its members, Nedd4-2s, a natural isoform of Nedd4L/Nedd4-2 – which lacks most of the N-terminal C2 domain – was found to potently stimulate HIV-1 release. Remarkably, Nedd4-2s corrected the budding defects of HIV-1 mutants lacking all L domains and thus appeared to provide an additional access to the host cell ESCRT pathway. Nedd4-2s rescue of HIV production required both cellular Tsg101 and intact C2 and HECT domains, and further data supported the idea that Nedd4-2s is recruited to sites of virus assembly through specific interaction between its residual C2 domain and Gag. Nedd4-2-mediated ubiquitination of either the ESCRT-I complex or Gag itself was suggested to facilitate the recruitment of the ESCRT pathway and promote viral budding in absence of L domains. Further studies are needed to better understand how this ubiquitin ligase (and possibly other Nedd4-like members) stimulates HIV-1 release and decipher the molecular mechanisms involved.

Role of Ubiquitin in HIV-1 Budding

Ubiquitin plays a central role in cellular processes involving ESCRT proteins including virus budding and sorting of cargo proteins at the MVBs. Attachment of ubiquitin molecules to cargoes signals their entry into endosomal compartments

(Shields and Piper 2011). Therefore, it is not surprising that ubiquitin was found to play a role in ESCRT-mediated retroviral budding. Ubiquitin is a highly conserved protein composed of 76 amino acids, which can be covalently linked post-translationally to a lysine residue of a target protein. Ubiquitin itself has seven lysines (K6, K11, K27, K29, K33, K48, and K63) that all have the potential to engage in interaction with other ubiquitin molecules (poly-ubiquitination) allowing the formation of different types of chains. The biological role of poly-ubiquitination depends on the type of linkage and the length of the chain. For example, proteasomal degradation is signaled by poly-ubiquitin conjugation through lysine residues at position 48 (Lys-48-linked), whereas single or multiple mono-ubiquitination and Lys-63-linked poly-ubiquitination mediate protein entry in the ESCRT pathway. To enter the pathway, cargos are ubiquitinated by specific E3 ubiquitin ligases and as such recognized by members of the ESCRT-0, ESCRT-I, and ESCRT-II complexes that contain ubiquitin-binding domains (UBDs). The first evidence that ubiquitin plays a role in retrovirus release came from the finding that free ubiquitin is selectively incorporated into avian sarcoma leukosis virus particles. Subsequently a small subset of Gag molecules within HIV-1, simian immunodeficiency virus (SIV), and MoMLV viral particles were found to be mono-ubiquitinated, and the level of Gag ubiquitination appeared to be L domain dependent. In addition, depletion of soluble ubiquitin by treating cells with proteasome inhibitors induced a late budding defect. Whereas a role for ubiquitin in virus budding is accepted, the identity of the ubiquitinated protein(s) and the functional significance of ubiquitination at HIV-1 budding sites was (Vogt 2000) and still is a subject of debate. One possibility is that the relevant targets for ubiquitination are cellular Gag binding proteins involved in viral budding. In fact, Tsg101 and Alix are both ubiquitinated, a modification that might play a role in their facilitation of viral budding. Along this thought, an artificial lysine-free Gag protein from foamy virus that cannot be ubiquitinated was still able to promote viral budding and release. Aside from the importance of

ubiquitin conjugation to cellular factors in the vicinity of Gag, an alternative model is that Gag itself is the relevant target for ubiquitination, and several findings supported such a model. Indeed, cumulative mutations of lysine residues in the p6 region of HIV-1 Gag-arrested viral budding, although milder, were reminiscent of L domain disruption. Such defects however were alleviated by the physical fusion of ubiquitin to Gag including in the absence of L domains, suggesting that the mere presence of ubiquitin molecule near Gag (or onto Gag) is sufficient for the recruitment or utilization of the ESCRT machinery for virus release. In agreement with this notion, Gag ubiquitination appears to be highly dependent on its association with the plasma membrane, the site of virus budding, and deposition K63-linked ubiquitin chains to Gag by the ubiquitin ligase Nedd4.2s correlated with the stimulation of HIV-1 release. Although these observations and others support the notion that conjugation of ubiquitin chains to Gag is involved in ESCRT-mediated virus scission from producer cells (Sette et al. 2013), further studies are warranted to elucidate mechanisms of ubiquitin involvement in HIV-1 budding.

Conclusion

The last decade has brought a wealth of information on how HIV-1 assembles nascent virus and exit cells. Notably, the major finding was the discovery that budding is a tightly regulated step of the HIV-1 life cycle that requires the participation of a complex cellular membrane fission machinery, the ESCRT pathway. A significant progress has been made in understanding how HIV-1 hijacks a select number of ESCRT proteins to separate from cells. However, the possible involvement of additional host factors and mechanistic details in the sequence of events leading to the membrane fission step that ultimately releases

the newly formed HIV-1 virions are yet to be uncovered. More efforts are needed to gain a better understanding of the budding process at the molecular level, a prerequisite for the development of novel antiretroviral therapies (ART) (► [Antiretroviral Therapy and Cellular Cofactors as Drug Targets](#)) such as small-molecule inhibitors of HIV-1 production and spread.

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We cited reviews rather than individual papers to abide by space constraints. We apologize to our colleagues for the inability to cite their individual valuable contributions to the field.

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Burkitt and Burkitt-Like Lymphoma

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Definition

Burkitt lymphoma (BL) is a rare tumor of mature B lymphocytes. Clinically, it develops rapidly and runs an aggressive course. Tumor can develop in

any body organ, but most often involves sites other than lymph nodes, especially in the abdomen, pharynx, or the jaw. BL is relatively more common in sub-Saharan Africa, where it is endemic. Patients with HIV infection are also at increased risk of BL, and in this setting it is called epidemic BL. Endemic and epidemic BL are both associated with Epstein-Barr virus, a common virus that infects 90% of the global population. Although the tumor is highly aggressive, being the most rapidly growing cancer known and doubling every 24 h, it is also very responsive to modern chemotherapy. With currently available regimens, 90% of patients can expect to be cured when properly treated.

Introduction

In 1982, reports of Kaposi sarcoma and *Pneumocystis carinii* infections in ► **men who have sex with men (MSM)** heralded the advent of the AIDS epidemic in the USA. Soon afterward, a cluster of cases of aggressive B-cell non-Hodgkin lymphoma (NHL) was noted in the same population. As the epidemic unfolded, it was noted that these AIDS-associated lymphomas included cases of Burkitt lymphoma (BL) or Burkitt-like lymphomas (BLL). Burkitt lymphoma (BL), a rapidly growing B-cell non-Hodgkin lymphoma (NHL), was known prior to the HIV epidemic. The pathology of BL was described as small non-cleaved cell lymphoma in the Lukes and Collin's classification system of lymphomas, and prior to the AIDS epidemic, it was relatively rare outside sub-Saharan Africa, where it was known to be endemic. First described by Denis Burkitt in 1958, the tumor was known to affect mostly children who developed tumors in the jaw or in the abdomen. Shortly after, the pathology of the tumors was described, allowing sporadic cases to be described worldwide and leading to the disease to be named after Burkitt as a specific clinical and pathologic entity.

Three epidemiological variants of BL are now recognized: endemic, sporadic, and immunodeficiency related (or epidemic). These variants differ

with respect to their incidence, geographic areas, and associated factors, but they are currently not distinguishable by routine histopathological, immunohistochemical, or molecular criteria. All three variants of BL share the starry-sky pattern under the microscope and a morphology of uniform medium-sized cells with cytoplasmic vacuoles, small nucleoli, and a non-cleaved nucleus. The immunophenotype typically shows positivity for monotypic surface IgM, B-cell antigens (CD20, CD10, CD79a), and a high proliferation index. Cytogenetic studies show the presence of a rearranged *c-myc* gene. The rearrangement is often the result of translocation of *c-myc* on chromosome 8 into the vicinity of heavy chain immunoglobulin (IgH) sequences on chromosome 14, or light-chain immunoglobulin genes on chromosomes 2 and 22 as well. The cell of origin for BL is a germinal center B cell, and BL thus expresses *bcl-6* but not *bcl-2*, CD-138, or TdT (Swerdlow et al. 2008).

Epidemiology

The two major variants of BL described prior to the HIV epidemic mainly differ by geography and incidence rates. Endemic BL occurs in sub-Saharan Africa and Papua New Guinea, in regions that are also holoendemic for *Plasmodium falciparum* malaria. In sub-Saharan Africa, patients with endemic BL frequently present with a rapidly growing tumor involving the jaw or abdomen. The peak age is 5–9 years, in part because of early acquisition of ► [Epstein-Barr virus \(EBV\)](#) infection in sub-Saharan Africa coupled with repeated infection with *Plasmodium falciparum*, the two generally accepted key risk factors. EBV is detected in more than 95% of endemic BL cases. In regions of sub-Saharan Africa that have holoendemic malaria, BL constitutes the majority of pediatric lymphomas and accounts for as many as half of all childhood cancers. Accurate estimates of the incidence of endemic BL are lacking, but the annual incidence is between 40 and 50 cases per million children (Molyneux et al. 2012). Boys are affected more than girls, particularly for tumors involving the

jaw where the male-to-female ratio may be higher than 10:1. BL occurring outside the malarial regions of Africa and Papua New Guinea and outside the setting of HIV infection is referred to as sporadic BL.

The clinical presentation of sporadic BL differs from that of endemic BL by more frequently involving the lymph nodes, the abdomen, and the bone marrow. In addition, sporadic cases generally occur later during adolescence and young adult age, although cases have been reported in the elderly. The incidence of sporadic BL is at least ten times lower than endemic BL – about two per million in the USA, Europe, and some parts of Asia. Unlike endemic BL, no specific risk factors have been identified for sporadic BL. The association of EBV in sporadic BL varies considerably from country to country. In the USA and Western Europe, only about 20–30% of BL are EBV positive. However, in Brazil, Argentina, Turkey, and Egypt, where the cases are considered to be sporadic, EBV positivity ranges from 50% to 70% (Magrath et al. 1992). It is not clear whether EBV-positive BL in the USA differs by race.

Risk for BL is very high in individuals with immunodeficiency, either as a result of infection with human immunodeficiency virus (HIV) or following solid-organ transplantation. Patients with AIDS have about a 50- to 60-fold greater risk of developing BL than people without AIDS of a similar age and sex. The burden of AIDS-associated BL in the USA from 1980 to 2007 has been estimated to be about 3,452 cases (Shiels et al. 2011b), and these cases account for one-fifth of all BL cases diagnosed in the USA during the same period. A distinct age-associated peak of BL at age 40 has been identified in persons with HIV infection, perhaps influenced by the fact that most HIV-infected individuals are less than 50 years of age. More recent analyses of cancer registry datasets now suggest that the age-specific pattern of BL in most countries (except Africa, for which data are sparse and/or incomplete) is trimodal. Separate incidence peaks near ages 10, 40, and 70 are clearly apparent. While the middle peak overlaps with cases of AIDS-BL, this trimodal age-specific incidence of BL is also apparent in the general population. The multiple

incidence peaks of BL raise the possibility that conditions currently diagnosed and classified as BL comprise a mixture of entities with distinct biology or etiology and distinct age peaks. It remains to be seen if variation in BL immunophenotype, molecular, and viral profiles corresponds with the distinct age peaks identified and/or with the immune status of tumors.

How immunodeficiency increases the risk for BL is not entirely clear. It is likely that immunocompromised patients are unable to control EBV infection, which might increase the risk of EBV-associated BL. EBV is indeed found in 40–60% of HIV-associated BL from the USA and Western Europe, but changes in control of EBV alone may not explain all of the observed increase in HIV-associated BL because a substantial proportion of cases are not EBV positive. Although HIV has been associated with dramatically increased BL incidence in the USA and Western Europe, data are conflicting about the impact of HIV on BL in sub-Saharan Africa, where the HIV epidemic is substantial and BL was previously endemic. For example, comparison of rates of incidence of BL in Uganda, between pre-HIV (1960–1971) and HIV era (1991–1997), suggests only slight increase in BL incidence, and while some of the increase might be attributable to HIV, it is not possible to be certain because cancer registry studies do not collect HIV status on their patients. Two case-control studies conducted in Uganda showed a modest but statistically significant increase in BL risk with HIV (with the odds ratio in one study of 7.5 and the other 2.2), raising doubt about the significance of the overall association. Similarly conflicting results were reported in two analyses from a study conducted in Malawi, with significant results reported in one analysis (OR = 12.4) and a non-significant result in the other (OR = 2.2). There are sparse data on the association between HIV and BL in adults in Africa. A study from Kenya (Otieno et al. 2001) showed the median age of BL was 35 years, suggesting an impact HIV on the age of BL diagnosis, but this study was small, and it did not estimate the fold increase. Therefore, it is quite unlikely that a substantial effect of HIV on AIDS-associated BL

in sub-Saharan African BL belt has been missed. Why this difference? One reason is that competing mortality – death from other, more common conditions – contributes. This may be supported by one study that has reported a stronger impact in South Africa, where survival rates for children with HIV may be better than in Uganda and Malawi, although this has not been shown. In that study, the BL risk in children with HIV was increased nearly 46-fold. However, since BL in South Africa is more likely the sporadic type, i.e., not associated with holoendemic malaria, the substantially higher risk of BL with HIV infection may simply indicate that the impact of HIV on BL is greater for sporadic than endemic BL. Following the widespread availability of ► [combination antiretroviral therapy \(cART\)](#), the overall incidence of AIDS-related NHLs has significantly decreased with the largest decrease seen for primary central nervous system (CNS) lymphomas (Shiels et al. 2011). There are conflicting data on the incidence of BL in particular during the cART era. A large meta-analysis of 23 cohort studies did not support a decline in BL (International Collaboration on HIV and Cancer 2000).

BL accounts for a substantial proportion of AIDS-associated NHL in the cART era. The relationship between degree of immunosuppression and risk to BL contrasts with that of the correlation between degree of immunosuppression and risk for primary CNS lymphoma. While the latter occurs at very low CD4 counts, there is a clear deficit of BL cases in people with very low CD4, suggesting a requirement of a threshold of CD4 for BL pathogenesis (Guech-Ongey et al. 2010). Although access to antiretroviral therapy has expanded significantly in recent periods in sub-Saharan Africa, there are sparse data to understand whether cART has impacted incidence of BL in these regions.

Molecular Features of BL

Irrespective of the epidemiological variant, almost all BL are characterized by a signature translocation that juxtaposes and thereby deregulates the

c-myc gene, bringing it under the regulatory control of the B-cell-specific immunoglobulin locus. Most frequently this translocation is between c-myc on chromosome 8 and the IgH gene on chromosome 14 [t (8:14)]. Variant translocations involving c-myc and antibody light-chain genes, either Immunoglobulin Kappa (IgK) [t (2:8)] or Immunoglobulin Lambda (IgL) [t (8:22)], occur in about one-fifth of BL cases. Some reports of lymphomas that otherwise are immunophenotypically identical to BL but do not carry these translocations have also been described. Such myc-negative cases occur both in adults and children. It is likely that alternate pathways with the end result similar to a deregulated myc might be in play in these lymphomas. The proportion of AIDS-related BL that similarly shows absence of a c-myc translocation is not known. Molecular translocations of chromosome 8 and 14 differ with respect to their breakpoints between sporadic and endemic BL and also differ between sporadic BL from different regions. In endemic BL the breakpoint often leaves the transcriptional unit of c-myc intact and occurs several hundred kilobases upstream or downstream of the c-myc gene. On the other hand, in sporadic BL cases in the USA, the translocation often involves breaks between exons 1 and 2 of the c-myc gene. Sporadic BL and AIDS-associated BL from the USA do not appear to be different at the molecular level of the translocations. The breakpoints in sporadic BL cases from other world regions such as Argentina and Brazil differ and often cluster immediately upstream of c-myc gene (Magrath et al. 1992). There is insufficient data on breakpoints from AIDS-associated BL from countries outside the USA. The significance of these regional differences in breakpoints is unclear. However with the exception of BL from Algeria (where the breakpoints are often within the c-myc transcriptional unit), there appears to be a gradient of association between the proportion of EBV positivity and breakpoint location among BL from other regions. Patients with EBV-negative BL are relatively more likely to have breaks occurring within the transcriptional structure of c-myc gene, suggesting the possibility that EBV may contribute to deregulation of translocated

c-myc with breakpoints far upstream or far downstream of c-myc.

There is considerable evidence that the Ig-myc translocations lead functionally to myc deregulation, although this in itself is insufficient to trigger onset of clinical BL. Some data suggest that proliferation in normal germinal center cells is not myc driven and thus a forced expression of c-myc in germinal center B cells is the most likely central component of the pathway to B-cell lymphomagenesis in BL. In addition to the translocation, a contribution to the deregulation of c-myc is also provided in AIDS-associated BL by mutations that are present in a small 500 base pair regulatory region in the junction of the first exon and first intron of the c-myc gene. Most BL, including AIDS-associated BL, carry no or only a few other cytogenetic abnormalities. Abnormalities involving chromosomes 1q, 7q21, and 12p13 have been reported in sporadic BL.

However, like other cancers, additional genetic alterations are required to circumvent the homeostatic feedback loops triggered by deregulated c-myc to prevent progression to malignancy. Among the additional molecular lesions described in BL, those that incapacitate apoptotic pathways are prominent. Inactivation of p53 is frequently found in BL including AIDS-associated BL. Other molecular abnormalities include mutations in proapoptotic proteins such as Bak and diminished expression of proteins that inhibit apoptosis such as cIAP-1 and cIAP-2. The presence of inactivating mutations of RBL2 has been reported in endemic and some sporadic BL, while HIV-positive BL cases have been found to demonstrate an overexpression of wild-type RBL2. The tumor suppressor functions of RBL2 have been shown to be neutralized by physical interactions with HIV-1 Tat protein suggesting that HIV-1 may directly interact in the pathogenesis of BL. In addition to mediating proliferation, constitutional expression of c-myc also blocks cell differentiation and induces apoptosis. The oncogenic conversion of c-myc in HIV-associated BL often incorporates mutations within the c-myc coding regions (Bhatia et al. 1993). Mutations affecting c-myc are most

likely a result of abnormal somatic hypermutations. Aberrant somatic hypermutation also results in a wide range of mutations in other oncogenes including BCL6. Epigenetic alterations are also frequently associated with both HIV and non-HIV BL and target expression of MGMT, DAPK, and P73.

Phosphoinositide-3-kinase (PI3K) signaling plays a critical role downstream of B-cell receptor signaling in B cells. More recent data implicate activation of PI3K pathway as an important pathogenic event in BL that might synergize with deregulated c-myc (Schmitz et al. 2012). Aberrations in this pathway result from somatic mutations that deregulate the activity of E2A or inactivate its negative regulator ID3 and also from mutations in cyclin D3, a gene that is the direct target of E2A and is intricately involved in the control of germinal center B-cell proliferation. It is not yet clear whether there are differences in the proportion of HIV and non-HIV BL with respect to the synergistic involvement of the PI3K pathway in conjunction with a deregulated c-myc.

Role of EBV

Most of the cancers associated with HIV infection have a viral etiology. ► [Hodgkin lymphoma](#) and primary CNS lymphomas arising in the context of immunodeficiency are almost always associated with EBV, and approximately 30–50% of ► [diffuse large B-cell lymphomas \(DLBCL\)](#) in people with HIV are EBV positive compared to less than 5% of EBV-positive DLBCL in the HIV-negative setting. Also, approximately 30–60% of cases of HIV-associated Burkitt lymphoma are EBV associated. This is more than what is observed in sporadic BL but less than what is found in endemic BL, in which 95% or so of cases are EBV associated.

As in other lymphomas, EBV is generally latent in BL tumor cells. In latent EBV infection, a very restricted pattern of gene expression is observed. There are three programs of EBV latency, ranging from latency I, in which only EBV nuclear antigen 1 (EBNA-1) and EBV-encoded RNAs (EBERs) are expressed, to

latency III in which all the latent genes are expressed. Many latently expressed EBV proteins are immunogenic. Latency III is only found in tumors in immunodeficient hosts such as in HIV-associated DLBCL and the polymorphic posttransplant lymphoproliferations. Interestingly, in BL that occurs in the context of immunodeficiency, the pattern of latency expression is latency I, in which only EBNA-1 and EBERs are expressed. This is similar to the latency pattern in non-HIV BL. Also similar to the non-HIV BL, EBV is monoclonal in BL that arises in AIDS patients. Both these observations are consistent with the hypothesis that EBV is not merely an opportunistic passenger as a result of diminished immunosurveillance but plays a pathogenic role in HIV-associated BL.

Several possible roles for EBV in the pathogenesis of BL have been suggested. The expression of EBERs might support survival by inducing the expression of IL-10. Both EBNA-1 and EBERs also confer antiapoptotic activity and thereby promote survival of clones with a deregulated c-myc. The restriction of expression of latency genes to latency pattern I in immunodeficiency-associated BL is nonetheless intriguing and suggests that even in the context of reduced immune pressures, there is a need for downregulating the expression of the majority of EBV latent genes. It is possible that the expressions of certain latent EBV genes, such as EBNA-2, are incompatible with pathways that support BL lymphomagenesis. A scenario that emerges therefore is that HIV-positive BL cells are derived from an amplified pool of EBV-transformed B cells that initially expressed the full repertoire of latent genes. It might be that some of these genes such as latent membrane protein-1 (LMP-1) helped orchestrate immunoglobulin class switching and somatic hypermutation through the induction of activation-induced deaminase (AID) pathways, as will be discussed below.

The contribution of EBV to antiapoptotic mechanisms in BL along with the incompatibility of EBNA-2 with BL lymphomagenesis is also supported by recent observations of a variant latency pattern in a small proportion of BL. This

latency pattern, called Wp-restricted latency, results from the use of the Wp promoter in mutant EBV genomes and is associated with about 15% of BL. The mutant EBV genomes carry a deletion of the EBNA-2 region and allow expression of EBNA 3A, 3B, and 3C and the EBV antiapoptotic gene BHRF1 (Bornkamm 2009). It is yet not known how often mutated EBV genomes are associated with HIV-positive BL in comparison to non-HIV BLs. There is a greater genomic diversity associated with EBV in HIV-infected individuals, and HIV-positive BL are more often associated with type B EBV. The theoretical possibility therefore exists that a wider genomic diversity of EBV strains and subtypes prevalent in the context of a reduced EBV immunosurveillance in people with HIV infection allows for more “oncogenic” variants of EBV to enhance the likelihood of lymphomagenesis including the development of Burkitt lymphomas.

Role of HIV

HIV does not directly infect BL clones, and thus its influence in enhancing risk for BL in people with HIV infection is mediated indirectly. T-cell immunodeficiency resulting from HIV infection plays a central role in increasing the risk of certain cancers, particularly those caused by oncogenic viruses. Thus both the degree and the duration of immunosuppression measured by deficit in CD4 T cells correlate with the risk of lymphomas. A decrease in incidence of some lymphomas such as primary CNS lymphomas in the cART era supports the importance of such immune surveillance, directed against the virus and/or viral antigens expressed on the tumor cells. By contrast with primary CNS lymphoma, the correlation between the degree of immunosuppression and the risk for BL is more complex. The incidence of BL is only modestly affected in the cART era, and BL arises even in the context of a relatively recovered immune status in patients receiving cART therapy. A deficit of BL has been documented in patients with extremely low CD4, such as those with CD4 counts between 100 and 50/ul, suggesting a

threshold requirement of T cells in BL lymphomagenesis. In addition to decrease and dysfunction of T lymphocyte as a result of HIV infection, there is a parallel increase in B-cell activation and an overproduction of B-cell stimulatory cytokines combining to allow chronic B-cell hyperactivation, contributing indirectly to risk for lymphomas. As noted above, one postulated mechanism of HIV-driven lymphomagenesis is the activation and expression of the DNA-editing enzyme-induced cytidine deaminase (AID). AID expression has been shown to be elevated in B cells of HIV-positive individuals prior to the diagnosis of NHL. It is possible thus that this overexpression of AID, which is known to promote *c-MYC/IgH* translocations, induces aberrant (*Ig*) class-switch recombination (CSR) and somatic hypermutation (SHM) contributing to increased frequency of B cells with lymphoma-specific translocations (Epeldegui et al. 2006).

Other mechanisms whereby HIV-related proteins may directly influence BL development also need to be considered. HIV-infected cells secrete ► *tat* protein which is present in sera and thus is capable of exerting biological effects when taken up by other cells including B cells. *Tat* has potential oncogenic activity and can inactivate tumor suppressor genes including RB2, which has been shown to play a contributory role in development of some BL.

Presentation and Pathology

Endemic BL often presents with localized disease, frequently affecting the jaw or the abdomen. In some cases, other extranodal sites including breasts, ovaries, and kidneys may be involved. Bone marrow involvement and nodal involvement are rare in endemic BL. Sporadic BL particularly in children also frequently involves the abdomen, while facial involvement is rare. A greater proportion of sporadic BL than endemic BL involves the bone marrow. By contrast to endemic BL, AIDS-BL often presents with lymphadenopathy. CNS involvement and advanced stage at presentation are also seen

more frequently with AIDS-BL than in other types of BL, both in children and adults.

Most cases of endemic and pediatric BL present a classical cytologic appearance. The tumor is comprised of monomorphic, uniform cells of medium size with round nuclei, moderately clumped chromatin, and multiple basophilic nucleoli. The cytoplasm is abundant and basophilic and contains many lipid vacuoles. The characteristic “starry-sky” pattern of BL results from multiple infiltrating macrophages that ingested apoptotic tumor cells. Compared to classic BL, AIDS-associated BL and a proportion of adult BL have a variant appearance and depict less uniformity. In AIDS-associated BL there is a tendency for plasmacytoid differentiation. Irrespective of the type of BL, several important immunophenotypic characteristics are common. These include positivity for surface IgM, CD10, and CD79a, a high score of proliferation identified by Ki67 staining of all or nearly all (>95%) of tumor cells, and an absence of BCL-2 and TdT staining.

In patients with HIV and particularly in adults, distinguishing between cases that are true BL from morphologically similar other aggressive B-cell lymphomas such as those that are diffuse large B-cell lymphomas (DLBCL) is prognostically important. A fraction of BL cases remain difficult to classify because they have features intermediate between diffuse large B-cell lymphomas and BL. Recent data using molecular gene expression profiles have provided a characteristic molecular signature of BL. Therefore application of such molecular diagnostics might provide added diagnostic tools in the future. In the more recent WHO classification of lymphomas, a category of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (B-UNC/BL/DLBCL) has been introduced to characterize the lymphomas that are difficult to distinguish between BL and DLBCL (Jaffe and Pittaluga 2011).

Treatment

Endemic BL was one of the first malignancies that was shown to respond to chemotherapy. Since

then, chemotherapy has remained the mainstay for therapeutic management of all forms of BL. Surgery and radiotherapy have little to offer to BL treatment. Early studies demonstrated a high degree of chemosensitivity of endemic BL, which responds particularly well to cyclophosphamide, methotrexate, and vincristine. Long durations of remissions were noted with these drugs, and late relapsing tumors were found to retain chemosensitivity to the initial treatment and to respond fully to second-line treatment. The use of simultaneous and upfront combination chemotherapy, particularly using drugs that were not cross-reactive, along with intrathecal chemotherapy, improved long-term outcomes and reduced relapse of BL in the central nervous system. The experience gained from these early treatment protocols continues to influence the management of BL in Africa.

The recommended first-line treatment of BL is intravenous treatment with cyclophosphamide, vincristine, and methotrexate (COM) and intrathecal methotrexate and cytarabine. At least three cycles, given every 2–3 weeks, are recommended for early stage disease and 6 cycles for late-stage disease. The second-line regimen, for patients who fail the first line, is a four-drug intravenous regimen including etoposide, ifosfamide, mesna, and cytarabine, along with intrathecal methotrexate and cytarabine. While these treatments are likely very efficacious (80% response in African settings), many patients with endemic BL die soon after diagnosis from tumor lysis syndrome, late presentation, and the patients choosing to stop receiving treatment.

Treatment of sporadic BL in the USA and other western countries was informed by the experience in treating endemic BL. Cyclophosphamide, vincristine, and methotrexate formed the backbone of initial treatment regimens, which over time became increasingly intensive. An important step to optimal treatment of both adult and pediatric BL was the design of the CODOX-M/IVAC regimen, sometimes called the Magrath regimen, which achieves remission rates of up to 100% and 92% event-free survival at 5 years in children and adolescents (relapses in BL are very rare after 1 year). Subsequent

clinical studies have evaluated this protocol in multi-institutional settings, and some modifications of the protocol have been introduced to preserve efficacy while decreasing neurotoxicity. These modifications include a reduction in the dose of methotrexate, limiting the dose vincristine, and increasing the dose of doxorubicin. Utilizing data from preclinical studies that suggested diminished tumor resistance when sustained concentrations of doxorubicin, vincristine, and etoposide are used, Wyndham Wilson and colleagues in the National Cancer Institute intramural program introduced infusion dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (DA-EPOCH) for the treatment of adult lymphomas, and this regimen was found to have utility in the treatment of sporadic BL.

Early in the AIDS epidemic, most AIDS-associated lymphomas, irrespective of histological subtype and stage, were treated with less aggressive therapy as compared to standard regimens, and the general outcome of these initial approaches in HIV-associated BL was inferior compared to non-HIV BL. With the introduction of combination antiretroviral therapy (cART), there was an appreciation that HIV patients were better able to tolerate standard chemotherapeutic regimens, generally along with cART. The AIDS Malignancy Consortium has studied modified CODOX/IMVAC regimen for AIDS-related BL. DA-EPOCH is another option that has been shown to be effective for treating AIDS-BL and AIDS-BL-like lymphomas. Because BL and BL-like lymphomas, both in HIV-positive and HIV-negative patients, frequently express CD20, the use of rituximab, a monoclonal anti-CD20 antibody, has also been tested and found to have activity. Rituximab has been incorporated into a modified Magrath regimen as well as with the infusion dose-adjusted EPOCH regimens (Mwamba and Remick 2012).

The treatment of HIV-related BL in sub-Saharan Africa poses special challenges. In general, the use of highly intensive therapy is difficult in Africa because of limited drug supply, as well as a lack of high-quality supportive care. Modified regimens that are practical in the

locoregional setting, including oral-based regimens and modified CHOP-like regimens, are being studied in Africa; similarly incorporating cART to standard regimens used to treat BL in sub-Saharan Africa such as the COM regimen is a possible alternative for use in sub-Saharan HIV-related BL (Magrath 2012).

Conclusion

The discovery of the entity now known as Burkitt lymphoma was first made by Dr Dennis Burkitt in equatorial Africa. The study of what is now called endemic BL proved to be immensely important and established several landmarks in biology and treatment of cancers worldwide. These included the discovery of the first human virus associated with cancers – EBV – and the first demonstration of the use of chemotherapy in the treatment of solid tumors. The risk for BL is increased about 50- to 60-fold in people with AIDS in the USA; however, the magnitude of increase of BL is only about tenfold in HIV-infected children and adults in areas where BL is endemic. BL is a lymphoma derived from germinal center B cell and is variably associated with EBV. A defining feature of BL is the presence of a simple translocation involving the c-myc locus with an IgH, IgK, or IgL locus. It is a highly proliferating tumor with more than 95% of tumor cells staining for Ki67. Molecular analysis of gene expression studies has defined a BL signature, which distinguishes it from other aggressive B-cell lymphomas such as DLBCLs. However, some tumors are intermediate between BL and DLBCL and are now considered a separate category of tumor. A diagnosis of BL is clinically relevant since BL-specific therapy is essential for optimal outcome. The treatment of HIV-associated BL now yields a comparable response rate and outcome as compared to BL in the HIV-negative setting. Recent laboratory-based studies have identified new genetic lesions in BL which may provide novel targets for the development of small molecules capable of interfering with genetic circuits that sustain proliferation of BL cells.

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Cardiovascular Complications

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Definition

With the advent of widespread use of ART and associated immunologic recovery and viral suppression, there has been a concomitant reduction in AIDS-related mortality across multiple patient populations worldwide. This phenomenon has led to the emergence of noncommunicable diseases, such as non-AIDS malignancies, hepatic disease, and cardiovascular disease as major sources of morbidity and mortality in the HIV-infected population. Included among these complications are cardiovascular diseases that affect the heart and peripheral vasculature. During the early course of the HIV epidemic, the major cardiovascular complications included dilated cardiomyopathy and pericarditis. While these are present in the current era of HIV treatment, the successes of antiretroviral therapy (ART) and the aging of the HIV-infected population combined with unique predisposing factors have led to the emergence of coronary heart disease (CHD) as a major source of morbidity and mortality. Management

strategies for CHD in HIV-infected patients require attention to traditional coronary risk factors and novel, HIV-specific factors (Deeks et al. 2013).

Early Cardiovascular Complications

From the initial stages of the HIV epidemic even prior to the introduction of widespread ART, cardiomyopathy was recognized as an important comorbidity. HIV-infected patients were noted to have disproportionate rates of left ventricular dysfunction and dilated cardiomyopathy. Autopsy studies suggested myocarditis as the major cause of cardiac dysfunction, though the etiologic agent remained unknown in the vast majority of cases. Chronic viral infections including cytomegalovirus (CMV) or herpes simplex virus (HSV) in addition to bacterial infections such as *Mycobacterium tuberculosis* and *Toxoplasma gondii* and HIV itself were also implicated. Rates of dilated cardiomyopathy fell after the introduction of ART, likely in the setting of better virologic control and reduced rates of infections related to immunosuppression.

Pericarditis has also been identified as a cardiovascular complication of HIV infection since the early days of the epidemic, with patients presenting with pericardial effusions and cardiac tamponade. The underlying etiology, when able

to be identified, often related to opportunistic infections such as *Mycobacterium tuberculosis* or *Cryptococcus neoformans* or AIDS-associated malignancies such as lymphoma or Kaposi's sarcoma.

Endocarditis has remained a notable complication of HIV infection given the shared mode of transmission of intravenous drug use. Early autopsy reports frequently found nonbacterial thrombotic (marantic) endocarditis in HIV patients, but this association has not been seen in the ART era. Among cases of bacterial endocarditis, *Staphylococcus aureus* occurred most frequently, with other significant pathogens including *Streptococcus pneumoniae*, *Candida albicans*, *Haemophilus influenzae*, and *Cryptococcus neoformans*.

HIV and Coronary Heart Disease

As ART became more widely available in resource-rich settings, case reports emerged of myocardial infarction in HIV-infected patients, particularly those on protease inhibitor (PI)-based therapies. These events occurred with the recognition that patients on ART had increased rates of metabolic disorders such as diabetes mellitus, insulin resistance, and dyslipidemia. Initial reports subsequently led to larger, more systemic examination of rates of cardiovascular disease among HIV-infected patients compared to control patients. The result has been consistent evidence showing a 1.5- to 2-fold higher relative risk of myocardial infarction (MI) or coronary heart disease among HIV-infected patients across various time periods and a variety of clinical settings. Accumulated data indicates that the increased risk of CHD is independent of demographic factors or traditional cardiovascular risk factors. Specific subpopulations of HIV-infected patients bear a disproportionate amount of CHD risk. HIV-infected women appear to be at greater relative risk than men, and hepatitis C virus (HCV) coinfection also may increase CHD risk (Boccarda et al. 2013; Freiberg et al. 2013; Grinspoon et al. 2008; Triant et al. 2007).

Cardiovascular Risk Factors in HIV

The underlying reason behind the heightened risk of CHD in HIV infection likely reflects a complex array of factors including traditional cardiovascular risk factors, medication effects, and immunologic and inflammatory changes.

Traditional Cardiovascular Risk Factors

HIV-infected patients exhibit higher rates of traditional cardiovascular risk factors, including hypertension, smoking, diabetes, and dyslipidemia. These trends may explain a component of elevated CHD risk. Rates of smoking among HIV-infected patients have been reported to be double that of non-HIV-infected patients. Quitting smoking has been shown to decrease cardiovascular risk for HIV-infected patients, with greater risk reduction with longer times since quitting. Elevated rates of diabetes and dyslipidemia in HIV-infected patients highlight the interplay between ART and traditional risk factors. Some selected antiretroviral medications have been implicated in higher incidence rates of insulin resistance and diabetes among HIV-infected patients, although these metabolic effects were most widely seen with medications such as stavudine and indinavir, which are currently less commonly used. Prior to the ART era, patients with advanced AIDS were noted to have lower levels of favorable high-density lipoprotein (HDL) cholesterol and elevated levels of triglycerides. Treatment with antiretroviral therapy tends to raise all lipid indices, although the net result is an unfavorable lipid profile given lower increases in HDL relative to other lipid indices. In the current treatment era among the different classes of ART, PIs in particular have been associated with dyslipidemia. Ritonavir, even when used at lower doses for pharmacokinetic boosting, is associated with hypertriglyceridemia. Newer PIs, such as atazanavir and darunavir, demonstrate lower but still persistent levels of lipid abnormalities. Antiretroviral therapy, particularly medications used in the earlier stages of the epidemic, can cause fat redistribution with lipoatrophy in the limbs and face and lipohypertrophy of existing fat deposits.

The clinical features of this syndrome are similar to those of the metabolic syndrome of obese, non-HIV-infected patients (Grinspoon et al. 2008; Grunfeld et al. 2008; Grinspoon and Carr 2005).

The Role of Antiretroviral Therapy in Cardiovascular Risk

Several antiretroviral medications have been directly implicated in the development of cardiovascular events. Initial evidence indicated that the PI class of medications had a direct association with MI not entirely explained by changes in dyslipidemia. Further studies indicated that this finding may be driven by the particular effects of lopinavir/ritonavir, amprenavir/ritonavir, fosamprenavir/ritonavir, and indinavir, medications that are used with decreasing frequency. The nucleoside reverse transcriptase inhibitor (NRTI) abacavir has also been investigated regarding its possible association with MI. There is no consensus as to whether abacavir is associated with MI, although meta-analyses do not support an association (Ding et al. 2012; Friis-Moller et al. 2007).

Inflammation and Immune Dysfunction

While traditional cardiovascular risk factors are more prevalent in HIV and specific antiretroviral medications may contribute to risk, these factors do not fully account for the increased risk seen by HIV-infected patients. Subsequent efforts in outlining the relationship between HIV and CHD therefore focused on inflammatory and immunologic changes in HIV. Both advanced and moderate immunosuppression, with CD4+ T-cell counts less than 200/ μ L or less than 500/ μ L, have been associated with MI, with risk on the same order of magnitude as traditional CHD risk factors. These findings may be related to the importance of chronic inflammation and immune activation in HIV-associated CHD pathogenesis.

Chronic inflammation has been associated with CHD outcomes in HIV. Multiple inflammatory indices, including interleukin 6 (IL-6), D-dimer, and C-reactive protein (CRP), are elevated in HIV-infected patients. Initial interest in

the role of chronic inflammation stemmed from investigation showing that an interrupted ART strategy, when compared with one of continuous ART use, was associated with both increased cardiovascular event rates and with increased markers of chronic inflammation, including IL-6 and D-dimer. These markers were notably found to be elevated after treatment interruption, coincident with cardiovascular events. In addition to elevated levels in HIV infection, CRP has also been shown to be associated with MI among HIV patients. Activation markers also shed light into the immune-mediated pathogenesis of CHD. T-cell activation markers, which decrease with ART, are connected to cardiovascular disease progression as measured by surrogate markers of atherosclerosis including carotid intima-medial thickness (cIMT). Macrophage and monocyte activation markers, notably soluble CD163 and soluble CD14, have also been linked to CVD disease progression among HIV populations (Deeks 2011; El-Sadr et al. 2006).

While virologic suppression with ART reduces inflammatory and immune activation markers, it does not completely normalize them. Even elite controllers, HIV-infected patients who maintain viral suppression without ART, demonstrate evidence of an activated immune response, suggesting that patients with controlled HIV disease in the long term will still confront an elevated risk of cardiovascular disease. Other sources of chronic inflammation which disproportionately affect HIV-infected patients and may heighten cardiovascular risk include chronic infections such as CMV, HSV, or HCV (Hsue et al. 2009).

Acute Coronary Syndrome Characteristics

In addition to increased disease incidence, HIV-infected patients have altered presentation, management, and subsequent outcomes of cardiovascular events. HIV-infected patients are more likely to present with an ST-elevation MI (STEMI) as opposed to a non-ST-elevation MI (NSTEMI) or unstable angina. There also may

be altered angiographic presentation with HIV-infected patients more likely to have single-vessel disease or less complex lesions. The management of acute events also differs with HIV-infected patients receiving lower rates of anticoagulation, cardiac catheterization, and coronary artery bypass graft (CABG) surgery. Regarding outcomes, HIV-infected patients have higher rates of in-hospital cardiovascular mortality and recurrent MI. They also have higher rates of subsequent hospitalization for heart failure. It remains unclear whether outcome differences are driven by differing underlying pathophysiology or by differing rates of interventions.

Cardiovascular Outcomes and Surrogate Markers of Cardiovascular Disease

HIV infection may predispose to other cardiovascular complications beyond coronary heart disease. Stroke and peripheral arterial disease rates appear to be higher among HIV-infected patients compared with control populations, with similar underlying mechanism as ischemic coronary heart disease. Additionally, congestive heart failure, atrial fibrillation, and sudden cardiac death disproportionately affect HIV-infected patients. Whether ischemia-related cardiomyopathy and arrhythmias account for these findings remains unclear.

HIV-associated cardiovascular disease can also be demonstrated using surrogate markers of atherosclerosis including imaging studies. Carotid intima-medial thickness (cIMT) is a marker of subclinical atherosclerosis measured by ultrasound that is associated with cardiovascular events. HIV-infected patients generally have higher cIMT than non-HIV-infected patients. Additionally, cIMT progresses more rapidly among HIV-infected patients, notably at the carotid bifurcation. Coronary artery plaque burden, measured by computed tomography (CT), is greater among HIV-infected patients. This increased plaque burden is also more likely to be noncalcified, a characteristic associated with a higher likelihood of rupture. Imaging studies also show the heightened inflammation associated

with HIV. Positron emission tomography (PET) imaging shows that arterial, and in particular aortic, inflammation is elevated in HIV infection and associated with markers of monocyte and macrophage activation. Apart from imaging studies, HIV-infected patients demonstrate the downstream effects of cardiovascular risk factors through measurements of worsened flow-mediated dilation of the brachial artery, which is a proxy for endothelial function, and poorer measures of arterial elasticity.

Cardiovascular Risk Reduction in HIV

Optimal cardiovascular risk reduction in HIV infection involves aggressive management of traditional CHD risk factors as well as consideration of novel, HIV-specific risk reduction strategies. However, a major current challenge is the difficulty in accurate cardiovascular risk prediction. Current risk prediction models for the general population employ demographic and traditional CHD risk factors. These models designed for the general population have yet to be validated for use among HIV-infected patients. Additionally, methods of risk prediction which incorporate novel HIV-related risk factors such as ART use and markers of inflammation or immune activation have not been widely validated and have not been incorporated into clinical practice (Schambelan et al. 2008).

Regarding management of traditional CHD risk factors, smoking cessation may be one of the most important interventions given the disproportionate impact of smoking on the HIV-infected population. Recommended interventions include screening, counseling, participation in cessation groups, and adjunctive pharmacologic therapies and are based on recommendations for the general population. The cornerstone of dyslipidemia therapy is pharmacologic management with agents such as HMG co-A reductase inhibitors (statins) and fibrates. Statins may be less effective at LDL cholesterol reduction in the setting of HIV compared with the general population, but their anti-inflammatory properties may mitigate the effects of HIV-specific chronic inflammation. The indications for statin treatment in HIV have not been

established but are under active investigation. Vigilance is required given the interactions between selected statins and PIs through the cytochrome P450 pathway. New cholesterol guidelines released for the general population by the American College of Cardiology (ACC)/American Heart Association (AHA) in 2013 can be used to inform HIV practice until further HIV-specific recommendations become available. Blood pressure should be measured regularly and managed according to Joint National Committee (JNC 8) guidelines. Whether aspirin has a differing role for the primary and secondary prevention of CHD in HIV infection has yet to be elucidated; accordingly, its use should mirror that for the general population.

Apart from management of traditional risk factors, modification of chronic inflammation and immune activation is likely to be important as a preventive strategy. Initiation of antiretroviral therapy resulting in virologic suppression and immune reconstitution is a critical first step toward achieving these goals. Importantly, the net beneficial effects of antiretroviral therapy with concomitant virologic suppression are thought to outweigh any proatherogenic effects of individual antiretroviral medications. This shift in thinking has resulted in acceptance of the use of ART as a cardioprotective strategy, an approach reflected in the most recent HIV treatment guidelines. Given that inflammation and immune activation persist even after viral suppression, the role of additional immunomodulatory interventions will likely become important in minimizing CHD risk for HIV-infected patients even further (Aberg 2009).

Conclusion

HIV infection is associated with a wide range of cardiovascular complications. The early years of the HIV epidemic were characterized primarily by complications related to opportunistic infections and advanced immunosuppression. With the advent of ART and the prolonged life span of HIV-infected patients, CHD has emerged as the major cardiovascular complication, with presentation and sequelae that differ from those of the

general population. The pathogenesis of cardiovascular disease in HIV infection is distinct from that of the general population because of the unique contributions of ART, chronic inflammation, and immune activation. Treating HIV to achieve virologic suppression and aggressively controlling traditional risk factors should be employed to decrease cardiovascular risk among HIV populations. HIV-specific tailored prevention measures will likely help further diminish CHD risk among HIV-infected patients in the future.

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cART and Supportive Care for HIV-Associated Malignancies

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Definition

cART is an abbreviation for combined antiretroviral therapy and generally refers to a three- or four-drug combination regimen of antiretroviral medications, which is considered standard treatment for human immunodeficiency virus-1 (HIV-1) infection. Another name for cART is highly active antiretroviral therapy (HAART). This treatment is important for suppressing HIV-1 replication and has greatly increased the longevity of individuals with this infection. As part of the treatment of malignancies in HIV-infected patients, cART and other supportive measures such as antiemetics, hematopoietic growth factors, blood transfusions, prophylactic and therapeutic antibiotics, antidiarrheal medications, and psychosocial support for each individual undergoing the stressful therapy for these often life-threatening cancers have greatly enhanced both the efficacy and tolerability of these treatments and allowed patients to achieve remissions and/or cure of their cancers.

cART as Treatment for HIV and Cancer

Cancers have been a recognized as part of the acquired immune deficiency syndrome (AIDS)

since the initial recognition of this disease in 1981 (Hymes et al. 1981; Ziegler et al. 1982, 1984a). Initially reported mortality from Kaposi's sarcoma (KS) and non-Hodgkin's lymphoma (NHL), the two most common of these AIDS-associated malignancies (► [Cancers Related to HIV](#)), was extremely high, in the range of 40% at 2 years for KS and 80% at 1 year for NHL with what then was considered standard chemotherapy (Longo et al. 1984; Moss et al. 1984; Ziegler et al. 1984b). With the advent of combination antiretroviral therapy (cART), not only have HIV treatment outcomes improved but the ability to dose-intensify cancer treatment regimens has increased, allowing better control of cancer, fewer treatment-related serious adverse effects and complications, higher remission rates, more durable remissions, and longer survival. It has been reported that HIV-associated diffuse large B-cell lymphoma can be successfully treated with a regimen that involves HAART being temporarily stopped during therapy. However, there is a general consensus among most clinicians with expertise in this area that when possible, HIV should be controlled with HAART if possible during the treatment of HIV-related malignancies and other diagnosed cancers in patients with HIV/AIDS (Lim et al. 2005; Thiessard et al. 2000; Little et al. 2000). Single and multi-institutional studies have shown that HAART can be administered along with anticancer therapy as long as attention is paid to pharmacokinetic interactions and overlapping toxicities. The increasing occurrence of cancer as a major part of the HIV/AIDS epidemic and the recognition of cancer as a leading cause of death in HIV have led to a greater emphasis on initiating treatment with cART earlier and to continue its use when possible during management of patients who develop cancers during the course of their disease.

Drug Interactions Between cART and Chemotherapy

Of concern has been the potential for significant drug interactions between some of the currently used antiretroviral drugs (ARVs) and some

chemotherapeutic and other treatments for malignancies. Given the relatively small number of these patients and the need to get lifesaving ARVs out to the HIV-infected community as quickly as possible, with a few exceptions, little attention was focused on the interactions of cancer therapies with ARV, and few pharmacokinetic studies were undertaken early on in the AIDS epidemic to assess the degree and influence of these drug interactions on the outcome of patients with AIDS and cancers (Ratner et al. 2001). Some of these adverse drug interactions have been found empirically in the course of treating HIV-infected individuals with specific malignancies, such as the well-known enhanced myelosuppressive effects of many cytotoxic chemotherapy drugs with zidovudine and the serious increase in irinotecan gastrointestinal toxicities with certain protease inhibitors for HIV (Kaplan et al. 1997; Corona et al. 2005; Makinson et al. 2001). With the growing realization that cancer is now a growing problem in HIV, especially as the population of HIV-infected individuals ages and because of increased longevity in the setting of continued immunodeficiency, and with the ever-growing list of available new cancer treatment agents, it has become increasingly important that HIV-treating physicians and oncologists know and are able to make appropriate dosage adjustments for possible cancer therapy-ARV interactions and to avoid certain combinations of drugs which may lead to severely enhanced toxicities. Most clinical trial protocols for treatment with cytotoxic chemotherapies now require that patients not receive zidovudine when receiving myelosuppressive chemotherapy or will restrict the use of dideoxynucleosides or other drugs that may significantly induce peripheral neuropathy (PN) when using cancer therapies with high potential for PN. More recently the National Cancer Institute's (NCI) Clinical Therapy Evaluation Program (CTEP) has embarked on formally assessing the pharmacokinetic/pharmacodynamic (PK/PD) interactions of antiretroviral drugs with new investigational cancer agents in conjunction with the NCI-supported AIDS Malignancy Consortium (AMC). These phase I PK/PD studies will hopefully enable greater participation of

HIV-infected patients with cancers in general oncology clinical trials for various cancers, thus making the results of these phase II and III trials more widely applicable and avoiding the previous situation where HIV patients would be excluded from studies of new investigational cancer drugs, because of concerns about unknown and potentially harmful drug interactions with antiretroviral treatments.

Supportive Care for HIV Patients Receiving Cancer Treatments

Another important principle in the management of individuals with cancer and HIV is the need to provide adequate medical and logistical support for patients needing to receive therapy for their cancers. It is well known that patients with HIV, especially those with advanced HIV disease, e.g., those with lower CD4 counts or a history of prior opportunistic or other infectious diseases, generally have poorer marrow tolerance to the myelosuppressive effects of chemotherapy and, in some cases, radiotherapy as well. High rates of infections have been reported in trials of chemotherapy for HIV-associated lymphoma with regimens such as R-CHOP and R-EPOCH (Kaplan et al. 1997, 2005), which may be avoided to a large degree with the use of hematopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), blood transfusions, and prophylactic antibiotics (Kaplan et al. 1994; Scadden et al. 1991; Barta et al. 2012; Sparano et al. 2010). In a lymphoma trial conducted by the AMC, the routine use of prophylactic antibiotics dramatically reduced the incidence of severe infections in patients receiving intensive chemotherapy (Sparano et al. 2010).

Neurologic complications of HIV also may confound the management of patients with HIV and cancers, as peripheral neuropathy is a common complication of HIV and a not infrequent side effect of some ARVs and several anticancer drugs (e.g., vinca alkaloids, cis-platinum, oxaliplatin, bortezomib, etc.). In addition,

progressive cognitive impairment due to progressive and long-term effects of HIV or to central nervous system (CNS) involvement with malignancy may impact the patient's ability to adhere to HIV, cancer treatments, and associated therapies for complications or side effects of these treatments.

The management of nausea, vomiting, pain, diarrhea, rashes, headaches, and other side effects of cancer and HIV therapies is critical and can be very effectively controlled with prophylactic treatments or mitigated post hoc with standard antiemetics, antidiarrheals, analgesics, etc. to allow patients to better tolerate and continue to receive important drug treatments.

As with anyone diagnosed with a life-threatening disease such as cancer, psychological support and the ability to listen to the patient's and their caregiver's concern are critical for the successful outcome of treatment. With the added burden of having HIV and the many financial, social, and logistical issues for providing the care that this engenders, it is particularly important to assure that a working support system is in place to assist the patient in maneuvering the healthcare system and getting the help they need to receive optimal care. In this regard, the concept of patient navigators (PNs) has received considerable attention as a means of facilitating patient access and their ability to navigate the complex medical and social-financial situation caused by diseases such as cancer and HIV. With the assistance of the Center to Reduce Cancer Health Disparities (CRCHD) within the NCI, the AMC has piloted and is investigating the usefulness of PNs as a means of improving access of HIV cancer patients to AMC clinical trials and for increasing the ability of individuals from racial/ethnic minorities and other underrepresented groups (such as women) to take part in these clinical trials and to maneuver the complexities of obtaining required diagnostic and therapeutic procedures and services for their malignancies. Understanding the special burdens faced by cancer patients with HIV, especially as it relates to obtaining needed social and psychiatric services to help deal with social ostracism and

isolation, lack of transportation, loss of insurance, unemployment, poverty, homelessness, drug use, under or poor nutritional state, and multiple comorbid diseases, is critical in allowing patients to fully benefit from the many recent advances in cancer diagnostics and therapy which have occurred in the past few decades. Unfortunately, faced with severe budget deficits, many state- and locally funded organizations and programs set up to provide social services, case managements, and other support services have had their funding dramatically reduced, making it more difficult for individuals to adhere to the strict treatment schedules required for some of the more complicated treatment regimens.

Cancer Screening in HIV

Early diagnosis and established prevention strategies for many cancers should also be considered in the routine management and follow-up of the HIV-infected individual. Age-appropriate American Cancer Society (ACS), NCI, and US Preventive Services Task Force (USPSTF) cancer screening guidelines should, as a minimum, be followed for screening HIV-infected individuals for cancer and/or precancerous lesions, as they would be for non-HIV-infected adults. In addition, routine monitoring for cervical and anal dysplasia and intervention for high-grade squamous intraepithelial lesions (SIL) have been recommended or are under evaluation to determine the best frequency for monitoring in HIV-infected patients, where the relative risk of cervical (► [Cervical Cancer](#)) and/or invasive anal cancer (► [Anal Cancer](#)) is higher than in the general population. Whether HIV patients would benefit or should be checked and monitored more closely or more frequently for certain AIDS-related cancers (ADC) or non-AIDS-related cancer (NADC) because of their higher risk of developing these malignancies remains to be determined. Future prospective studies will be needed to assess the efficacy and cost effectiveness of some of these routine screening procedures in HIV.

Conclusion

Significant advances in understanding and treating cancers have occurred in the past several years. Similarly, the ability to treat HIV and to provide a normal life expectancy to individuals with HIV infection has improved dramatically. Subsequently, malignancies in HIV are now emerging as a growing problem and will require greater vigilance on the part of the primary care providers to identify these cancers and a heightened awareness on the part of HIV physicians and oncologists about the many complications and special needs of individuals who have these diseases concurrently.

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Case Management for Linkage

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Definition

Case Management

Case management is a collaborative process that includes client identification, outreach, engagement, assessment, planning, coordination,

linkage, monitoring, reassessment, and discharge planning to meet the health and social service needs of clients.

Linkage to HIV Care

Conceptually, HIV care is challenging to define and can best be illustrated along a continuum that ranges from people not aware of their status to people who are fully engaged in care and have achieved viral suppression (Gardner et al. 2011). Linkage to HIV care represents a point along this continuum after a person is diagnosed with HIV and initially seeks care from an HIV provider (Mugavero et al. 2013).

Main Text

Background

In the United States, over 1.2 million people currently live with human immunodeficiency virus (HIV), and approximately 1 in 6 of those infected is not aware they have the virus (Centers for Disease Control 2016). While the incidence of infection has remained relatively stable, disparities exist among racial, ethnic, and sexual minorities, and some regions, such as the Southeast, have been disproportionately impacted (Centers for Disease Control 2016).

Treatment advances with antiretroviral therapy (ART) have contributed to both population and individual benefits. At the individual level, those who are in care and receiving ART are more likely to experience decreased mortality, decreased viral load, and decreased morbidity and have life expectancies now equal to that of the general population (Quinn et al. 2000; Anglemyer et al. 2013; Loutfy et al. 2013; Samji et al. 2013). At the population level, people with HIV who take therapeutic levels of ART are less likely to transmit the virus compared with those who delay ART (Anglemyer et al. 2013). They are also more likely to abstain from having unprotected sex with someone who is HIV negative or status in unknown (Metsch et al. 2008); thus, getting people into HIV care is a public health priority for preventing transmission of the virus.

With research supporting the individual and population benefits of being in HIV care, there has been increased focus on helping people become aware of their HIV status through expanded testing efforts and getting them into treatment as soon as possible (test and treat). However, just because someone knows their HIV status does not mean they will seek care, and approximately 25–31% of persons with HIV/AIDS fail to seek treatment after initial diagnosis (Gardner et al. 2011).

There are numerous barriers to linkage to HIV care, and these can be organized by social ecological levels and may include individual (mental health, substance use, stigma, housing, transportation, and access to care), relationships (partner, family, friends, providers), community (poverty, social norms, stigma), healthcare system (appointment and service availability), and policy (funding, quality, service coordination, workforce) (Mugavero et al. 2013). Many people with HIV each face multiple and unique combinations of barriers necessitating a patient-centered approach to linkage to care.

Linkage to care has been identified as a priority by the Presidential National HIV/AIDS Strategy (NHAS), the Centers for Disease Control (CDC), and the Health Resources and Services Administration (HRSA). Several interventions exist that help facilitate linkage to HIV care after someone receives an HIV diagnosis.

Case Management Interventions

Case management can be an integral component for the treatment and management of acute and chronic disease, and, according to the International Advisory Panel on AIDS Care guidelines, it is a recommended intervention to increase linkage to HIV care (IAPAC Guidelines 2015). There are many models of case management (i.e., broker, strengths, assertive community treatment, clinical/rehabilitative), and it is practiced among a variety of disciplines (nursing, social work, rehabilitation) and in a variety of settings (hospital, community, insurance, and private industry). The Health Resources and Services Administration HIV/AIDS Bureau (HAB) outlines the concepts of HIV medical case

management as being client centered, focused on linkages, coordination, and continuity of care and services (Health Resources and Services Administration 2008). Six core functions serve as the foundation for HIV case management practice in federally funded programs including client identification, outreach and engagement, assessment, planning, coordination and linkage, monitoring and reassessment, and discharge.

The most well-known case management intervention for linking newly diagnosed people to HIV care is Antiretroviral Treatment and Access to Services (ARTAS). The ARTAS intervention is one of the few interventions that has been shown effective in a randomized controlled trial to increase the percentage of people newly diagnosed with HIV linked to care (Gardner et al. 2005) and further tested for effectiveness in community settings (Craw et al. 2010). ARTAS is grounded in a strength-based case management approach and typically consists of a limited number of sessions with a linkage coordinator. During the sessions, the linkage coordinator assesses the client's strengths and, with input from the client, identifies goals and develops a structured plan to meet those goals. Often linkage coordinators are partners with community-based organizations, support groups, and other providers to aid in referrals to formal and informal services that may be needed by the client to promote a successful transition into care. Once the client engages in HIV care or the limited number of sessions have been used, the linkage coordinator's relationship with the client is terminated. Implementing this intervention in sites with co-located care yielded the best success in linking to HIV care (Craw et al. 2010).

Navigation and Outreach Models

Other models of care utilizing case management approaches have been evaluated in the Special Projects of National Significance (SPNS). System navigation, an intervention modality that is based on a social work model, assists patients to overcome barriers (structural, financial, and personal), improve mediators (efficacy of treatment, quality of treatment, adherence), and improve outcomes (morbidity, mortality, well-being, and functioning)

(Bradford et al. 2007). If delivered by peers with HIV, this type of model requires assistance from trained personnel to help patients navigate through the system and treatment, but peers are not always adequately trained for the challenging process (Bradford et al. 2007) and the efficacy of navigation programs is not well known (Koester et al. 2014).

Recommendations for Successful Linkage to Care Programs

There are several characteristics, core components, and operational strategies for successful linkage to HIV care. Successful linkage programs are low cost, intensive, time limited, and flexible; however, the interventions specifically focus on linkage to care instead of multiple points in the HIV care continuum (Liau et al. 2013; Gilman et al. 2012). They will utilize dedicated linkage staff, employ active referral strategies, maintain a patient-centered approach, and strive for cultural concordance between staff and the population served (Gilman et al. 2012). From the systems perspective, these programs will develop and adhere to protocols for the linkage process, select staff that are appropriately trained and effective in working with the population, seek to coordinate and integrate services, and strive to maintain consistent funding for sustainability.

Limitations of Current Intervention Strategies

Geographic area of residence, funding, and effectiveness with certain populations present limitations to current case management intervention strategies. While the interventions enhancing linkage to care have shown promise in large metropolitan areas, implementation in smaller targeted metropolitan/rural areas has met with challenges in funding and feasibility. National funding shortages for HIV care challenge implementation of linkage to care strategies that require additional personnel support (Mugavero et al. 2011). Finally, several population subgroups have not responded as well to case management interventions. In the ARTAS studies, people with mental illness or substance use problems did not respond as well to the intervention (Gardner et al. 2009) although referring to a case manager soon

after diagnosis is associated with improved health outcomes (Kenya et al. 2014). In the prison population, an intensive, strength-based case management intervention designed to help the transition into HIV care was no more effective than the standard of care, which consisted of intensive dedicated pre-discharge planning by a prison nurse (Wohl et al. 2011). However, patient navigation could be a potential approach to improve linkage in this population (Koester et al. 2014). To optimally engage people in care, further research is needed in these hard to engage populations.

Conclusion

It is essential that we develop strategies to prioritize and target interventions to promote linking to HIV care in a timely manner that are effective, feasible, and efficiently use available resources. Developing strong partnerships within communities may help with implementation of case management interventions such as ARTAS. The successful linkage to care case management interventions presented in this section all include assessment, referral, navigation, and the provision of social support. Linkage to HIV care remains an important concern among healthcare providers and public health officials in both prevention and care goals. In the design of interventions for linkage to HIV care, it is important that the intervention include (1) coordination between public health agency and medical providers, (2) continuity of care from the time of diagnosis until the person is situated in HIV care, and (3) advocacy for the client to ensure appropriate services are obtained.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Behavioral Aspects of HIV Treatment as Prevention](#)
- ▶ [Behavioral Science Highlights of Evidence and Research](#)

- ▶ [Clinical Ethics in HIV/AIDS Prevention, Care, and Research](#)
- ▶ [Combination Approaches to HIV Prevention](#)
- ▶ [Comorbidity: Opioids](#)
- ▶ [Healthcare Workers, Shortage and Task Shifting of](#)
- ▶ [HIV Counseling and Testing, Prevention of HIV](#)
- ▶ [HIV Prevention Efforts Within Substance Use Disorder Treatment Settings](#)
- ▶ [HIV Prevention for Serodiscordant Couples](#)
- ▶ [HIV Prevention in the Correctional System](#)
- ▶ [HIV Testing and Counseling](#)
- ▶ [Multilevel Interventions/Structural Approaches to HIV Prevention](#)
- ▶ [Peer-Based Intervention Approaches](#)
- ▶ [Positive Health, Dignity, and Prevention \(PHDP\)](#)
- ▶ [Prevention Counseling and Other Strategies in the HIV Care Setting](#)
- ▶ [Prevention for People Living with HIV](#)
- ▶ [Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#)

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Cell-Intrinsic Immunity

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Definition

Eukaryotes have been under attack of retroviruses for millions of years; thus different mechanisms evolved to protect cells immediately after infection from dissemination. These mechanisms include a diverse array of proteins that are constitutively expressed in cells, but the expression of many can be augmented at the first signs of evasion. The potent enzymatic activities that can abort or inhibit to some extent viral replication are the component of the cellular intrinsic immunity. This system confers the first defense line of the organism and also signals for the innate and adaptive immune system to mobilize their effectors.

Introduction

Human immunodeficiency type 1 (HIV) is a lentivirus that infects and replicates in immune cells that express the CD4 cell surface receptor and a chemokine coreceptor, namely, CD4⁺ T helper, macrophages, and likely dendritic cell subsets. Although the host employs multiple cell-intrinsic restriction mechanisms against HIV, none is effective enough to completely block viral replication,

whereas the virus has evolved a number of strategies to effectively circumvent most if not all of these mechanisms. Thus, despite the activation of cell-intrinsic immunity and its numerous effectors, HIV continues to replicate in these cells, resulting in high-level viremia in most infected individuals and eventual exhaustion and depletion of the CD4⁺ T-cell population. In the absence of antiretroviral medications, over time HIV⁺ individuals will invariably progress to AIDS and death.

Antiretroviral therapy (ART) includes a combination of medications that typically target different steps in HIV life cycle: virus binding to host cells, cell fusion, reverse transcription, genomic integration, and viral particle maturation. Unfortunately, although ART can halt and often reverse the immunological failure induced by HIV, it does not result in cure or virus eradication and necessitates lifelong treatment. Understanding the host restriction factors and the counteracting viral evasion mechanisms may provide insight into novel approaches to manipulating the immune system in controlling viral replication and combating this devastating disease.

The expression of many, if not most, of the recently identified intracellular restriction factors, including IFITMs, Trim5 α , APOBEC3G, SAMHD1, MX2, Schlafen-11, KAP1, HERC5, and Tetherin, is induced by type I interferons. We will first focus on the interferon response induced by HIV and subsequently we will review the scientific data that have been accumulated for a subset of the restriction factors. It should be emphasized that thus far there is no convincing data *in vivo* in humans that any of these restriction factors plays a critical role in controlling the replication of HIV.

The Type I Interferon Response Suppresses HIV Replication

As the role of interferons in the antiviral response was known since the early 1960s, the effect of interferon upon HIV replication was investigated soon after the virus was identified as the causative agent of AIDS. The suppression of HIV

replication in human PBMCs (peripheral blood mononuclear cells), measured by a reduction in reverse transcriptase activity of 50–75%, was demonstrated to be due to interferon- α and interferon- β , but not interferon- γ , by Yamamoto et al. in 1986. Subsequent work by Wong showed that pretreatment of cells with either interferon- γ or TNF- β reduced susceptibility to HIV infection and inhibited the production of HIV mRNA, CA protein, Capsid, and infectious virions (Wong et al. 1988). The observed effects of both host factors were synergistic, suggesting independent mechanisms were at play.

Evidence of HIV suppression by interferon in the clinic accumulated slowly, originating from observations made during treatment of HIV/HBV co infected patients and also from treatment of patients with advanced HIV and Kaposi's sarcoma (KS), in which circulating levels of CA were reduced by interferon. These encouraging results led to the combined use of interferon and zidovudine for the treatment of AIDS patients suffering from KS (Kovacs et al. 1989).

The effectiveness of interferon- α therapy for asymptomatic HIV⁺ adults was tested in a 12-week randomized, placebo-controlled clinical trial. Significant differences were found between the interferon- α -treated and control group with respect to positivity of HIV cultures obtained from peripheral blood (59% vs. 87%), respectively stabilization of peripheral CD4⁺ T-cell counts, and slowing the progression to AIDS. These promising effects, however, were accompanied by a high dropout rate of 35% in the interferon-treated arm, due to toxicity and adverse events (Lane et al. 1990). Other early studies evaluated the effects of interferon- α , β , and γ upon HIV in man, most commonly combining the interferon with early antiretroviral medications that were in different stages of development. The necessity of parenteral administration, the high incidence of adverse events, the questionable additive effects in comparison to antiretroviral drugs alone, and the high efficacy of combination antiretroviral medications left interferon out of the armamentarium and off the shelves, excluding it as a major treatment option by the late 1990s. Some insight into dual effects of IFN in humans

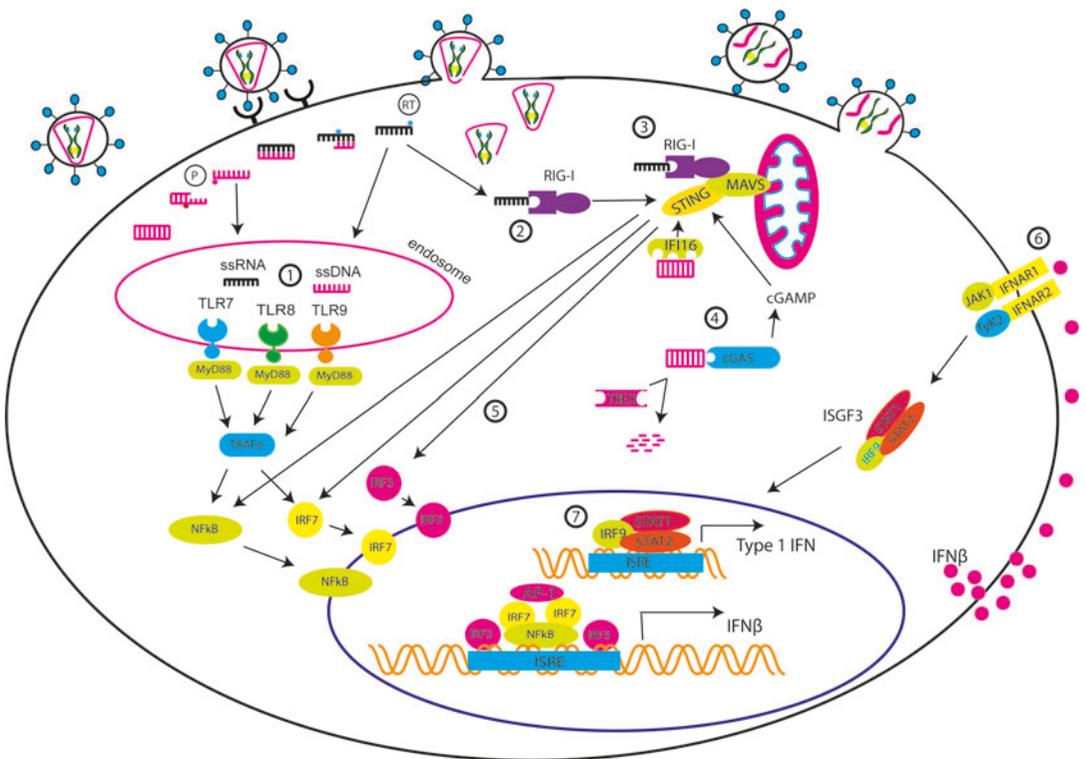
can be gleaned from recent experiments in rhesus macaques that demonstrated the effects of IFN upon resistance to SIV infection and also disease progression. Blockade of the IFN-I receptor reduced antiviral gene expression and accelerated CD4 T-cell depletion, resulting in rapid progression to simian AIDS. Although IFN- α 2a administration induced transient resistance to SIV infection, surprisingly it also led to the same unfortunate accelerated disease progression as a result of induction of a negative feedback mechanism mediated by FOXO3a (Sandler et al. 2014). The hope of identifying antiviral host factors and how the virus counteracts each one was the driving force for further exploring the interferon-

induced anti-HIV response. If precise mechanisms could be uncovered as to how the interferon-induced host response is evaded, that could be the impetus driving the development of novel antiviral therapeutics.

Detection of HIV Infection by the Host

See (Fig. 1)

The cascade of events that results in IFN production is complex and is highly regulated by both positive and negative feedback mechanisms. Many of the components and regulators of the cascade are currently known, but it is likely that many others will be identified in the future. The initiation and activation of this signaling pathway



Cell-Intrinsic Immunity, Fig. 1 Virus detection by intracellular receptors and induction of type 1 IFN. (1) Viral ssRNA and ssDNA are detected in the endosome by TLRs and induce IRF3, IRF7, and NFkB activation via MyD88 and TRAF6. (2) After RIG-I detects ssRNA, it relocates to the mitochondrion where it interacts with MAVS (3). (4) dsDNA is detected by cGAS and IFI16, activating STING. TREX1 degrades cytosolic DNA, inhibiting STING activation. (5) Activation of MAVS and STING results in NFkB signaling and formation of the enhanceosome

(composed of NFkB, IRF3, IRF7, and AP-1), which induces transcription of IFN- β . (6) IFN- β is secreted from the cell and binds to the IFN receptor in both a paracrine and autocrine manner, thus activating the IFN pathway. JAK/STAT activation by receptor binding results in the formation of ISGF3 (composed of phosphorylated STAT1 and IRF9), which binds to ISRE (IFN-stimulated responsive element), and stimulates transcription of type 1 IFN and hundreds of ISGs (including those shown in Fig. 1). RT reverse transcriptase, P DNA polymerase



first requires identifying and discriminating viral components called PAMPs (pathogen-associated molecular patterns) as nonself by PRRs (pattern recognition receptors). When they accumulate in the cytoplasm, single- and double-stranded RNA, uncapped RNA, and various forms of DNA are all PAMPs that induce immune signaling. The major classes of cellular molecules relevant to HIV that serve as PRRs include TLRs (toll-like receptors), RLRs (RIG-I-like receptors), or retinoic acid inducible gene I-like receptor NLRs (NOD-like receptors), or nucleotide binding oligomerization domain receptor and DNA sensors. Each of these PRR classes activates different adaptor proteins and pathways to induce the production and release of interferon, which in turn acts in a paracrine and autocrine manner to induce the expression of hundreds of interferon-stimulated genes or ISGs. These ISGs then act by various mechanisms to limit or curtail viral replication, including that of HIV (Blanco-Melo et al. 2012; Malim and Bieniasz 2012).

The TLRs, or toll-like receptors, are transmembrane proteins located at the plasma or endosomal membrane and play a major role in pathogen sensing and signal transduction. The TLRs that act as sensors for the presence of HIV in the cell are TLR7 sensing (ssRNA), TLR9 recognizing (ssDNA), and to lesser extent TLR8 identifying (ssRNA) (Buitendijk et al. 2014). In the case of HIV, these various forms of nucleic acids may be released from dead or dying cells. The role of TLR3 in HIV recognition, which is a major intracellular viral sensor that recognizes dsRNA and possibly short loop hairpin RNAs, is not established. All these three TLRs are expressed at the endosomal membrane, and they utilize TIRAP/MyD88 to activate the NF κ B pathway (Chakrabarti and Simon 2010; Rustagi and Gale 2014).

The RLRs, RIG-I-like receptors, are a family of cytosolic PRRs that include RIG-I, MDA5, and LGP2 which are DexD/H box RNA helicases that bind RNA, hydrolyze ATP, and scan RNA substrates for recognition motifs. RIG-I and MDA5 both contain a caspase activation and recruitment domain (CARD) that is essential for their antiviral activity. LGP2, which lacks this domain, is a

negative regulator of RIG-I and MDA5. RIG-I and MDA5 bind to different PAMPs, but after binding RNA, RIG-I and MDA5 relocate to the mitochondrial-associated endoplasmic reticulum membrane. There they interact with MAVS (mitochondrial antiviral signaling protein) and assemble into a protein complex together with STING (stimulator of interferon genes) to activate NF κ B, IRF3, and IRF7. Despite numerous reports describing the inhibitory effects of type I IFN (including subtypes of IFN- α , IFN- β , and IFN- ω) on retroviral replication, there is little evidence that HIV RNA triggers RLR signaling. RIG-I, but not MDA5, may detect incoming HIV RNA after particle uncoating in the cytosol, which results in the activation of the RIG-I pathway and possibly the suppression HIV replication (Berg 2012).

The NLR proteins (NOD-like receptors) are another diverse group of cytosolic PRRs, the prototypes of which are NOD1 and NOD2. These PRRs can identify both bacterial products and viral ssRNA and activate NF κ B and IRF3. Although the NLRs could potentially play a role in the anti-HIV cellular response, this has yet to be shown.

The DNA sensors are a very heterogeneous group; two of the members are TLR-9 and RIG-I, mentioned above. Other DNA sensors include IFI16, DNA-dependent activator of IRFs (DAI), DDX41, LRRFIP1, Ku70, and cGAS. Due to the diversity of this group of sensors, there is no characteristic mechanism and no common pathway that is triggered upon the activation of these proteins. Thus, while DAI activates IRF3, RIG-I signals through MAVS, DDX41, and IFI16 stimulate STING for the activation of IRF3 and NF κ B. LRRFIP1 binds to and activates β -catenin, which then binds to the C-terminus of IRF3, and Ku70 signals by activating the promoter of IFN- λ 1 after binding to cytosolic DNA. The ability of IFI16 to restrict HIV replication, in addition to its role as a DNA sensor, was shown recently in macrophages but not in T cells, and there is limited evidence that it is counteracted by Vpr (Jakobsen et al. 2013). cGAS was demonstrated to sense HIV DNA after reverse transcription and then synthesize cGAMP (cyclic guanosine

monophosphate-adenosine monophosphate) in order to activate STING and cause IFN- β production (Gao et al. 2013). Surprisingly, Ku70 was shown to enhance HIV replication, due to the protection of HIV integrase from proteasomal degradation (Rustagi and Gale 2014; Zheng et al. 2011).

Recent work has shown that HIV not only uses viral proteins to circumvent or inactivate the IFN-induced restriction factors but also cellular proteins. For example, HIV recruits cyclophilin A and possibly CPSF6 to camouflage and escape detection by PRRs (viz., TRIM5 α) (Rasaiyaah et al. 2013).

Transcription of Type I IFN and Subsequent Genes

As described above, sensing and identifying the various components of HIV by multiple PRRs results in elevated levels of phosphorylated IRF3, IRF7, and NF κ B, all of which are transcriptional activators of IFN- β . The assembled complex of proteins, which includes NF κ B (composed of subunits p50 and p65/RelA), an IRF3 dimer, an IRF7 dimer, and AP-1 (composed of subunits ATF-2 and c-Jun), is termed an enhanceosome. This complex binds to DNA promoters and other sequences that contain an ISRE (IFN-stimulated responsive element) to facilitate the transcription of IFN- β . IFN- β augments the transcription of IRF7 and thus further boosts its own transcription in a feed-forward manner. Although even a partial complex can enhance transcription, the fully assembled enhanceosome induces robust and rapid transcription of IFN- β and different isoforms of IFN- α that establish intrinsic cellular anti-HIV resistance. The main effect of IFN- γ upon HIV infection, on the other hand, is mediating NK and CD8 $^+$ T-cell activity against HIV-infected CD4 $^+$ T cells and macrophages, and it does not play a role in the intracellular restriction of HIV.

Type I IFN induces expression of multiple genes at the transcriptional level, some of which are specialized components of the conventional innate immune system that inhibits HIV replication at different levels of the HIV life cycle. To achieve this, the IFN signal transduction pathway

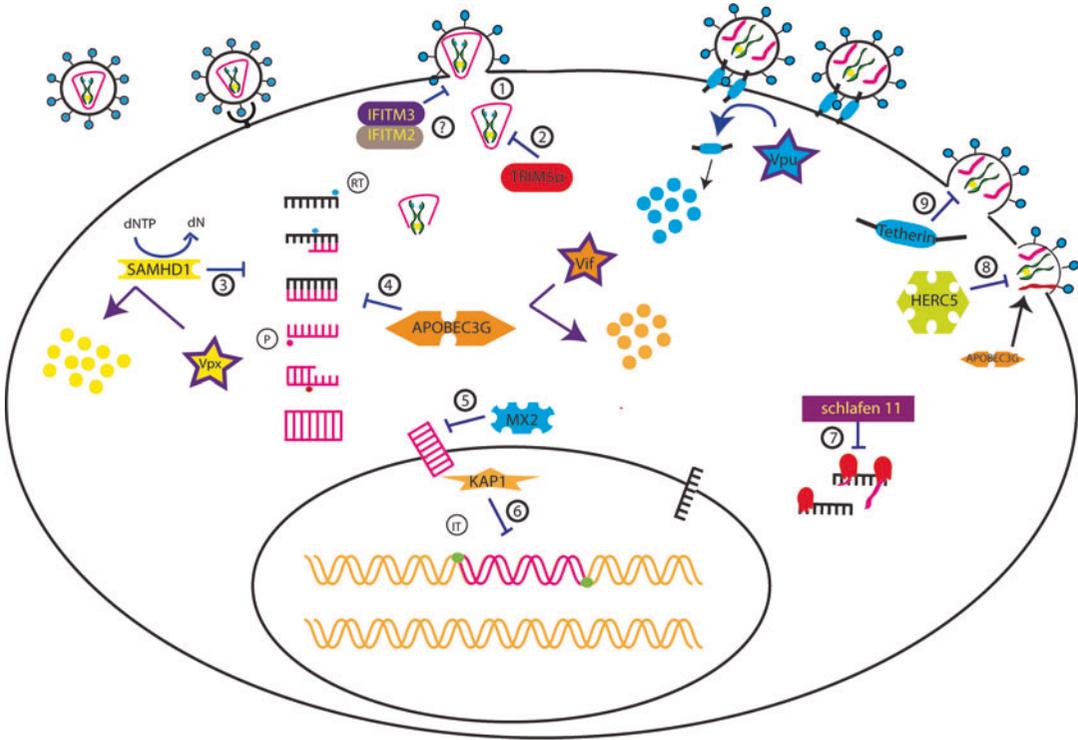
must be activated. IFN- α /IFN- β binding to IFNAR, composed of the two subunits IFNAR1 and IFNAR2, stimulates the JAK1-STAT pathway. This leads to the assembly of the ISGF3 complex (IFN-stimulated gene factor 3), which is composed of STAT1-STAT2 dimers and IRF9. ISGF3 binds to the ISREs located in the promoters of hundreds of ISGs in order to positively regulate their expression. Numerous ISGs have already been thoroughly investigated, and as might be expected, a substantial number of them can inhibit HIV replication, although most of them do not exclusively block HIV. As additional inhibitory factors become uncovered, it appears that almost every discrete step in the viral life cycle is targeted, with some of these factors counteracted by the various accessory proteins encoded by the virus. In the next section, some of these restriction factors that are induced by IFN and how they inhibit each step of HIV replication will be reviewed.

Intracellular Anti-HIV restriction factors

See (Fig. 2)

Virus-Cellular Fusion: IFITM1, IFITM2, and IFITM3

The interferon-inducible transmembrane proteins have been shown to restrict the replication of multiple viruses. This family of approximately 15 kd proteins was reidentified using a genome-wide RNA interference (RNAi) screen of influenza A (IAV) replication. This family of proteins was found to block very early stages of IAV and JSRV (Jaagsiekte sheep retrovirus) replication by somehow inhibiting virus-cell fusion (Lu et al. 2011). IFITM2 and IFITM3 were identified as HIV restriction factors in a large-scale antiviral ISG screen, but the inhibitory effect was fairly minimal (Schoggings et al. 2011). Although the precise resistance mechanism is not known, it has been suggested that the IFITMs bind to double-stranded viral RNAs such as RRE and TAR and interfere with viral protein expression (Chutiwitoonchai et al. 2013).



Cell-Intrinsic Immunity, Fig. 2 Inhibition of HIV replication by intracellular factors. (1) IFITM2 and IFITM3 inhibit virus particle-cell membrane fusion; ? indicates this is controversial. (2) TRIM5 α accelerates uncoating and likely signals as well. (3) SAMHD1 enzymatically depletes dNTPs, thus blocking reverse transcription. SAMHD1 is targeted by SIV/HIV-2 Vpx for ubiquitination and proteasome-mediated degradation. (4) APOBEC3G (and others) inhibit reverse transcription by deaminating single-stranded DNA, causing mutations and virus catastrophe. APOBEC3s are degraded by Vif, also via the proteasome. In general, APOBECs are incorporated into virus particles in the producer cell (shown) but act in the

target cell (also shown). (5) Perinuclear MX2 interferes with viral pre-integration complex transport into the nucleus in an unknown manner. (6) KAP1 induces integrase deacetylation and reduces integration efficiency. (7) Schlafen 11 specifically inhibits viral mRNA translation. (8) HERC5 affects Gag assembly. (9) Tetherin blocks fully mature virus from detaching from the cell. Tetherin is removed from the cell surface by Vpu in order to enhance viral replication. Tetherin also signals, similar to TRIM5 α . Here, viral RNA is indicated in black, DNA pink, ribosomes red, cellular DNA orange. RT reverse transcriptase, P DNA polymerase, IT integrase

Capsid Dissociation: TRIM5 α

A decade ago, TRIM5 α , a member of the TRIM family that contains a carboxy-terminal B30.2 (SPRY) domain, was shown to restrict HIV replication in old-world monkey cells (rhesus macaques and owl monkeys). While rhesus macaque TRIM5 α markedly inhibited HIV replication, the effect upon SIV was less pronounced. Human TRIM5 α has minimal activity against HIV due to an amino acid change in the SPRY domain. Although TRIM5 α is thought to directly interact with CA to induce premature disruption of the CA multimeric structure and block reverse

transcription and at the same time induce intracellular signaling, its exact mechanism of action is not yet known. Certainly, a major role of TRIM5 α , like other restriction factors, is to prevent cross-species retroviral transmission.

Reverse Transcription: APOBEC3G

The first HIV restriction factor identified (Sheehy et al. 2002) is a cellular protein that is incorporated into virions and deaminates cDNA after first-strand reverse transcription, causing G to A mutations in the sense HIV genome and viral catastrophe (Harris et al. 2003). Its activity is

inhibited by the viral protein Vif. This restriction factor is reviewed elsewhere and will not be covered further here.

Reverse Transcription: SAMHD1

SAMHD1 was first identified as a protein induced by IFN- γ in dendritic cells. Its role in cellular immunity was further investigated as it was found that specific, loss-of-function mutations in SAMHD1 can result in Aicardi-Goutières syndrome (AGS). The phenotype of AGS is congenital chronic encephalopathy and lupus-like phenomena, accompanied by high levels of IFN- α . In some ways AGS resembles a congenital viral infection. AGS can result from mutations in TREX1, RNASEH2A, RNASEH2B, RNASE2C, ADAR1, or SAMHD1: the consequences of these mutations are high levels of cytosolic DNA. Thus, it was postulated that SAMHD1, along with the other proteins, are negative regulators of the innate immune system, offering some protection against autoimmunity.

The SAMHD1 gene is located on chromosome 20 and encodes a 626 amino acid protein that is composed of two main motifs – the SAM (sterile alpha motif) and the HD domain (an amino acid doublet of divalent cation-coordinating histidine and aspartic acid residues). SAM likely functions as a protein-protein interacting domain, whereas the HD domain is a potent dGTP-stimulated triphosphohydrolase, which converts deoxynucleoside triphosphates to the constituent deoxynucleoside and inorganic triphosphate. Data to suggest that SAMHD1 is also a nuclease are not yet convincing and have been refuted.

SAMHD1 is highly expressed in dendritic cells, moderately in MDM (monocyte-derived macrophages) and resting T cells, and at low levels in activated T cells. Because of its enzymatic activity, there is an inverse correlation between the amount of SAMHD1 and the level of dNTPs. In terminally differentiated MDM, dNTP levels are 20–40 nM, 1% of the levels in activated CD4⁺ T cells (2–4 μ M). These low levels of dNTPs impede reverse transcription.

Lahouassa et al. demonstrated using knock-down assays and overexpression studies in MDM that SAMHD1 regulates the intracellular dNTP pool and that the reduction of the dNTPs

below the K_m (Michaelis coefficient) of HIV RT (reverse transcriptase) inhibits viral replication in these nonpermissive cells (Lahouassa et al. 2012). In permissive cells, such as activated T cells, despite the expression of SAMHD1, high levels of dNTPs allow reverse transcription to progress. This may be due to posttranslational regulation of SAMHD1's enzymatic activity. Both the teams of Lahouassa and Hrecka (Hrecka et al. 2011) showed that Vpx, an accessory protein encoded by HIV-2 and some SIVs (simian immunodeficiency viruses), counteracts SAMHD1. Vpx targets SAMHD1 for proteasome-mediated degradation via CRL4^{DCAF11} E3 ubiquitin ligase, thus increasing dNTP pools and enabling these viruses to replicate in monocytic and dendritic cells. Why doesn't HIV-1 encode a homologue of Vpx? Perhaps it is because infecting dendritic cells would stimulate the PRRs described above, thus initiating an antiviral response. Since dendritic cells express high levels of SAMHD1 and are largely resistant to HIV infection, the triggering of an antiviral innate immune response is largely avoided. Evidence supporting this hypothesis is the milder clinical course of individuals infected with HIV-2, in comparison to those infected with HIV-1. Of course that begs the question as to why Vpx evolved in the SIV lineage in the first place, a conundrum still under active investigation. TREX, another host protein that when mutated clinically manifests as AGS similarly to SAMHD1, is an example of a host protein that facilitates HIV replication indirectly by degrading cytosolic HIV cDNA (which of course should be inhibitory to the virus). The nuclease activity of TREX prevents the stimulation and the production of INF- β since the cytosolic DNA sensors are not activated. Thus, knockdown of TREX mRNA results in cytosolic DNA sensing, high levels of IFN- β , and eventual suppression of viral replication. The physiological role of TREX in the HIV replicative cycle is still an area of active investigation. TREX, in contrast to SAMHD1 and others, is not considered a restriction factor since it does not directly inhibit HIV replication, there is no evidence of positive selection of the gene, and no HIV accessory gene counteracts TREX.

Nuclear Transport: MX2

MX2 is a recently discovered intracellular HIV restriction factor. It is closely related to human MX1 (63% amino acid sequence identity), which inhibits a variety of RNA and DNA viruses, including IAV, La Crosse encephalitis virus, and hepatitis B virus. MX2 does not inhibit influenza virus, and MX1 has no effect upon HIV. The role of MX2 in HIV restriction was first identified by Kane et al. and Goujon et al. simultaneously in 2013 (Goujon et al. 2013; Kane et al. 2013).

Both groups used high-throughput screening to identify factors whose levels of induction by IFN correlated closely to the levels of inhibition of HIV infection observed in monocytoid cell lines. Kane et al. used microarrays to quantify mRNA transcripts, whereas Goujon et al. performed transcriptional profiling of RNA isolated from 15 different cell lines. MX2 was the only factor with validated inhibitory effect upon HIV titers. Although the mechanism of inhibition is not yet established, both groups showed that viral cDNA levels were not reduced in the presence of MX2, whereas levels of 2-LTR circles were decreased. This suggests that MX2 blocks nuclear import of the pre-integration complex (PIC) or causes a decrease in PIC stability. This is consistent with the perinuclear localization of MX2. Mutation of CA amino residues that are important for interaction with host proteins such as cyclophilin A, CPFS6, NUP358, or NUP153 abrogated or reduced the effects of MX2. The observation that modifying CA can modulate MX2 susceptibility or escape suggests that the CA is a specific target of MX2, which remains to be fully explored. Given the inner nuclear envelope localization of Vpr in infected cells and its potential role in HIV nuclear entry in macrophages, it is intriguing to speculate that Vpr could counteract this host gene.

Integration: KAP1

KAP1 was previously known as a transcription corepressor which interacts with HDAC, SETDB1, or HP-1. As a TRIM protein family member, it was also known to restrict murine

leukemia virus. KAP1 was identified by using the yeast two-hybrid assay system as an integrase (IN)-interacting protein (Allouch et al. 2011). KAP1 overexpression and knockdown experiments were consistent with the role of KAP1 as a restriction factor and mechanistic investigations revealed that by binding to acetylated integrase, KAP1 induces HDAC1 complex formation, leading to integrase deacetylation and reduced integration efficiency.

Translation: Schlafen 11

The SLFN family includes six human proteins that are induced by IFN- β , and SLFN5 and SLFN11 are the most prominent family members.

Li et al. investigated the effect of knockdown and overexpression of SLFN11 and demonstrated that this protein blocked HIV replication (Li et al. 2012). SLFN selectively decreased viral protein production without any reduction in viral RNA levels, consistent with inhibition of mRNA translation. Plasmid reporter constructs, encoding HIV Gag open reading frame using the virally preferred codons (Gag^{vir}, which has low guanine and cytosine content, and in which adenine/uracil are preferred in third position) or human-preferred codons (Gag^{opt}), were used to investigate the role of codon usage in the inhibition induced by SLFN11. SLFN11 strongly affected expression of Gag^{vir} but had little effect on Gag^{opt} expression. Using a tRNA array, the investigators demonstrated that HIV triggered substantial changes in tRNA concentrations in SLFN11-knockdown cells, and this effect was abrogated by SLFN11. Further work will help clarify how SLFN11 counteracts alterations in tRNA levels induced by HIV.

Gag Assembly: HERC5

The HERC5 gene is located on chromosome 4 and encodes a 1,024 amino acid protein. HERC5 is ubiquitously expressed, with highest levels of expression within the testis. HERC5 is a ligase that targets newly synthesized proteins for ISG15 conjugation and facilitates many cellular pathways. Woods et al. showed that overexpression

of HERC5 induced a fourfold decrease in HIV production in 293T cells and HERC5 knockdown doubled viral production (Woods et al. 2011). By transmission electron microscopy, it was shown that HERC5 blocks an early step of HIV-1 particle assembly at the plasma membrane. HERC5 and Gag proteins were found to associate with each other in vitro and to co-localize within cells. These interactions correlated with ISG15 modification of Gag. As expression of HERC5 is induced by IFN, HERC5 and ISG15 levels are increased in viremic patients, both in lymphatic tissue and in monocytes. Further work should help elucidate whether HERC5 plays a critical restrictive role in HIV replication.

Budding: Tetherin

This protein was identified as a result of a long search for the target of viral accessory protein Vpu (Neil et al. 2008). As its name implies, this IFN-induced transmembrane host protein anchors and traps virions extracellularly, thus blocking the release of nascent, fully mature viral particles from the cell membrane. Tetherin is also known to signal through the NFkB pathway. This restriction factor is discussed at length elsewhere in this series.

Conclusions

Taken together, the multifaceted innate immune response to HIV infection of cells may be well orchestrated but certainly appears somewhat chaotic, with numerous contributing host factors and multiple feedback mechanisms, with intersecting and overlapping pathways. The primary role of this system is to rapidly respond to exogenous pathogens, as the adaptive immune system begins to react, thus limiting microbial and viral dissemination and preventing catastrophic consequences to the infected organism. The innate immune system thus acts as a gatekeeper mechanism that controls the damage inflicted by pathogen invasion at its earliest stages. As an integral part of the innate immune response, PRRs identify microbial and viral

PAMPs and elicit a robust cellular response, as well as instruct the more specific yet slower adaptive cellular and humoral immune response against the invading pathogen (Chakrabarti and Simon 2010). HIV elicits a potent innate immune response, including the activation of multiple signaling pathways, including the type I IFNs. Elevated IFN levels induce the expression of numerous downstream effectors, some of which are quite well characterized, whereas others remain elusive. Many of these proteins can inhibit HIV replication at multiple stages of the viral life cycle. At the same time, HIV expresses accessory proteins that can block or render ineffective at least some, if not most, of these intracellular protective mechanisms, while circumventing the natural innate immune “cocktail” of antiretroviral proteins. Targeting or disrupting these viral counterstrategies, perhaps as an adjunct to cART, may augment the immune response and provide an additional level of virologic control and improve patient outcomes.

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Cellular and Soluble Immune Activation Markers in HIV-Infected Subjects

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Introduction

Chronic and generalized immune activation characterized by an elevated frequency of immune cells with an activated phenotype, increased lymphocyte turnover, and high serum levels of pro-inflammatory cytokines has been established as a key feature and a main driver of HIV disease progression. Indeed, the increased frequency of T cells expressing the T-cell activation markers, CD38 and HLA-DR, is an independent and better predictor of HIV disease progression than CD4 T-cell count or viral load (Liu et al. 1997, 1998) in untreated HIV-infected subjects, and in patients on antiretroviral therapy (ART), persistent immune activation (predominantly inflammation) is a significant driver of non-AIDS comorbidities. Further, in primate HIV infection models, elevated levels of immune activation/inflammation are detectable only in pathogenic SIV infections (rhesus and pigtail macaques) while absent in nonpathogenic SIV infection of natural hosts (sooty mangabeys and African green monkeys) (Silvestri et al. 2003). Finally, data suggest that the potential of ART to effectively reconstitute the immune system is dependent on how efficient ART reduces immune activation. Though ART is effective in suppressing viral replication to levels below detection, restoring the functionality of the immune system and reducing immune activation, abnormally high levels of inflammation and immune activation persist in comparison to uninfected individuals (Deeks and Phillips 2009). This residual immune activation may importantly contribute to non-AIDS comorbidities including cardiovascular diseases, metabolic disorders, neurocognitive decline, increased risk of malignancies, and loss of bone density. Considering that immune activation is a highly pertinent predictor of disease progression, evaluation of cellular and

soluble markers of immune activation is essential for the clinical management of infected ART-naïve subjects and in subjects on ART.

What Is the Mechanism Underlying Immune Activation?

The specific mechanisms underlying the generation of HIV-associated immune activation are still largely unclear, but data from a large body of work suggest the mechanisms entail multifactorial, complex, molecular, and cellular factors. The fact that the introduction of ART leads to a reduction in immune activation provides significant evidence that the virus itself is a driver of immune activation. The presence of the virus invariably elicits host antiviral innate and adaptive responses; further, HIV viral proteins such as gp120, Tat, and Nef directly activate immune cells (Juno and Fowke 2010). Additionally, immune cells expressing the appropriate pattern recognition receptors (PRR) including Toll-like receptors (TLR) are likely activated by the recognition of pathogen-associated molecular patterns (PAMPS) within the HIV proteome. HIV encodes TLR7 and TLR9 ligands that can directly activate plasmacytoid dendritic cells (pDCs) and B cells expressing TLR7 and TLR9. However, viral load alone cannot account for HIV pathogenesis considering that in nonpathogenic SIV infection in natural hosts, high viral loads are not coincident with high levels of immune activation (Silvestri et al. 2003). Another likely key driver of immune activation is the dramatic depletion of CD4+ T cells in gut-associated lymphoid tissue (GALT) and the resulting loss of epithelial barrier (due to depletion of a subset of T cells; Th17 + CD4+ T cells) (Dandekar et al. 2010). Loss of the epithelial barrier contributes to the translocation of microbial products including TLR ligands from the lumen into the circulation, and results from multiple studies indicate that the presence of these microbial products in the systemic circulation triggers broad and persistent activation of immune cells, especially innate cells and B lymphocytes expressing a range of TLRs (Brenchley et al. 2006). In addition to loss of epithelial barrier, other causes of microbial translocation include impaired clearance of bacterial products by

Kupffer cells in the liver. Kupffer cells are monocyte-derived macrophages, whose function is to phagocytose bacteria and microbial products. HIV infection is associated with a decreased density of Kupffer cells possibly leading to impaired clearance of bacteria and microbial products. Further evidence for the role of systemic translocated microbial products in triggering immune activation is provided by data from studies, in which lipopolysaccharide (LPS) administration led to increased immune activation in SIV natural hosts. HIV infection-associated CD4⁺ T-cell depletion can also indirectly lead to immune activation. In healthy individuals, the detection of cytomegalovirus (CMV)-specific T cells is an indication of persistent CMV reactivation. In HIV-infected individuals, CD4⁺ T-cell loss results in the inability to mount a competent immune response against reactivated CMV, which contributes to immune activation.

Finally, disruptions in the immunoregulatory machinery likely contribute to immune activation in chronic HIV infection. The most studied regulatory cell subset remains regulatory T cells (Tregs) that dampen excessive T-cell activation; however, during HIV pathogenesis, studies show conflicting data regarding the role of Tregs in dampening HIV-associated immune activation. Another regulatory subset is the myeloid-derived suppressor cells (MDSC, CD11b⁺CD14⁺CD33⁺CD15⁺) that suppress T-cell functions and have elevated frequencies in untreated HIV infection (Vollbrecht et al. 2012). Finally, the frequency of IL-10-secreting regulatory B cells (Bregs, CD19⁺CD24^{hi}CD38^{hi}) has been shown to correlate with immune activation in HIV-infected subjects (Siewe et al. 2013). Bregs inhibit anti-HIV cytotoxic T-lymphocyte activity, leading to viral persistence and likely contributing to immune activation.

Cellular Markers of Immune Activation

Markers of Dendritic Cell Activation

Dendritic cells (DCs) are innate cells, patrolling mucosal, lymphoid tissue, and blood, acting as the first line of host defense by recognizing PAMPs

on invading pathogens. Further, DCs create a link between the innate and adaptive immunity by processing and presenting microbial antigens to T and B cells thus exerting a critical host immune function against invading pathogens. There are two major DC subsets – myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) – differing in morphology, phenotype, and function. mDCs are largely responsible for antigen presentation, and although they express the HIV coreceptors CD4, CCR5, and CXCR5 (mDCs can also bind and endocytose HIV via c-type lectins), mDCs are largely resistant to productive HIV infection. mDCs from HIV-infected individuals express increased levels of the activation marker and T-cell cognate ligand, CD40, but expression normalizes during ART to some extent. pDCs specifically recognize pathogens expressing TLR7 and TLR9 ligands, producing copious amounts of interferon-alpha (IFN- α) (McKenna et al. 2005). Because of the potent response (IFN- α production) to TLR stimulation, pDCs have been intensely studied in HIV infection (the role of IFN- α in driving HIV disease progression is discussed below in detail). In vitro studies have determined that exposure of HIV to pDCs leads to upregulation of co-stimulatory molecules HLA-DR, CD80, CD86, and CD83, though pDCs from HIV-infected individuals exhibit marginally elevated expression of co-stimulatory molecules. Finally, TLR7-stimulated pDCs express the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL). Activated TRAIL-expressing pDCs can bind the receptors TRAIL R1 or R2 on to CD4⁺ T cells, leading to CD4⁺ T-cell apoptosis via the caspase pathway.

Markers of Monocyte Activation

As discussed above, drivers of HIV infection-associated immune activation including viral replication and translocated microbial products directly activate innate cells. Monocytes express multiple TLRs including TLR4, the ligand for LPS, which is widely used as the marker of microbial translocation in HIV-infected subjects (Brenchley et al. 2006). During HIV infection, dysregulated TLR expression has been reported as a marker of monocyte activation. Monocytes

from HIV-infected individuals exhibit upregulated TLR2 and TLR4 expression, correlating with viral load in patients without ART. However, upon ART initiation, TLR2 expression remained elevated, while TLR4 expression returned to normal levels. A critical marker of monocyte activation in HIV-infected subjects is elevated expression of the procoagulant tissue factor (TF, also known as thromboplastin). In vitro studies have shown that LPS-stimulated monocytes upregulate TF expression, and monocytes from HIV-infected subjects exhibit higher expression of TF, which was reduced after ART initiation but remained higher than in uninfected individuals. Further, there was a positive correlation between the frequency of TF-expressing monocytes and immune activation (as determined by HLA-DR+ CD38+ expression on CD4+ T cells). Additional results suggest that compared to monocytes from uninfected individuals, monocytes from HIV-infected counterparts exhibit an increase in the expression of the inflammatory marker CD16+, which normalized after initiation of ART. Further, monocytes from HIV-infected subjects exhibit elevated CD11b expression (a component of the β 2-integrin macrophage-1 antigen). This marker is involved in recruitment of monocytes to atherosclerotic plaques and correlates with atherosclerosis in experimental animal models. Expression of CD11b is reduced during ART but still remains higher compared to age-matched HIV-uninfected controls. Further, monocytes from HIV-infected individuals exhibit reduced expression of the adhesion molecule CD62L as well as CD115 (a macrophage colony-stimulating factor, M-CSF receptor). Initiation of ART did not lead to restoration of CD62L or CD115 expression.

Markers of Natural Killer (NK) Cell Activation

Natural killer (NK) cells are innate lymphoid cells first identified by their potential to kill infected cells by releasing cytotoxic granules containing perforin and granzymes. However, this cytotoxic function has been attributed to the major CD56dimCD16+ subset. A minor CD56brightCD16- subset has been identified that in response to IL-12 stimulation secretes

cytokines including IFN- γ . Activation of NK cells, as identified by the co-expression of HLA-DR and CD38, is associated with impaired NK cell function. Elevated frequencies of activated NK cells occur frequently in untreated HIV-1 infection and persist even during ART.

Markers of B-Cell Activation

Though there is limited evidence that HIV replicates in B cells, data from multiple studies indicate that in vivo B cells interact with HIV via the complement receptor CD21 and complement proteins on HIV (Moir et al. 2000). Some studies suggest that B cells also interact with HIV virions expressing CD40 ligand. Further, B cells likely interact with HIV viral proteins. Finally, as discussed above, TLR stimulation plays a critical role in driving immune activation during HIV infection; B cells express a range of TLRs suggesting that B cells can be directly activated via TLR signaling. The result of these diverse B-cell interactions during HIV infection is B-cell hyperactivation, characterized by the heightened expression of activation markers including CD70 and CD71 and the co-stimulatory molecules CD40, CD80, and CD86. Although most B-cell defects are readily reversed with ART (Moir and Fauci 2009), recent findings suggest that the elevated expression of activation markers persists during ART; this may have implications for the development of B-cell malignancies during HIV-1 infection (Guo et al. 2013).

Markers of T-Cell Activation

Depletion of CD4 T cells is a hallmark of HIV infection with memory CCR5 + CD4+-activated T cells being the prime targets of the virus. Further, up to 80% of gut lymphoid tissue-associated (GALT) CD4+ T cells are activated and express CCR5 and are likely depleted in the first 3 weeks of HIV infection. The mechanisms underlying CD4+ T-cell depletion have been intensely investigated, and the data strongly indicate that during HIV infection, disease progression is largely a result of persistent chronic T-cell activation and increased T-cell turnover, ultimately resulting in CD4+ T-cell loss. Accordingly, T-cell activation markers predict the rate of disease progression,

and after initiation of ART, the reduction in T-cell activation indicates a critical role for virus in causing T-cell activation. Further, some data suggest that the efficacy of ART in restoring the damage to the immune system is dependent on its ability to dampen immune activation. T-cell activation markers including CD38, HLA-DR, CD25, CD69, and CD70 have been extensively studied in HIV-infected subjects; however, CD38 remains the most thoroughly characterized marker of immune activation. CD38 is a transmembrane protein upregulated early in T-cell activation, coincident with cell-to-cell adhesion, increased cytokine expression, and rapid cell turnover. During HIV infection, T-cell CD38 expression positively correlates with other T-cell activation markers, and accordingly, the frequency of T cells coexpressing HLA-DR and CD38 is the most validated marker of immune activation during HIV infection (Liu et al. 1997, 1998).

Soluble Markers of Immune Activation

Markers of Microbial Translocation

As discussed above, microbial translocation is a critical driver of immune activation, and biomarkers of microbial translocation often correlate with disease progression. Since breach of the intestinal epithelial barrier leads to microbial translocation, assessing damage to epithelial cells (enterocytes) is an indicator of microbial translocation. Citrulline secreted by the epithelial cells of the small intestine is a by-product of glutamine and arginine metabolism. During HIV infection, a reduction in citrulline levels is an indication of the dysfunction of the epithelial cells and associated with malabsorption observed in HIV-infected individuals. An additional marker of enterocyte loss is the elevated plasma level of intestinal fatty acid-binding protein (I-FABP), expressed exclusively by enterocytes. However, the most critical marker of microbial translocation remains the detection of microbial products in serum of HIV-infected individuals. Elevated systemic levels of LPS, bacterial 16 s ribosomal (r) DNA, and lipoteichoic acid (LTA, part of cell wall of gram-positive bacteria) likely result from

microbial translocation; such changes have been frequently documented in HIV-infected subjects and serve as critical serum biomarkers of HIV-associated immune activation. As described above, the presence of microbial products in the systemic circulation leads to activation of immune cells (mostly innate) via TLR-TLR ligand interactions. The soluble markers secreted by these activated innate and adaptive cells are described below.

Soluble Activation Markers of Innate Immunity

As discussed above, innate cells represent the first line of defense against invading pathogens. During HIV infection, the outcome of pDC interaction with HIV in driving immune activation is a defining aspect of disease progression. pDCs are most susceptible to HIV due to the expression of CD4 and TLRs, especially TLR7 and TLR9, whose ligands are encoded by HIV. A model of HIV disease progression postulates that stimulated pDCs express high amounts of type I interferon (IFN- α/β) and indoleamine 2,3-dioxygenase (IDO) which modulate antiviral properties of T cells. Normally, type I interferons activate host restriction mechanisms, effectively exerting an antiviral function. However, systemic and persistent IFN- α/β expression as observed during HIV infection has deleterious effects on the immune system and drives HIV disease progression. The importance of IFN- α/β production by pDC in driving immune activation was demonstrated by studies showing that ex vivo, pDCs from nonpathogenic SIV-infected sooty mangabeys produce significantly less IFN- α/β compared to pDCs from pathogenic SIV-infected animals. This was corroborated by findings that in vivo, nonpathogenic SIV infection was characterized by a downmodulation of the initial IFN- α/β response, whereas in pathogenic infection, IFN- α/β production was uncontrolled and associated with chronic immune activation and disease progression. Further, during HIV infection, there is a correlation between disease progression and high titers of IFN- α . Additionally, women progress more rapidly to AIDS and ex vivo; pDCs from women produce more IFN- α after HIV

stimulation. An important downstream effect of HIV-induced IFN production is the activation of the interferon-stimulated genes (ISG) in immune cells especially CD4+ T cells that contribute to immune dysfunction and HIV disease progression. IFN- α/β and direct TLR7 stimulation are potent inducers of IDO, though, in vitro, HIV viral proteins have been shown to induce IDO expression in innate cells. IDO is involved in tryptophan catabolism, by cleaving tryptophan to kynurenine. During HIV infection, IDO contributes to immune dysfunction via two different mechanisms: (1) by suppressing T-cell functions either by depleting the important amino acid tryptophan or by producing metabolites that are toxic to T cells and (2) by altering the Th17/Treg ratio, thus increasing immune activation and HIV disease progression.

As discussed above, the presence of systemic bacterial LPS during HIV infection is a critical consequence of microbial translocation. Monocytes express high levels of TLR4, the receptor for LPS. LPS stimulation of monocytes is a significant driver of monocyte activation. Activated monocytes in turn secrete copious amounts of pro-inflammatory cytokines including IL-6 and tumor necrosis factor (TNF)- α . The most critical pro-inflammatory cytokine secreted by monocytes is interleukin (IL)-6. Serum IL-6 levels often correlate with viral load and even after ART-elevated serum levels of IL-6 persist and represent key biomarkers of non-AIDS-associated comorbidities and mortality (Deeks 2011). LPS-stimulated monocytes also express TNF- α , which in turn drives HIV replication. In the SIV model, nonpathogenic infection is characterized by dampening of TNF- α production by monocytes. In pathogenic infection, TNF- α production by LPS-stimulated monocytes is uncontrolled and associated with immune activation and disease progression. Activated monocytes also elevate the expression of surface CD14, and these activated monocytes shed CD14 (soluble, sCD14), which binds LPS. In some cohorts of HIV-infected individuals, serum levels of sCD14 have been shown to correlate with disease progression (Deeks 2011). Elevated serum levels of sCD14 persist during ART, and this is associated

with comorbidities including atherosclerosis progression and neurocognitive impairment (Deeks 2011).

In addition to TLR4, monocytes express TLR7/8, and the stimulation of monocytes by HIV-encoded TLR7/8 ligands as well as IFN- γ drives monocyte secretion of the chemokine C-X-C motif chemokine 10 (CXCL10) also known as IFN- γ -induced protein 10 (IP-10). IP-10 serves as a chemoattractant by binding to its receptor CXCR3 expressed by other immune cells including T cells, monocytes, and NK cells, effectively recruiting these cells to sites of inflammation. IP-10 is an important biomarker of disease progression, because serum IP-10 levels positively correlate with viral load and inversely with CD4 counts. Further, in patients on ART, IP-10 levels correlate with the frequency of CD16+ monocytes, suggesting monocytes are likely a significant source of IP-10.

Another marker of monocyte activation is soluble CD163 (sCD163). sCD163 is the shed form of the hemoglobin scavenger receptor. Serum levels of sCD163 are not normalized even in patients on ART correlating with aortic inflammation and a predictor of noncalcified coronary plaques.

Soluble Activation Markers of Adaptive Immunity

During HIV infection, activated adaptive immune cells secrete multiple proteins, some of which correlate with disease progression. Moreover, in patients on ART, some of these soluble markers are associated with the development of B-cell malignancies.

CD27 is a transmembrane glycoprotein, a member of the TNF receptor family and expressed on the surface of some T cells and antigen-experienced B cells. The receptor for CD27 is CD70, which is transiently expressed by activated B and T cells. Chronic activation leads to the shedding of CD27 and to the binding of soluble CD27 to B-lymphocyte-induced protein I (BLIMP-I) and X-box binding protein I (XBP-I) ultimately leading to increased IgG production. In HIV-infected individuals, sCD27 levels correlate with IgG concentrations. Even in

patients on suppressive ART, elevated serum levels of sCD27 persist and are associated with the development of non-Hodgkin lymphoma (NHL), a common malignancy in HIV-infected individuals on ART.

CD30 is another member of the TNF receptor superfamily, originally described as a marker for malignant B cells. CD30 is expressed on activated B- and T-helper 2 (Th2) cells. The ligand for CD30 is CD30L expressed by B and T cells, and CD30-CD30L interaction leads to the cleavage of the extracellular portion of CD30 resulting in the soluble form sCD30. In HIV-infected individuals, elevated serum levels of sCD30 correlate with other soluble markers of immune activation including sCD27 and sCD44 and, in some studies, with viral load. Similar to sCD27, elevated serum levels of sCD30 in ART-treated HIV-infected individuals are associated with the development of NHL.

CD23 is the low-affinity receptor for IgE expressed on IgD+ IgM+ B cells, though these B cells lose IgE after isotype switching. HIV viral protein gp160 has been shown to induce CD23 upregulation, and cleavage of CD23 (sCD23) further stimulates B cells. Elevated serum levels of sCD23 are also associated with the development of NHL.

The loss of memory B cells and the resulting impairment of antibody production are major hallmarks of HIV infection. It has been suggested that damage to the lymph nodes and the resulting impairment of B-cell trafficking contribute to B-cell deficiencies observed in HIV-infected subjects. The chemokine CXCL13 plays a key role in B-cell trafficking and is expressed by B cells in the secondary lymph nodes. After differentiation into plasma cells, B cells in the lymph nodes down-regulate CXCL13 expression and exit the follicles. HIV infection is associated with an elevated expression of CXCL13 on B cells, and some studies have shown a correlation with IP-10. However, B-cell CXCL13 expression is reduced during ART, though elevated levels are correlated to the development of NHL in some studies.

As previously discussed, monocytes are a major source of pro-inflammatory cytokines IL-6

and TNF- α ; however, TLR-stimulated B cells also secrete these cytokines, and B cells from HIV-infected subjects on ART express higher levels of IL-6 compared to healthy controls. This suggests that B cells likely contribute to the inflammatory milieu during HIV infection and even during ART.

During HIV infection, activated T cells also secrete important soluble biomarkers of immune activation. Activated Th1 helper cells express IFN- γ , and during HIV infection, IFN- γ -activated macrophages express elevated levels of neopterin, a catabolic product of guanosine triphosphate (GTP). In untreated HIV-infected subjects, serum levels of neopterin correlate directly with viral load and indirectly with CD4 count. Data from multiple studies suggest that during ART, the neopterin serum levels are not completely normalized indicating persistent immune activation.

Activated T cells as well as some innate cells upregulate CD87. CD87 is a urokinase-type plasminogen activator receptor, a transmembrane protein which can be cleaved and shed in serum (suPAR). Similar to neopterin, in untreated HIV-infected subjects, serum levels of suPAR correlate directly with viral load and indirectly with CD4 counts. In these untreated HIV-infected subjects, serum levels of suPAR are an independent marker of HIV-1-related early mortality. ART results in reduction of serum levels of suPAR, but these levels are still higher than in HIV-uninfected subjects.

Other Soluble Markers of Immune Activation

Some soluble markers of immune activation are secreted by a number of different immune cells, and these markers are described here.

Most nucleated cells and especially lymphocytes express the major histocompatibility complex I comprising beta-2 microglobulin (β -2 m) light chain and an alpha heavy chain. Activated cells shed β -2 m (s β -2 m), and in untreated HIV-infected subjects, the serum level of s β -2 m is an independent predictor of disease progression correlating positively with viral load and negatively with CD4 counts. These elevated serum

levels of β -2 m persist despite ART, reflecting the systemic immune activation and inflammation even in ART.

As discussed above, during HIV infection, activated monocytes express the pro-inflammatory cytokine TNF- α . Most immune cells express the receptors for TNF- α , the 55-kDa type I (TNFR-I) and 75-kDa type II (TNFR-II). Activation of these receptors leads to cleavage of the extracellular domains, sTNFR-I and sTNFR-II. In untreated HIV-infected individuals, elevated serum levels of sTNFR-I and sTNFR-II are predictive of disease progression, and despite ART, the levels remain elevated compared to HIV-uninfected subjects.

Conclusion

In HIV-infected individuals, ART is very successful in controlling viral replication leading to significant reduction in mortality due to AIDS. However, despite ART, persistent immune activation and specifically inflammation significantly increase the risk for non-AIDS comorbidities.

Multiple biomarkers of HIV infection are linked to clinical outcomes, and larger clinical trials are needed to confirm that these biomarkers can safely be employed as markers of a clinical outcome. Moreover, it may be possible to use our current knowledge about biomarkers of HIV-associated immune activation for tailoring antiretroviral treatment decision to individual needs of HIV-1-infected persons.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [Microbial Translocation](#)
- ▶ [NKT Cells: Bridging Innate and Adaptive Immunity](#)

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Cellular Cofactors for HIV-1 Transcription

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Definition

Several host cell factors, the chromatin structure of the viral LTR promoter, and the RNA-binding Tat transactivator regulate the transcription of the integrated HIV-1 genome. Transcriptional latency of the provirus in metabolically resting T cells generates cell reservoirs that are refractory to current combination antiretroviral therapy (cART).

Overview of Regulation of HIV-1 Transcription

Following ► [integration](#) into the host cell genome, the molecular mechanisms that regulate transcription from the HIV-1 provirus recapitulate the events that control the expression of most cellular genes transcribed by RNA polymerase II (RNAPII). Transcription is controlled by the U3 region of the long terminal repeat (LTR) positioned at the 5' of the provirus, which acts as an inducible promoter, with the essential involvement of several cellular specific transcription factors, the general transcription factor-RNAPII machinery, and the essential function of the virus-encoded Tat protein. Of note, the HIV-1 LTR sequence acts as a very strong promoter when analyzed as naked DNA in *in vitro* transcription experiments, while it is almost silent when integrated into the cellular genome. In the absence of cellular stimulation or the viral Tat

protein, the chromatin structure of the provirus bears marks of repressed, facultative heterochromatin, while RNAPII is still present in proximity to the LTR transcription start site, but stalled after the transcription of only a few tens of nucleotides.

Transcriptional activation of the LTR can be triggered by a variety of extracellular stimuli that lead to cellular activation, including phorbol esters, different cytokines, incubation with anti-CD3 antibodies, or inhibitors of histone deacetylases. In infected CD4+ T lymphocytes, the most relevant of these stimuli is the engagement of the T-cell receptor by its specific antigen. Following cell activation, different cellular histone acetyltransferases (HATs) are recruited to the promoter, chromatin acquires active chromatin marks, and RNAPII becomes elongation competent, the latter event being essentially catalyzed by the P-TEFb general transcription factor.

The Tat protein of HIV-1 is a small polypeptide of 101 aa – with the exception of the widely studied HXB2 strain, in which a point mutation truncates the protein at 86 aa – which is essential for the transcription of viral genes and for viral replication. The protein acts as a highly unusual transactivator that binds TAR (transactivation response element), a 59 nt long, structured RNA element positioned at the 5' end of the proviral transcript. Through this interaction, the protein activates HIV-1 transcription by promoting the assembly of transcriptionally active complexes at the LTR by multiple protein-RNA and protein-protein interactions. Tat was reported to bind specific transcription factors (e.g., Sp1), general transcription factors (including TBP, TAFII250, and P-TEFb), the RNAPII itself, and various transcriptional coactivators possessing HAT activity. A hallmark of Tat function is its interaction with the Cyclin T1 component of P-TEFb and its recruitment to the LTR to promote transcriptional elongation.

Regulation of HIV-1 Transcription by Cellular Transcription Factors

The viral LTR U3 region has a structure typical of promoters associated with cellular RNAPII,

which includes multiple upstream DNA regulatory elements that serve as binding sites for cellular transcription factors (Marcello et al. 2004). The basal promoter, which contains an initiator element in correspondence with the transcription start site, an upstream TATA box directing TFIID binding, and three further upstream tandem binding sites for the constitutively expressed Sp1 transcription factor, directs basal levels of LTR-directed RNA synthesis (Mbonye and Karn 2011). This basal promoter is very efficient *in vitro*, in the absence of chromatin, and very weak *in vivo*, when the provirus is assembled in nucleosomes. Functional analyses of LTR-driven reporter constructs have shown that the mutation of individual or pairs of Sp1 sites has little, if any, effect on these basal or Tat-transactivated levels of expression. However, the mutation of all three Sp1 sites markedly reduces the response to Tat.

Transcription initiation from the HIV-1 LTR is highly inducible by a variety of stimuli linked to the host T-cell or macrophage activation. Response to these stimuli is mediated by a short region which lies immediately upstream of the basal promoter, therefore named the “enhancer” region. This sequence contains two tandemly arranged binding sites for the dimeric transcription factors composed of several combinations of members of the Rel/NF- κ B or the NFAT families of polypeptides. Because their recognition sequences overlap, binding of these factors is mutually exclusive. The predominant complex that binds to these LTR κ B sites in activated cells is NF- κ B (p50/p65 heterodimer). In cells that are not stimulated, the p65 subunit of NF- κ B is retained in the cytoplasm through interaction with inhibitor proteins belonging to the I κ B family. The activation of NF- κ B occurs through the phosphorylation and proteolysis of the I κ B inhibitor and the subsequent translocation of p65 to join p50 in the nucleus where the factor binds to its cognate binding sites (Hoffmann and Baltimore 2006).

In the prototype HXB2 HIV-1 strain, different members of several other cellular transcription factor families (AP-1, COUP, USF, Ets, LEF-1) can bind the regulatory region upstream of the enhancer region. The arrangement of these

transcription factor binding sites in the LTR may vary in different HIV-1 subtypes. Most of these other factors binding to the LTR have a more modest effect on transcription compared to Tat or NF- κ B, and their primary function is most likely the modulation of transcriptional activity in different cell types.

Functions of P-TEFb

A unique feature of mammalian RNA polymerase II (RNAPII) is the presence of an extended C-terminal domain (CTD) in its largest subunit RPB1, which contains 52 heptad repeats with a consensus sequence, YSPTSPS. Phosphorylation of serines at positions 2 and 5 (Ser2 and Ser5) in these repeats is one of the essential molecular events that regulate gene expression in eukaryotic cells (Phatnani and Greenleaf 2006). The RNAPII CTD is hypo-phosphorylated when initially recruited to genes and undergoes sequential phosphorylation at Ser5 during promoter clearance and at Ser2 at the start of elongation (Core and Lis 2008). The former modification is imparted by the Cdk7 subunit of TFIIF; the nascent transcription complex, containing Ser5-phosphorylated RNAPII, however, is incompetent for processive elongation. The positive transcription elongation factor b (P-TEFb) is so far the best known characterized factor that can rescue the paused polymerase by inducing Ser2 phosphorylation (Peterlin and Price 2006). In the absence of P-TEFb, RNAPII complexes, phosphorylated on Ser5 only, accumulate 20–40 nt downstream of the transcription start site, due to the action of the inhibitory complexes NELF (negative-acting elongation factor) and DSIF (DRB sensitivity-inducing factor) (Core and Lis 2008). It is likely that this checkpoint serves to ensure proper capping of pre-mRNA, since the Ser5-phosphorylated CTD helps recruit capping enzymes to the 5' end of the nascent transcript. When P-TEFb is present, it relieves polymerase stalling by phosphorylating both NELF and DSIF; the former dissociate from the RNAPII, while the latter is converted into a positive elongation factor (Zhou and Yik 2006).

P-TEFb was originally discovered in the early 1990s as a kinase that phosphorylates the RNAPII CTD and is inhibited by 5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole (DRB) at concentrations that have no effect on the initiation of transcription. Subsequent gene cloning from flies and humans revealed that P-TEFb was a cyclin-dependent kinase. The *cdc2*-type catalytic subunit, first identified as PITALRE, was later renamed Cdk9 and can be produced in two isoforms of 42- and 55-Kda (Cdk9-42 and Cdk9-55). Cdk9 associates with a C-type cyclin subunit; flies have one Cyclin T, while humans express Cyclin T1 and two forms of Cyclin T2 (T2a and T2b) (Peterlin and Price 2006). Possibly, human Cdk9 protein also binds Cyclin K, a partner of the Cdk12 and Cdk13 kinases, which also phosphorylate Ser2 in the CTD of RNAPII.

In addition to the role of P-TEFb in stimulating transcriptional elongation, multiple evidence indicates that this general transcription factor also assists in regulating pre-mRNA processing. In particular, the Cyclin T1 subunit of P-TEFb is known to interact with Tat-SF1, a factor taking part in the formation of a nuclear complex, together with spliceosomal U snRNPs, which stimulates both RNAPII elongation and splicing. Additionally, the splicing factor SKIP (c-SKi-interacting protein) also binds P-TEFb together with Tat-TAR. This factor is essential for Tat transactivation and couples transcription with pre-mRNA splicing (Zhou and Yik 2006).

Regulation of P-TEFb

Studies performed using either RNA interference or highly specific inhibitors have indicated that P-TEFb acts as a global cofactor important for most RNAPII-mediated transcription (Peterlin and Price 2006). Not surprisingly, therefore, its activity appears to be tightly regulated in different cell types, throughout differentiation, and as part of the cell response to various stimuli. The most relevant mechanism of P-TEFb regulation derives from its interaction with an inhibitory complex formed by 7SK RNA and one of the HEXIM proteins.

The 7SK RNA is a relatively abundant (2×10^5 copies per cell), noncoding RNA of 331 nt,

transcribed by RNA polymerase III. This RNA localizes in the nucleoplasm where, together with the HEXIM1 or HEXIM2 proteins, it sequesters P-TEFb in an inactive form. The mammalian HEMIX1 protein was originally identified as a hexamethylene bisacetamide (HMBA)-inducible protein in vascular smooth muscle cells (hence the name) and was later found to be inducible in several other cell types, acting as a potent cell growth suppressor and inducer of differentiation (Zhou and Yik 2006). A smaller homologue, HEXIM2, was later described, showing high homology to HEXIM1 in the region binding to 7SK while diverging significantly at the N-terminus.

Both 7SK RNA and a HEXIM protein are required to inhibit P-TEFb. HEXIM1/2 proteins form stable homo- or heterodimers that associate with two P-TEFb complexes, one 7SK RNA and, most likely, other cellular proteins, among which LARP7 and MePCE (Diribarne and Bensaude 2009). The 7SK RNA exerts a scaffolding role in this complex: by binding an arginine-rich basic region of HEXIM1, it determines an allosteric change that allows HEXIM1 to specifically bind P-TEFb, which culminates in the inhibition of transcription.

As a consequence of these interactions inside the cells, P-TEFb can be found in two forms: a “free,” active form and a “sequestered,” inactive, P-TEFb/7SK/HEXIM1/2 form. Physiological stimulations such as those occurring during cardiac hypertrophy or upon cell treatment with inhibitors of transcription or other cellular stresses induce the rapid release of HEXIM1/7SK from P-TEFb and the consequent switch toward the active P-TEFb form. Active P-TEFb associates with other cellular proteins to form the so-called super elongation complex (SEC), also containing other elongation factors and coactivators (Ott et al. 2011).

The enzymatically active P-TEFb complex can also be found in association with the bromodomain protein Brd4 (Zhou and Yik 2006). This is a ubiquitously expressed, nuclear protein belonging to the BET (bromodomains and extraterminal) family of proteins (Wu and Chiang 2007). Brd4 was found to bind to actively

transcribed genomic regions by directly associating to acetylated histone H3 and H4, through its bromodomain, and to be part of the mediator complex, a multicomponent complex bridging specific transcription factors to the general transcription machinery. The interaction with Brd4 might thus contribute to the transcriptional activation promoted by P-TEFb, in particular by tethering P-TEFb to gene regions bearing active chromatin marks (Zhou and Yik 2006).

Given the central role of P-TEFb in transcriptional activation, it is not surprising that several layers of additional mechanisms for its regulation exist. In different cell types, modulation of P-TEFb function is also attained by controlling the amounts of Cyclin T1, Cdk9, and HEXIM1 proteins, by modulating their function by post-translational modification by phosphorylation, ubiquitination, and acetylation and by regulating Cdk9 nucleocytoplasmic shuttling (Cho et al. 2010).

P-TEFb and HIV-1 Transcription

The cyclin cofactor of P-TEFb, Cyclin T1, was originally identified for its direct binding to the HIV-1 Tat protein in HeLa nuclear extracts (Wei et al. 1998). A current model for Tat transactivation envisions that Tat, through its specific binding to Cyclin T1, determines the recruitment of P-TEFb to the 5' trans-acting response (TAR) RNA sequence (nucleotides 1–59 of the nascent viral transcripts) to stimulate proviral transcription (Core and Lis 2008). In the absence of Tat, transcription from the HIV-1 LTR generates only short transcripts; however, the presence of Tat dramatically stimulates the efficiency of elongation and results in a large increase in the level of full-length transcripts that extend through the more-than-9-kb HIV-1 genome.

Tat, TAR, and Cyclin T1 form a stable, ternary complex, where Tat specifically binds the TAR RNA at a three-nucleotide bulge and several flanking nucleotides in the double-stranded RNA stem just below the apical loop and Cyclin T1 recognizes the TAR apical loop itself, while Tat adopts a structure complementary to the surface of P-TEFb and makes extensive contacts, mainly with the Cyclin T1 cyclin box but also with the

T loop of Cdk9 (Tahirov et al. 2010). These cooperative interactions allow the recruitment of P-TEFb in proximity to the stalled RNAPII and its consequent activation. Interestingly, rodent Cyclin T1 lacks a critical cysteine in the Tat recognition motif (TRM) that is required to interact with Tat. As a consequence, Tat is poorly active in terms of HIV-1 LTR transactivation in murine cells compared to primate cells (Peterlin and Price 2006; Zhou and Yik 2006). For a similar reason, human Cyclin T2 is also unable to bind Tat.

The Tat sequence involved in TAR binding, highly enriched in basic amino acids, is highly similar to the basic HEXIM1 sequence binding the 7SK RNA; in addition, Tat is capable of competing with HEXIM1 for 7SK association. These observations suggest that the Tat/TAR viral system has evolved in a manner that is architecturally similar to that of the cellular HEXIM1/7SK pair, with the evolutionary purpose to decoy HEXIM1/7SK and hijack P-TEFb for specific HIV-1 transcription (Zhou and Yik 2006).

Chromatin Architecture at the HIV-1 Provirus

In the nucleus of the infected cells, chromatin assembly essentially represses transcription (Marcello et al. 2004). Experiments performed both *in vivo* and *in vitro* using the HIV promoter reconstituted into chromatin have shown that, independent from the integration site, nucleosomes in the 5' LTR are precisely positioned with respect to *cis*-acting regulatory elements. In the transcriptionally silent provirus, these nucleosomes define two large nucleosome-free areas. The first is composed of the basal promoter, containing three tandem Sp-1 binding sites and the TATA box sequence, and the LTR enhancer, which is the target for the p50/p65 NF- κ B heterodimer; the same region also contains the binding sites for other transcription factors including Ets-1 and USF. The second open area spans the primer-binding site immediately downstream of the 5' LTR. These two open regions are separated by a single nucleosome called *nuc-1* that is

destabilized during transcriptional activation (Colin and Van Lint 2009). Genomic footprinting and chromatin immunoprecipitation (ChIP) experiments performed in both activated and in silently infected cells have indicated that most of the transcription factor binding sites at the promoter, including the TATA box, the Sp1 sites, the enhancer region, and the USF site, are occupied by cellular proteins independent from the state of activation (Marcello et al. 2004). ChIP experiments have also revealed that the modification of chromatin in proximity of the HIV-1 transcription start site precedes the onset of RNAPII transcription and is essential for viral gene expression to occur. Together, these are indications that the transcriptional activation of the integrated LTR is not primarily restricted by DNA target site accessibility but occurs through the modulation of chromatin conformation.

Transcriptional silencing of the HIV-1 provirus is achieved through mechanisms involving epigenetic changes of chromatin. Chromatin is maintained in a deacetylated state by different class I histone deacetylases (HDACs), which can be recruited to the LTR by the p50/p50 NF- κ B family homodimer, binding to the enhancer region, or the CBF-1 factor (Mbonye and Karn 2011). At the same time, latent HIV proviruses carry trimethylated histone H3 on Lysine 9 (H3K9met3) and Lysine 27 (H3K27met3), as well as dimethylated Lysine 9 (H3K9met2), and can be found in association with the respective histone methylases. Of note, H3K9met2 is a modification associated with facultative heterochromatin that marks the genes poised for transcription.

On the contrary, transcriptional activation coincides with acetylation of H3 and H4 histones in the LTR region, which precedes the actual onset of transcription. Histone acetylation is paralleled by the recruitment of specific HATs to the promoter, which is cell-type specific and depends on the stimulus used for transcriptional activation. Tat itself was shown to associate with different HATs, including the transcriptional coactivators p300 and the highly homologous cAMP-responsive binding protein (CREB) (CBP), the p300/CBP-associated factor (P/CAF), the general

control non-derepressible-5 (GCN5) protein, and the general transcription factor TFII250 (Marcello et al. 2004). Complexes containing HATs assist transcriptional activation through acetylation of the N-terminal tails of histones, an event inducing the destabilization of histone-DNA interactions.

Finally, it is of interest that, quite apart from histones, Tat itself is the subject of various post-translational modifications. Tat acetylation by P/CAF on Lysine 28 favors interaction of Tat with P-TEFb, while acetylation of Lysine 50 by p300 and GCN5 favors dissociation of the complex and release of P-TEFb from TAR. In addition, acetylation of Lysine 50 permits interaction of Tat with the subunits BRM and Ini1 of the SWI/SNF chromatin-remodeling complex, which is consequently recruited at the 3' end of nuc-1 in the 5' LTR to facilitate transcriptional elongation. In contrast, methylation of Tat by the arginine methyltransferases PRMT6 on its arginines at positions 52 and 53 decreases interaction with TAR and counteracts P-TEFb complex formation. Finally, one or several protein lysine methyltransferases, at least SETDB1, were demonstrated to methylate Tat on Lysines 50 and 51, thereby competing with acetylation of the same residues (Colin and Van Lint 2009). Together, these results again underline the strict molecular interplay connecting Tat with the molecular modifications regulating cellular gene transcription.

Conclusions

When considered collectively, the molecular interactions described above clearly indicate that transcription of the HIV-1 provirus, an essential step in viral replication, has evolved to perfectly adapt to its host cell environment. The regulation of transcription initiation relies on a patchwork of cellular transcription factors recruited by the viral LTR, including NF- κ B and NFAT that render the promoter highly responsive to the same stimuli that normally activate the host cell. On the other hand, proficient transcriptional elongation is achieved by exploiting the cellular P-TEFb complex, as with many cellular genes. The HIV-1 Tat

protein appears to be a central player in orchestrating these mechanisms, since it appears able to both mediate the recruitment of chromatin-modifying enzymes to the LTR for transcriptional initiation and to hijack P-TEFb to overcome polymerase stalling.

The peculiar property of the HIV-1 LTR to adapt to its cellular environment and to become transcriptionally silent in inactive T cells bears important practical consequences. HIV-1 efficiently infects metabolically active, CD4⁺ T cells, in particular activated T lymphocytes and macrophages. Once CD4⁺ T lymphocytes revert to a resting, memory state, HIV-1 undergoes reversible transcriptional silencing. Latently infected cells represent a major source of HIV-1 reservoirs, since these cells, not expressing any viral protein, are refractory to current combination antiretroviral therapy (cART), which only targets the replicative fraction of the virus and are not identifiable by the immune response. Therefore, understanding the molecular mechanisms controlling silencing and reactivation of the HIV-1 provirus at its integration site within the host cell genome has profound implications for both elucidation of the pathogenesis of HIV disease and for its pharmacological control.

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Cellular Cofactors of HIV as Drug Targets

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Definition

Infection with the human immunodeficiency virus type 1 (HIV-1) remains a substantial public health as well as a socioeconomic problem worldwide. Although highly active antiretroviral therapy (HAART) effectively halts HIV replication and profoundly increases survival of patients, it has not been possible yet to achieve a cure. Interruption of HAART typically results in a rebound of virus replication. This is primarily due to the fact that HIV ingeniously escapes from the continuous immune surveillance in a small pool of latently infected cells that are not susceptible to drug

therapy. Moreover, the rapid replication rate and the generation of an extensive genetic diversity fuel the emergence of drug-resistant viral strains resulting in treatment failure. Therefore, there is a continuous demand to search for novel and better ARVs for a better control of the HIV pandemic with the hope to eventually induce permanent remission of the disease.

HIV relies on the host cellular machinery to complete its replication cycle. HIV hijacks several biological processes and protein complexes of the host cell through distinct virus-host protein-protein interactions (PPIs) (Van Maele et al. 2006). Since these host-pathogen interactions directly mediate viral replication and disease progression, their specific disruption can provide alternative targets for therapeutic intervention. PPIs represent an attractive group of biologically relevant targets for the development of small-molecule protein-protein interaction inhibitors (SMIPPIs) (Arkin and Wells 2004). Since protein-protein interfaces are often based on extended, flat, barely defined, and large hydrophobic surfaces, overcoming binding energy with small molecules is hard to achieve. Therefore, obtaining validated starting points for chemical optimization of SMIPPIs has been difficult. Moreover, the applicability of PPIs as therapeutic targets is not only defined by their physicochemical properties but also by the biological properties of the protein-protein interaction and requires meticulous target validation prior to drug discovery (see entry “► [Identification and Validation of HIV Cofactors](#)”).

Cofactors of HIV as Antiviral Targets

In recent years our understanding of the interactomics of HIV proteins has dramatically increased, opening the possibility for the discovery of novel classes of therapeutics (see entry “► [Identification and Validation of HIV Cofactors](#)”). Not surprisingly, there are numerous interactions between HIV and cellular proteins involved in all stages of virus replication (Jager et al. 2012). In principle, any distinct

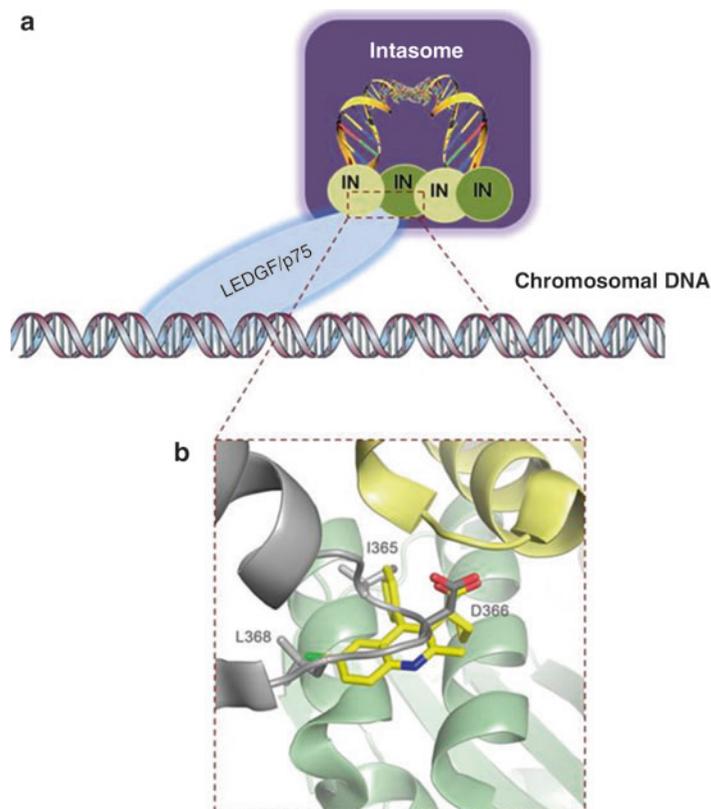
interaction between virus-encoded proteins and host cofactors has the potential to be a target for drug design.

The CCR5 antagonist, maraviroc, was approved as the first ARV targeting a host factor (Dorr et al. 2005). Maraviroc binds to the CCR5 coreceptor on the surface of cells and prevents interaction with the Gp120 envelop protein of the virus (Sayana and Khanlou 2009). Successful targeting of host-virus PPIs demonstrates that HIV-1 therapeutic drug targets are not limited to virus-encoded enzymes and that understanding of the virus-host interactome can be the basis for future HIV therapeutics (Greene et al. 2008; Busschots et al. 2009). In theory, this antiviral strategy is expected to make it more difficult for the virus to develop resistance. Since the host factor is genetically conserved in a biologically relevant host-virus interaction, resistance is less likely to occur, increasing the clinical potential of these drugs.

Cofactors of HIV Integrase as Potential Antiviral Targets

In recent years HIV-1 integrase (IN) joined the selection of important therapeutic targets to treat HIV infection. The enzyme orchestrates the insertion of the viral DNA into the host chromatin. HIV IN is a 32-kDa protein containing three canonical structural domains connected by flexible linkers: the N-terminal (NTD, residues 1–50), the catalytic core (CCD, residues 51–212), and the C-terminal domain (CTD, 213–270) (Fig. 1). All three domains are required for 3' processing and DNA strand transfer. The solution structure of the N-terminal HHCC domain revealed a three-helix bundle stabilized by zinc. The central catalytic core domain contains the DD(35)E motif conserved among retroviruses and retrotransposons. D64, D116, and E152 residues coordinate 2 Mg²⁺ ions necessary for catalysis. The C-terminal domain has a SH3-like fold. Full-length HIV-1 IN is a multimeric enzyme and forms stable tetramers in solution.

Despite the recent release of the crystal structure of full-length IN of the prototype foamy virus



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Fig. 1 LEDGINs bind to the LEDGF/p75 binding pocket in HIV-1 integrase. (a) LEDGF/p75 has an N-terminal chromosomal DNA-binding region. The C-terminus contains the well-characterized integrase-binding domain (IBD) and acts as a protein interaction playground. The interaction of the catalytic core domain (CCD) of HIV integrase with the IBD of LEDGF/p75 is

crucial to facilitate the tethering of the HIV intasome on the chromatin. (b) Cartoon representation of the IN CCD dimer (*pale green* and *pale yellow*) with a LEDGIN superimposed with the IBD (PDB entry 2B4J, *gray*) reveals mimicry of the protein-protein interaction. LEDGIN phenyl, acid, and chlorine groups substitute for LEDGF/p75 residues I365, D366, and L368 side chains, respectively

(PFV) (Hare et al. 2010), there is no crystal structure of full-length HIV-1 IN. The main obstacle for structural studies of HIV IN is its propensity to aggregate. The published two-domain crystal structures of HIV-1 IN (comprising the N-terminal and the catalytic core or the catalytic core and the C-terminal domain) as well as the crystal and NMR structures of individual domains represent valuable but incomplete information on the functional structure of the HIV intasome. HIV integrase was the last HIV enzyme to be effectively targeted with small molecules. Reasons were the lack of homologous disease targets, as opposed to well-studied DNA polymerases and aspartyl proteases and the absence of a crystal

structure. Indeed, nowadays structural information is playing a central role in successful drug development.

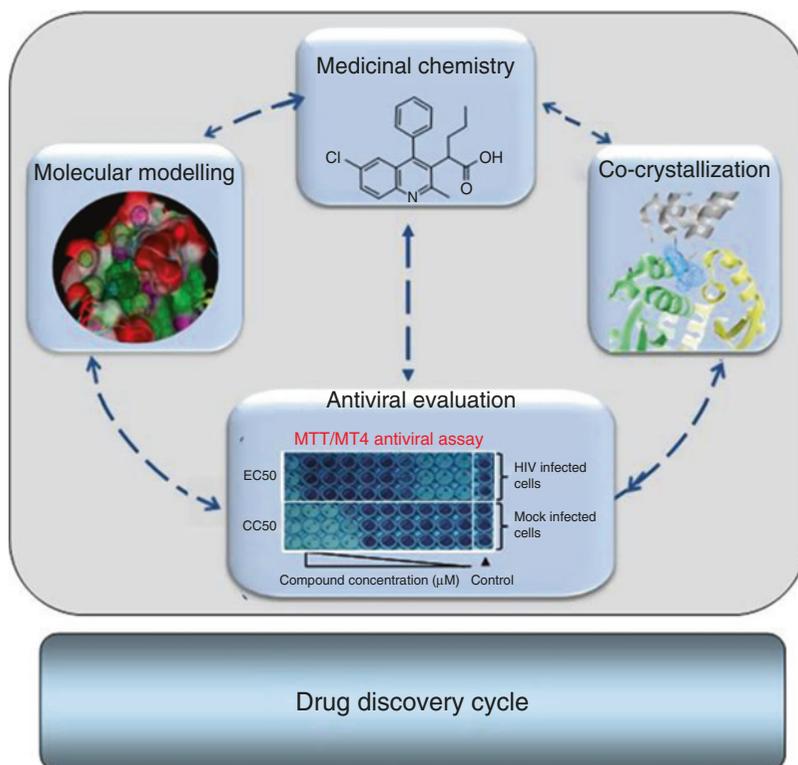
After completion of reverse transcription, the so-called pre-integration complex (PIC) is formed. Along with viral cDNA and IN, the PIC contains viral reverse transcriptase (RT), nucleocapsid (NC), matrix (MA), and Vpr. RT and NC are involved in the synthesis of viral cDNA, while MA and Vpr may affect nuclear import of the PIC. The PIC also contains host cell proteins, and nuclear import is mediated by the interaction with transport factors and nucleoporins. In the nucleus HIV IN catalyzes the stable insertion of the viral cDNA into a host chromosome.

The recent success in the application of structure-based rational drug design in the discovery and development of allosteric HIV-1 integrase (IN) inhibitors, the LEDGINs (Christ et al. 2010), was possible due to 7 years of intensive basic research on the cofactor lens epithelium-derived growth factor/p75 (LEDGF/p75). LEDGINs inhibit the interaction between LEDGF/p75 and HIV-1 IN and will be used as an example to discuss approaches, challenges, and future perspectives of SMIPPIIs.

Rational Design of LEDGF/p75-IN Interaction Inhibitors

Different approaches have been employed to design and identify small-molecule inhibitors of the LEDGF/p75-IN interaction. These include large-scale screening of chemical libraries (Du et al. 2008; Hou et al. 2008), computational three-dimensional (3D) database screening of chemical libraries, and structure-based de novo design (Christ et al. 2010; De Luca et al. 2009). High-throughput screening of large libraries of chemicals against a biological target is the prevailing method for the identification of new hit compounds in modern drug discovery. Alternatively, virtual screening is based on a computer-aided survey of large libraries of chemicals that complement targets of known structure and on experimentally testing of a limited set of compounds predicted to bind well. In order to obtain *bona fide* LEDGF/p75-IN interaction inhibitors, a structure-based drug design effort was initiated in 2007 (Christ et al. 2010). Drug design is based on a virtual screen of large libraries of small molecules to fit a consensus pharmacophore docked into the region of interest. The consensus pharmacophore consists of chemical groups critical for interaction with amino acid residues or peptide backbones in the proposed drug-binding pocket. In our case the pharmacophore was designed to bind to the LEDGF/p75 binding pocket located at the interface of a dimer of the CCD of HIV-1 IN. In principle, any drug discovery project requires design, prioritization,

analysis, and interpretation of results of consecutive experiments to ultimately facilitate the development of new therapeutic compounds. Often, it is the combination of methods, rather than a single experiment, that moves a drug discovery project forward. The scheme of the rational drug design work flow used during the discovery and hit-to-lead optimization process of LEDGINs is depicted in Fig. 2. The *in silico* screen for LEDGINs integrates a multidisciplinary approach where existing structural bioinformatics and chemoinformatics were employed in combination with a validated target-based PPI assay (Christ et al. 2010). Different crystal structures of the HIV-1 CCD and co-crystal structures with the IBD of LEDGF/p75 (Cherepanov et al. 2005) or ligand bound to the CCD were superpositioned to refine and construct more precisely a consensus pharmacophore model. Most important features in the final predictive pharmacophore model were a “hydrophobic/aromatic” moiety overlapping with Ile365 of the IBD, a “hydrophobic/aromatic” feature overlapping with Leu368 of the IBD, “acceptor” features mimicking the acid functionality of Asp366, and a “hydrophobic/aromatic” feature overlapping with the Lys364 side chain of LEDGF/p75. 200,000 commercially available and structurally diverse compounds were filtered using the established pharmacophore query. After serial filtering of the initial libraries, 25 promising molecules were ordered for biological evaluation in a bead-based *in vitro* LEDGF/p75-HIV-1 IN protein-protein interaction assay in the AlphaScreen™ format (Fig. 2). AlphaScreen™ is a bead-based medium throughput assay optimized to measure the interaction between LEDGF/p75 and HIV-1 IN (Christ et al. 2010). Of the 25 molecules retained from the initial screening, four hit molecules inhibited the LEDGF/p75-HIV-1 IN interaction. One of the hit molecules, LEDGIN 1, inhibited the PPI by 36% at 100 μM and served as a starting point for structure-activity relationship (SAR) investigations aimed at the identification of more potent LEDGINs (Christ et al. 2010). Medicinal chemistry optimization, aided by structural information provided by high-resolution co-crystals of LEDGIN 3 soaked into the



Cellular Cofactors of HIV as Drug Targets, Fig. 2 Work flow for rational design of LEDGINs. LEDGINs result from a rational drug discovery strategy involving a multidisciplinary effort. A 3D pharmacophore query was constructed for virtual screening of 200,000 molecules in commercial libraries. After performing stringent sequential scoring and filtering of the best scoring chemical entities, 25 molecules were retained and tested in the *in vitro* AlphaScreen™ assay. Hits emerging from

the screening were optimized by reiterative chemical refining and biological profiling in AlphaScreen™ and in a cell-based antiviral assay, MTT/MT4. Structure-activity relationships were deduced and used to guide synthesis of analogues with enhanced activity. The resulting early lead compounds were then further optimized in an integrated lead optimization strategy, while the molecular mechanism of action was investigated in cell culture. A similar approach may be used for other HIV cofactors

HIV-1 CCD, generated congeners of LEDGIN 3 (including LEDGINs 6 and 7) with improved biological activity.

Furthermore, LEDGINs did not interfere with the interaction between LEDGF/p75 and its cellular binding partners JPO2 or pogZ, confirming their specificity. Of note Hou et al. (Hou et al. 2008) identified several compounds inhibiting the LEDGF/p75-IN interaction through high-throughput screening of a compound library of more than 700,000 small molecules with AlphaScreen™. However, the quinolinylacetic acid derivatives are the first examples of potent and specific inhibitors of HIV-1 replication which

have been extensively evaluated for their therapeutic potential and mechanism of action in cell-based antiviral assays (including in primary cells) (Christ et al. 2010).

LEDGINs as Therapeutics

A critical evaluation of the mechanism of action and therapeutic potential of LEDGINs requires investigation of different drug characteristics: (a) a high binding affinity and specificity to HIV-1 IN, (b) potent and broad-spectrum anti-HIV activities in cell-based antiviral assays,

(c) lack of toxicity, and (d) an optimal pharmacokinetic (PK) and pharmacodynamic (PD) profile allowing a once a day administration in patients. Inhibition of the LEDGF/p75-HIV-1 IN interaction by LEDGINS blocks HIV integration (Christ et al. 2010). Integration inhibitors are characterized by a typical pattern of viral DNA species as measured by Q-PCR. 2-LTR circles are the dead-end by-product of non-integrated viral DNA; their number is increased upon integration block if steps upstream are not hampered. Both the classical integrase strand transfer inhibitor (INSTI) raltegravir and LEDGINS reduce the number of integrated proviral DNA and increase the number of 2-LTR circles without effect on reverse transcription. Resistance selection in cell culture against a new class of antiviral agents ultimately corroborates the antiviral target. By serial passaging of HIV-1 in increasing concentrations of LEDGIN 6, a resistant strain with the A128T substitution in IN was selected. The A128 residue is a hot spot of the IN-LEDGF/p75 interface and was included in the predictive pharmacophore model for the virtual screen. The resistance mutation thus corroborates the specificity of LEDGINS. The A128T mutation in integrase is not associated with resistance to INSTIs, and LEDGINS lack cross-resistance with other ARV classes corroborating their novel mode of action.

Other Cellular Cofactors of HIV with Potential as Antiviral Target

CCR5 and CXCR4, chemokine receptors, were identified as coreceptors of HIV in the 1990s (Deng et al. 1996). Natural resistance of individuals against HIV infection helped to identify the CCR5 coreceptor, whereas the antiviral bicyclam, developed in a random cell-based HIV replication screen, helped to understand the CXCR4 receptor.

DDX3 is an HIV cofactor that functions as an RNA-dependent ATPase/helicase in the Rev-RRE/CRM1 pathway for the export of unspliced/partially spliced HIV-1 transcripts (see entry “► [DDX3, Cofactors, and RNA Export](#)”). During screening for cellular factors induced by

Tat, the upregulation of DDX3 in human cell lines was observed (Yedavalli et al. 2004). Functional validation of a role of DDX3 in HIV nuclear export and HIV replication was obtained using antisense and dominant negative inhibition of DDX3 function. Co-immunoprecipitation showed interaction between DDX3 and CRM1 and DDX3 and Rev. Since DDX3 is a DEAD box helicase, it constitutes a possible antiviral target (Yedavalli et al. 2004).

Conclusion

PPIs represent an attractive group of biologically relevant targets for the development of small-molecule protein-protein interaction inhibitors (SMIPPIs). Direct interactions between HIV and intracellular cofactors provide novel druggable PPIs. This strategy is well exemplified by the discovery of drugs targeting LEDGF/p75-IN interaction. LEDGINS show a pathway of resistance development that is different from that of the INSTIs and lack cross-resistance with ARV in the clinic. Discovery of LEDGINS is a good example of structure-based rational drug design targeting a well-defined and biologically relevant PPI. Coreceptors and other cofactors presented in this entry may represent novel HIV drug targets.

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Cellular Immune Response to HIV-2 Infection

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Definition

T-cells play a central role in both the control and pathogenesis of HIV infection. While T-cell responses against HIV-1 infection have been studied in great detail, much less is known about T-cells in the context of HIV-2. This entry summarizes current understanding of the quantitative and qualitative characteristics of HIV-2-specific CD8⁺ and CD4⁺ T-cell responses, highlighting key areas in which they may differ from responses against HIV-1, and the clinical implications of such disparities.

Introduction

Although HIV-1 and HIV-2 share substantial similarity in terms of genome and life cycle, there are clear epidemiological and prognostic distinctions between them. An important distinguishing feature of infection is the distribution and time to clinical progression (Marlink et al. 1994). In contrast to HIV-1 infection, where the overwhelming majority of treatment-naïve patients develop AIDS within approximately a decade, the outcome of HIV-2 infection follows an unusual bimodal distribution, with more than half of untreated patients exerting strong control over the virus and the remaining patients progressing toward clinically indistinguishable end-stage disease (Brun-Vezinet et al. 1987; Clavel et al. 1987). Uniquely, the patients that do progress to AIDS

maintain significantly higher CD4⁺ lymphocyte counts and longer survival times following symptomatic onset than HIV-1 patients at the equivalent stage (Martinez-Steele et al. 2007). Additionally, long-term nonprogressors (LTNPs) and elite controllers (ECs) are significantly over-represented in HIV-2 infection, with rates as high as 37% in some cohorts (Thiebaut et al. 2011; van der Loeff et al. 2010) – markedly higher than the values below 1% typically recorded in the case of HIV-1.

The associated mortality rates of HIV-1 and HIV-2 infection are therefore vastly different. Relative to the uninfected population, HIV-1 patients present with a 10–20-fold increase in mortality, while in HIV-2 patients it is only twofold (Mulder et al. 1994; Poulsen et al. 1997; van Tienen et al. 2011). HIV-2-associated mortality risk is also significantly correlated with plasma viral load, with low levels of circulating viremia being strongly predictive of long-term survival and good clinical outcome (Ariyoshi et al. 2000; Berry et al. 2002; van der Loeff et al. 2010). It has been well established in the case of HIV-1 that the control of acute viremia and determination of the viral set point are themselves associated with efficient and timely T-cellular responses (Borrow et al. 1994; Koup et al. 1994; Ransinghe et al. 2012). Furthermore, recent studies have since revealed that robust T-cell responses are also elicited in seropositive HIV-2 patients. From these observations there is mounting evidence for an important role of T-cells in HIV-2 control and disease progression.

Background

T-cells are important players in the context of HIV-1 infection. The roles of CD4⁺ and CD8⁺ T-cells in both viral control and pathogenesis are extensive and will be discussed in detail in other entries (► [HIV and SIV, CD4 T-Cell Responses to](#), ► [HIV and SIV, CD8 T Cell Responses to](#)). Briefly, CD4⁺ lymphocytes orchestrate the immune response by activating a diverse range of innate and adaptive effector cells, while CD8⁺ lymphocytes lyse infected cells and secrete

antiviral factors that suppress infection through noncytotoxic mechanisms.

As a result of intense and long-standing investigation, much is known about the role of T-cells in HIV-1 infection. However, it is arguably less clear for HIV-2, due largely to the relative paucity of studies into this viral infection. Furthermore, investigations that draw direct comparisons between HIV-1 and HIV-2 often fail to stratify findings based on important clinical parameters, having substantial implications for proper interpretation of the data. For example, despite the characteristically lower viral load of HIV-2 patients, they are often directly compared to HIV-1 patients *as a whole*, rather than more appropriate subsets such as LTNPs.

Despite these limitations, a clear picture is being developed of not only the function of T-cells in the context of HIV-2, but also how the response differs from HIV-1 infection, and the potential consequences for viral control and patient prognosis.

CD8⁺ Virus-Specific T-Lymphocyte Responses: HIV-1 Versus HIV-2

The earliest published evidence for a role of CD8⁺ virus-specific T-cell responses in the control of HIV-2 infection came from observations in the macaque model that gag-, pol-, and env-specific cytotoxic responses could be detected in the blood of animals infected with HIV-2_{BEN} (Voss et al. 1992). However, the specific epitopes recognized by the generated lymphocytes were not identified at the time.

Soon after, the first description of HIV-2-specific T-cell responses in a human study was reported, following the observation that gag-specific cytotoxic T-lymphocytes against an identifiable epitope (1A; TPYDINQML) could be extracted from the peripheral blood mononuclear cells (PBMCs) of over 55% of study participants in a cohort of Gambian HIV-2-infected asymptomatic patients, without the need to first restimulate the cells in vitro (Gotch et al. 1993). The study had exciting implications, in that strong HIV-2 gag-specific responses were demonstrable

at a much higher prevalence than the detection in approximately 25% of healthy HIV-1 seropositive patients the group had reported previously (Gotch et al. 1990), indicating that the increased frequency and strength of HIV-2-specific responses could be responsible for mediating enhanced control of this virus, thereby explaining its unique pathology.

However, the sample size in the study was small, limited to only nine patients, and subsequent larger studies comparing HIV-1 and HIV-2 responses failed to corroborate any substantial difference in either frequency or magnitude of response in patients matched for disease stage (Gillespie et al. 2005; Jaye et al. 2004; Zheng et al. 2004). Instead, it has been suggested that the relatively reduced viral load of HIV-2 infection may be the result of either more efficient control exerted by a response of equivalent magnitude or conversely by reduced replication of the virus itself (Jaye et al. 2004).

An in-depth study of the functionality of HIV-1-/HIV-2-specific lymphocytes has since provided further support to the hypothesis that HIV-2-specific responses of equivalent magnitude may exert more efficient control on viral replication (Duvall et al. 2008). By measuring the production of cytokines and chemokines (IFN- γ , IL-2, TNF- α , & MIP-1 β), as well as degranulation (CD107a) and memory function by specific viral peptide-stimulated cells, it was determined that the degree of polyfunctionality exhibited by HIV-2-specific CD8⁺ T-lymphocytes is markedly higher than that of cells specific for HIV-1. Furthermore, polyfunctional HIV-2-specific lymphocytes release higher levels of IFN- γ and TNF- α than their monofunctional equivalents.

The more efficient response generated in HIV-2 patients has been linked to increased promiscuity of T-cell receptor (TCR) usage, leading to greater functional flexibility (Lopes et al. 2003). While the CD8⁺ cells of HIV-1 patients are known to undergo oligoclonal expansion in terms of TCR repertoire, HIV-2 patient cells were found to be much more heterogeneous. The authors inferred this to be functionally important as it suggests a greater ability to tolerate variation in recognized epitopes and, by

extension, generate protection against a broader range of HIV-2 strains. This is consistent with previous observations that highly oligoclonal TCR usage by HIV-1-specific cytotoxic lymphocytes is predictive of poor clinical outcome (Pantaleo et al. 1997). However, the study was not without its limitations, as the authors considered overall TCR usage, rather than performed a direct comparison between virus-specific T-cells in HIV-1 and HIV-2 infection.

A more recent study by Leligidowicz et al. – who set out to describe the TCR usage and broader characteristics of CTLs in the control of HIV-2 infection – identified a distinct differentiation phenotype from cells associated with HIV-1 infection (Leligidowicz et al. 2010). The cells were found to be CD27⁺ and CD28⁺, characteristic of early differentiation (Appay et al. 2002). The level of CD27⁺CD28⁺ cells was positively associated with CD4 count, suggestive of overrepresentation in patients with delayed progression. Furthermore, the patients unexpectedly demonstrated oligoclonal TCR V β segment usage in HLA-B*3501-restricted CTLs – contrary to previous evidence (Lopes et al. 2003; Pantaleo et al. 1997) – and all CTLs specific to the study epitope (HIV-2 gag NY9) made use of V β 17. These findings are consistent with the selective expansion of high-affinity V β segments by affinity maturation. Furthermore, the study demonstrated greatly enhanced secretion of IFN γ and pro-inflammatory cytokines by these cells at low peptide concentrations, indicating that they have high functional avidity.

While the protective effect of CD8⁺ lymphocytes is largely attributed to their role in mediating cell killing, they are also able to control infection by noncytotoxic means such as the release of soluble antiviral factors. The production of high levels of β -chemokines such as MIP-1 α (CCL3), MIP-1 β (CCL4), and RANTES (CCL5) by CD8⁺ cells plays a role in the prevention and/or control of HIV-1 and SIV infections (Ahmed et al. 2002, 1999; Cocchi et al. 2000), by inhibiting binding of the M-tropic virus to the co-receptor CCR5 on entry (Alkhatib et al. 1996; Cocchi et al. 1996). The importance of CD8⁺ T-cell-soluble factors in the control of HIV-2 is also becoming increasingly

apparent. In 1997, it was observed that filtered cell-free culture fluid containing unknown soluble factors secreted by CD8⁺ lymphocytes was capable of suppressing HIV-2 replication in baboon CD4⁺ cells, in vitro (Blackbourn et al. 1997); but the authors concluded that MIP-1 α , MIP-1 β , and RANTES were not responsible in this study. More recently, β -chemokine production by CD8⁺ lymphocytes has been shown to be greatly elevated in human HIV-2 patients relative to uninfected controls and correlates strongly with CD4⁺ lymphocyte count in these individuals (Ahmed et al. 2005).

Taken together, these findings suggest that homologous HIV-2 CD8⁺ T-cell responses – while neither more frequent nor higher in magnitude – may exert more efficient control over infection than the equivalent HIV-1 response. This is likely to be due to increased avidity and polyfunctionality: including direct cytotoxicity and the production of high levels of soluble antiviral factors. It is therefore conceivable that the reduced viral set point characteristic of HIV-2 infection may be the result of an enhanced CD8⁺ lymphocyte response.

CD8⁺ T-Cell Epitopes and Cross-Reactivity in HIV-1 and HIV-2 Infection

The known CD8⁺ and CD4⁺ epitopes of the HIV-1 genome have been comprehensively mapped, and the Los Alamos National Laboratory (LANL) maintains an up-to-date record of these on its website (available: <http://www.hiv.lanl.gov/content/immunology/maps/maps.html>; accessed June 2015). However, far fewer HIV-2 epitopes have been described to date – as summarized in Table 1.

The degree to which HIV-2-directed CD8⁺ responses are cross-protective against HIV-1 is not well established. An early report demonstrated substantial cross-reactivity of HIV-2 patient-derived CD8⁺ lymphocytes against HIV-1 (Bertoletti et al. 1998). Here, the cytotoxic lymphocytes (CTLs) of 11 HIV-2 patients were tested for their ability to recognize gag-derived from four subtypes of HIV-1. Strikingly, nine of the patients responded to at least one subtype of HIV-1, while five of these patients were able to

respond to at least three subtypes. Four of the five patients demonstrating broad cross-reactivity carried HLA-B*5801: a class-I human leukocyte antigen (HLA) allele associated with slow progression in HIV-1 infection (Navis et al. 2007).

However, more recent evidence suggests that HIV-2-directed CD8⁺ responses may in fact be less cross-protective than those generated against HIV-1 (Jennes et al. 2008). The authors found that responses from HIV-2 patient T-cells against HIV-1 gag peptides were both weaker in magnitude and narrower in breadth than the cross-reactive responses elicited by HIV-1 patients. However, responses to *homologous* gag were decisively broader and stronger in HIV-2 patients than HIV-1, perhaps contributing to the greater immunological control and reduced viral load characteristic of this infection.

Surprisingly, HIV-2 patient responses to homologous nef are rare (Leligdowicz et al. 2007; Zheng et al. 2007). Nef is one of the most abundant viral proteins and one of the earliest to be expressed, making it a prime target for cellular responses in HIV-1 infection. It is also a major target for cytotoxic lymphocytes in acute SIV infection (Mothe et al. 2002). As such, it is very unexpected that an equivalent response is not detected against HIV-2 nef. Possible explanations that have been proposed for the lack of HIV-2 nef-specific responses include difficulties with epitope processing, HLA-binding, TCR recognition, and/or antigen presentation resulting from the low degree of sequence homology between HIV-1 and HIV-2 nef.

CD4⁺ Virus-Specific T-Lymphocyte Responses: HIV-1 Versus HIV-2

HIV-1 specific CD4⁺ T-lymphocytes are preferentially targeted by the virus and depleted from the host repertoire. Conversely, in the majority of HIV-2 infected individuals, the CD4⁺ T-lymphocyte compartment remains preferentially targeted but quantitatively preserved (Duvall et al. 2007). As such, qualitative changes in their performance are likely to have a significant impact on disease control.

Cellular Immune Response to HIV-2 Infection, Table 1 List of known HIV-2 CD4⁺ T-lymphocyte epitopes and epitope-containing peptides and their relative

position mapped to the HXB2 reference genome. Publications referring to the listed epitopes can be accessed through the associated PubMed ID

Protein	Position	Sequence	HLA restriction	Reference (PubMed ID)
gag	24–32	GGKKKYKMK	B*35	1721107, 10203028
gag	71–80	EIINEEAAEW	A*25	10203028, 8760412
gag	77–85	SLFNTVCIW	A*02	19016530
gag	77–85	SLFNTVCVI		12817012
gag	130–138	PPSGKGGNY	B*35	7584954
gag	173–188	QALSEGCTPYDINQML		17823657
gag	179–190	CTPYDINQMLNC	B*58	Bertoletti, personal communication 1998 via LANL
gag	180–188	TPYDINQML	B*53(01); B*58(01)	19016530, 15832290, 18562522, 7690804, 23558015, 18622680
gag	234–251	SDIAGTTSTVDEQIQWY		17823657
gag	240–249	TSTVDEQIQW	B*58(01)	23558015
gag	240–249	TSTVEEQIQW	B*57; B*58(01)	12817012, 9499105, 8959245, 15832290, 18622680
gag	249–266	MYRQQNPVPGVGNIRRWI		17823657
gag	254–262	NPVPGVNIY	B*35(01)	7584954, 19016530, 23558015
gag	257–274	PVGNIRRWIQIGLQKCV		17823657
gag	295–304	SYVDRFYKSL	A*24	18562522
gag	296–313	YVDRFYKSLRAEQTPAV		17823657
gag	298–306	DRFYKSLRA	B*14	23558015
gag	304–321	LRAEQTPAVKNWMTQTL		17823657
gag	308–317	QTPAVKNWM	B*53	18562522
pol	329–337	NPDVILIYQ	B*35	7584954
gp160	21–34	LNSWGCAFRQVCHT		21849066
gp160	376–383	TNCRGEFL	Cw*04	7677956, 10203028
gp160	584–592	EKYLQDQAR	B*14	1372650, 10203028
gp160	586–593	YLQDQARL	B*08	1372650, 10203028
nef	75–82	VPLRPMTY	B*35	7584954

The proportion of patients with detectable CD4⁺ T-lymphocyte responses against gag is higher in HIV-2 donors than in HIV-1 donors when subjects with equivalent CD4⁺ counts are compared; and HIV-2 specific CD4⁺ T-lymphocytes demonstrate higher proliferative capacity than those specific to HIV-1 (Duvall et al. 2006). HIV-2 gag-specific CD4⁺ T-lymphocytes also demonstrate higher poly-functionality than HIV-1 gag-specific cells when the production of IFN- γ , TNF- α , MIP-1 β , and IL-2 and mobilization of CD107a are considered (Duvall et al. 2008).

A characteristic feature of asymptomatic, non-progressive HIV-2 infection in humans is the maintenance of robust CD4⁺ T-lymphocyte help

(Zheng et al. 2004). Furthermore, macaques are less likely to develop early signs of Th dysfunction when infected with nonpathogenic HIV-2 than the more pathogenic SIVmac (Dittmer et al. 1994). In summary, the preservation and persistence of both qualitative and quantitative features of CD4⁺ T-lymphocyte function appear to contribute to HIV-2 nonprogression and may account for viremic control in nonprogressors.

T-Lymphocyte Responses in HIV-2: Controllers Versus Progressors

HIV-2 patients who develop AIDS progress in a manner and time frame that is clinically

indistinguishable from HIV-1 non-controllers (Hansmann et al. 2005; Martinez-Steele et al. 2007). It is therefore surprising that a substantial proportion of HIV-2 patients control the infection, despite it having a demonstrated potential to kill. Understanding how disease progression is avoided in these patients may provide insight as to how to control HIV-1 infection. This key interest in distinguishing LTNPs from progressors stems from a widely held hypothesis that LTNPs have acquired natural immunity against HIV and from the idea that HIV vaccines should ideally replicate the immune responses found in such patients. Unfortunately, LTNPs are extremely rare in HIV-1 infection, and it is in this regard that the paradigm of HIV-2 infection distinguishes itself (Rowland-Jones and Whittle 2007).

In HIV-2 infection, an inverse relationship has been demonstrated between CTL responses and proviral load, with stronger cytotoxic T-lymphocyte activity detected in donors with low viral load (Ariyoshi et al. 1995; Sarr et al. 2001). Moreover, type 1 Th (Th1) responses are more prolific in donors with undetectable HIV-2 RNA versus non-controllers (Alatrakchi et al. 2006). Similar to the case for CD4⁺ T-lymphocytes, cytotoxic and IFN- γ responses were more likely triggered by HIV-2 gag, and patients with undetectable viremia demonstrated increased competence in mounting such responses (de Silva et al. 2013; Leligdowicz et al. 2007). More specifically, CTLs from non-progressors were more likely to target gag, while equivalent responses were absent in the majority of progressors. Ultimately, the establishment of gag-specific CD8⁺ polyfunctionality shows a strong inverse relationship to HIV-2 viremia in infected subjects.

This leads to a further question that has remained relatively unanswered: what accounts for the more efficient T-cell responses that occur in HIV-2 controllers than in progressors? And furthermore, why are T-cell responses more effective against HIV-2 than HIV-1? How are high levels of antiviral T-cells maintained in controllers who have undetectable plasma viral load for prolonged periods? Studies have alluded to the enhanced infectivity of dendritic cells (Manel

et al. 2010) facilitated by the additional encoding of vpx by HIV-2, which antagonizes SAMHD1 (Laguette et al. 2011). The latter may have consequences for antigen processing and presentation and the elicitation of innate immune responses such as type I IFN and TRIM5 α which may be more effective against HIV-2 (Cordeil et al. 2013; Ylinen et al. 2005). Yet Duvall et al. showed the lack of permissibility of blood-derived mDCs and pDCs to HIV-2 infection in culture, which suggests the possibility of alternative protective mechanisms (Duvall et al. 2007). The increased susceptibility of the HIV-2 capsid to TRIM5 α recognition could potentially lead to more efficient proteasomal processing of HIV-2 proteins and thus facilitate antigen presentation.

HIV-2-Specific T-Lymphocyte Responses in Dual Infection

A highly controversial topic regarding HIV-2-specific T-cell responses is whether or not these responses confer a protective advantage against subsequent infection with HIV-1. Furthermore, in patients dually infected by both HIV-1 and HIV-2, do these responses reduce the rate of disease progression?

Dual infection of both HIV-1 and HIV-2 is extremely uncommon in developed nations, but has been reported at a prevalence of between around 0% and 3% of the population in some West African countries (Hamel et al. 2007; Mansson et al. 2009). A high-profile early study of Senegalese sex workers suggested that HIV-2 infection may have conferred a protective advantage against incident HIV-1 infection in these women (Travers et al. 1995). Conversely, subsequent studies failed to repeat this finding (Greenberg 2001; Norrgren et al. 1999), and the increased level of T-cell activation in dual infection is suggestive of more aggressive disease (Koblav-Deme et al. 2004). There is also evidence to suggest that over an extended time scale, HIV-1 may outcompete HIV-2 in dually infected individuals with low CD4 counts (Raugi et al. 2013).

However, a substantial limitation to studies of dual-infected patients – in addition to the scarcity

of suitable study candidates – is that often the patients recruited are those attending sexual health clinics with symptomatic HIV or coexisting sexually transmitted infections. As such, they are perhaps more likely to present with aggressive or late-stage illness. Supporting this claim, a study of individuals who were unaware of their positive status and were recruited from tuberculosis and occupational cohorts showed that the HIV-1 plasma viral load of dual-infected patients was significantly lower than patients infected with HIV-1 alone (Andersson et al. 2000).

In a comparative longitudinal study of patients either dually or singly infected, Esbjörnsson et al. found that dually infected patients progressed to AIDS on average 36 months slower than patients infected with HIV-1 alone (Esbjörnsson et al. 2012). The protective effect appeared strongest in patients first infected with HIV-2 who controlled the infection and subsequently became HIV-1 positive, suggestive of an inhibitory effect mediated by existing T-cellular responses against HIV-2 antigens (de Silva et al. 2012). The conclusion to be drawn here is that HIV-2 may provide a degree of protection against HIV-1 disease progression, rather than against infection.

The direct role of T-lymphocytes in the context of dual infection has not been extensively studied. However, a 2007 study by Zheng et al. compared the T-cellular responses of HIV-1, HIV-2, and dual-infected patients to different env, gag, and nef epitopes (Zheng et al. 2007). The study demonstrated that the magnitude and frequency of responses against HIV-1 proteins were greater than those against HIV-2 and that functional CD4⁺ T-cell responses could be detected in all dually infected patients. Moreover, in the dual-infected patients, the level of HIV-2 gag-specific CD4⁺ and CD8⁺ T-cell response showed a significant inverse correlation with HIV-1 plasma viral load, indicative of a protective effect.

Conclusions

While the role of the cellular immune response in the context of HIV-1 infection has been well established for some time, it is only recently that

a clearer picture of HIV-2-specific T-cell responses is developing, and even now we can see only the proverbial tip of the iceberg. However, with a growing number of studies comes growing evidence that T-lymphocytes play a major role in the control of the virus.

Virus-specific CD8⁺ cells are detectable in HIV-2 patients, but not at significantly higher frequency or magnitude than in HIV-1 infection: targeting of gag epitopes is a prominent feature of HIV-2 controllers that is frequently absent in progressors. However, T-cellular responses of equivalent magnitude may exert more efficient control on infection through increased avidity and broader functionality: for example, through direct cytotoxicity or the release of higher levels of inflammatory cytokines and soluble antiviral factors at low antigen concentrations. It is worth noting that high frequencies of virus-specific T-cells can be found in HIV-2-infected controllers who have maintained an undetectable viral load for many years.

Polyfunctional CD4⁺ lymphocytes may be detected in HIV-2 patients at higher levels than in HIV-1 and demonstrate substantially improved proliferative capacity. Furthermore, there is preliminary evidence from the macaque model that suggests Th cell dysfunction may be less likely to occur in HIV-2 infection than in infection with more strongly pathogenic retrovirus types, although this has not yet been corroborated in humans. The reason for the relative preservation of Th function in HIV-2 infection is not known, as HIV-2 shows the same preferential targeting of virus-specific Th cells as does HIV-1.

Collectively, these findings strongly suggest that efficient T-cellular responses may play at least a supporting role in explaining some of the significant prognostic differences between HIV-1 and HIV-2 infection. This hypothesis is supported by observations in dually infected individuals, which indicate that initial infection with HIV-2 may confer some degree of protection against subsequent infection with HIV-1, due to the presence of preexisting cellular responses.

While dissimilarities arguably exist between HIV-1- and HIV-2-specific T-cellular responses *in vivo*, the mechanisms by which these arise are

not currently well understood. Promiscuity of TCR usage and a unique differentiation phenotype have been proposed as explanations, which would allow functional flexibility and recognition of a broader range of antigens, giving rise to a response which may or may not be cross-reactive to some degree.

In summary, the cellular immune response to HIV-2 is a complex process, which appears to have an important role in controlling infection and promoting less aggressive disease progression than that seen in HIV-1 infection. The specific processes involved and the extent of the control that it exerts are currently not well established, but active research in this field is gradually filling these gaps and paving the way to the identification of potential therapeutic targets.

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Central Memory CD4 T Cells

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Definition

Memory CD4 T cells can be divided into central memory (T_{CM}) and effector memory (T_{EM}) subsets, which are endowed with different homing

and proliferative capacities as well as distinct effector functions (Sallusto et al. 1999). T_{CM} cells can directly home to secondary lymphoid organs and express the lymphoid homing molecules CD62L (L-selectin) and CCR7 (chemokine receptor 7). In contrast, T_{EM} cells home to tertiary sites and tissues and have direct effector functions upon stimulation, including secretion of inflammatory cytokines such as interferon gamma ($IFN-\gamma$) and tumor necrosis factor (TNF). Thus, T_{CM} cells define a lymphoid homing subset without immediate effector function, whereas T_{EM} cells are defined as a subpopulation that localize to tissues, with rapidly induced effector function and less proliferative potential. A dominant theory is that T_{CM} represent a precursor to T_{EM} as T_{CM} have greater proliferative potential, are longer lived, and can generate T_{EM} and T effector cells upon stimulation. Furthermore, T_{CM} and T_{EM} homeostatic proliferations are distinct as T_{CM} respond to interleukin 7 (IL-7), whereas T_{EM} respond to interleukin 15 (IL-15).

The description of the two T cell memory subsets led to different models of memory generation. In the “divergent” model of memory T cell formation, naïve T cells, after antigen engagement, can directly differentiate into an effector T cell or a memory T cell. A variation of this divergent model is the “bifurcation” model, wherein daughter cells of naive T cells have an unequal cell division yielding a memory and an effector cell. This is in contrast to a “linear differentiation” model where effector cells are generated first and subsequently yield memory populations (Ahmed et al. 2009). What controls the memory subset composition in response to infection is not completely understood; however, duration, location, strength, and dose of antigen are thought to play critical roles (Kaech et al. 2002). Later work confirmed the T cell self-renewing properties of T_{CM} previously described, although the exact lineage relationship between effector T cells and the memory subsets *in vivo* has not been proved.

Subsequently, additional subsets have been identified including a transitional memory T cell subset (T_{TM}), an intermediate between T_{CM} and T_{EM} , which does not express CCR7 although retains other markers of T_{CM} cells such as CD27.

A stem cell-like memory T cell population (T_{SCM}) that expresses some classical markers of naïve T cells (CD45RA+) and of memory T cells (CD95) has also been identified. T_{SCM} cells have multipotency and increased survival and proliferative capacity and can generate all memory T cell subsets including T_{CM} (Gattinoni et al. 2011).

T_{CM} as a Target for HIV Infection and Replication

Memory CD4 T cells play a critical role in immune responses to viral infections and are a key target in HIV infection. Depletion of these cells results in alteration of immune function and progression to AIDS. As mentioned above, the memory CD4 T cell compartment is heterogeneous, with T_{CM} , T_{TM} , and T_{EM} cells displaying differences in their homing capacity, their ability to mount immediate effector responses, and their longevity. The relative susceptibility to HIV infection of these three memory CD4 T cells subsets is driven by a variety of cellular factors including the level of expression of the major HIV co-receptors CCR5 and CXCR4, the cellular abundance of viral restriction factors, and the preferential localization and homing capacity of these cells to anatomical sites in which HIV replication is promoted.

HIV Co-receptors

CD4 T cells displaying a T_{CM} , T_{TM} , and T_{EM} phenotype can all be infected by HIV *in vitro* (Flynn et al. 2014). However, the levels of expression of the two major co-receptors for HIV greatly vary between these subsets, with CCR5 levels increasing with the differentiation status, whereas CXCR4 expression gradually diminishes from T_{CM} , T_{TM} , to T_{EM} . As a result of this differential expression pattern, T_{CM} cells are more susceptible to infection by CXCR4-using viruses than by CCR5-using isolates. Similarly, the relative levels of expression of the co-receptors on the respective T cell subsets are a decisive factor for dendritic cell-mediated HIV transmission of memory T cells: CCR5-using viruses are most efficiently transmitted to T_{EM} cells, and CXCR4-using HIV

are preferentially transmitted to T_{CM} cells (Groot et al. 2006).

Restriction Factors

The relative abundance of restriction factors among the different memory subsets represents another parameter that greatly influences the sensitivity of a subpopulation to HIV infection. Indeed, resting memory CD4 T cells with the highest APOBEC3G protein levels display the lowest levels of provirus in vivo. Another example of the critical importance of restriction factors in T cell susceptibility to HIV infection is provided by the recently discovered restriction factor SAMHD1. SAMHD1 is expressed at high levels in all memory CD4 T cells displaying a quiescent phenotype (naïve and resting memory CD4 T cells), suggesting that a minimal state of activation, which leads to the downregulation of SAMHD1, is required to achieve productive infection (Baldauf et al. 2012; Descours et al. 2012a). As activation often leads to cell proliferation in T cells, this may provide an explanation for the greater susceptibility of cycling T cells to HIV infection compared to resting, quiescent T cells. Of note, those cycling CD4 T cells that are more susceptible to infection usually display a T_{CM} phenotype during chronic HIV infection (Sieg et al. 2005). Altogether, these studies suggest that cycling T_{CM} cells may be the preferential target for the virus during untreated HIV infection, at least in the peripheral blood.

Homing Capacities

The susceptibility of CD4 T cells to HIV infection may also be greatly influenced by their capacity to migrate to secondary lymphoid organs, in which HIV replication predominantly takes place. Indeed, it is well established that the bulk of HIV replication occurs in lymphoid tissues such as the gut-associated lymphoid tissue (GALT) and lymph nodes. The relative susceptibility to HIV infection of memory CD4 T cell subsets residing in these compartments has mainly been studied in animal models, particularly in simian immunodeficiency virus (SIV)-infected nonhuman primates. Productive infection and depletion of activated

resident CCR5+ CD4+ T cells at the earliest stage of SIV infection have been repeatedly observed. This is likely to mainly concern T_{EM} cells, as these cells predominate in the gut, whereas T_{CM} cells are rare in this tissue. Although T_{CM} cells are unlikely to be directly infected in the gut, they play a critical role in this site. In an attempt to replenish the pool of tissue cells, CD4 T cells (including T_{CM} cells) are recruited from the periphery and home to the gut, where they differentiate into effector cells and are eventually eliminated. In other words, effector-site T_{EM} cell populations show a slow, continuous decline, and their homeostasis is dependent on the production of new cells from T_{CM} precursors (Okoye et al. 2007).

As they express CCR7, T_{CM} cells have the capacity to recirculate between the periphery and second lymphoid organs and constitute the predominant memory CD4 T cells found in lymph nodes. The unique microenvironment of this compartment during viral infection (inflammation stemming from viral replication and limited accessibility to HIV-specific cytotoxic T lymphocytes) may greatly facilitate infection of this subset, even though the frequency of infection of T_{CM} cells residing in the lymph node of chronically HIV-infected subjects has never been directly measured. Recently, follicular helper T cells (T_{fh}), which express CXCR5 and are found in the B cell follicles of secondary lymphoid organs, have been identified as the major CD4 T cell compartment for HIV infection, replication, and production (Perreau et al. 2013). A fraction of circulating T_{CM} cells express CXCR5, and a preferential recruitment of these cells to CXCL13-rich B cell follicles promotes a quick and efficient protective secondary humoral immune response. Therefore, T_{CM} cells with T_{fh} functions are endowed with optimal homing capacities, functions, and survival features and thus may serve as an ideal subset to sustain HIV production in lymphoid tissues.

Infection of T_{CM} Cells in Acute and Chronic HIV Infection

Despite the aforementioned predicted resistance of T_{CM} cells to HIV infection as a result of low

CCR5 expression and high levels of restriction factors, HIV DNA is readily detected in the three subsets of memory CD4 T cells during the earliest stage of infection. Of note, although acute HIV infections are usually characterized by high levels of replication of viruses preferentially using CCR5 (which may restrict infection in T_{CM} cells), almost a third of the pool of infected CD4 T cells consists of T_{CM} cells within 1 month post-infection, and all three memory CD4 subsets can produce replication-competent virus after stimulation *in vitro* (Bacchus et al. 2003). Therefore, it is clear that T_{CM} cells are infected early during the natural history of HIV pathogenesis. More importantly, high CCR5 density on T_{CM} cells during acute HIV infection is associated with rapid disease progression (Yang et al. 2012), suggesting an important role for this particular subset in the depletion of the CD4 compartment.

In chronically HIV-infected subjects (receiving ART or not), resting memory CD4 T cells are the predominantly infected cells. Infection of naïve cells and terminally differentiated cells is rare (Brenchley et al. 2004; Chomont et al. 2009). Within the memory compartment, the T_{CM} subset is the predominantly infected subset and harbors a more diverse viral population compared to the others. Altogether, these observations indicate that although T_{CM} cells may be intrinsically relatively resistant to HIV infection, these restrictions are largely alleviated *in vivo*. Infection of T_{CM} cells has been clearly demonstrated in both SIV and HIV infections and may have a critical impact on disease progression during lentiviral infections.

Relative Protection of T_{CM} Cells to HIV as a Mechanisms for Control

Several lines of evidence indicate that a lower relative contribution of the T_{CM} subset to the HIV reservoir and a greater preservation of T_{CM} cells are elements integrally correlated to control of HIV infection. Widely differing models of viral control uniformly demonstrate that T_{CM} parameters are correlated to viral control and/or improved prognosis during HIV infection.

Long-Term Nonprogressors and T_{CM} Preservation

Protective HLA alleles, namely, HLA-B27 and HLA-B57, are associated with reduced plasma viral RNA levels and cell-associated HIV DNA levels as well as increased polyfunctional HIV-specific CD8 T cell responses. Long-term nonprogressors (LTNP) include subjects with or without the protective HLA-B27 or HLA-B57 alleles (Descours et al. 2012b). LTNPs with the protective alleles have a significantly lower percentage of T_{CM} cells that contribute to the viral reservoir than those without the protective allele (20% vs. 40% of the total CD4 T cell HIV reservoir, respectively). This lower contribution of T_{CM} cells to the HIV reservoir correlates to a greater T_{CM} pool size. The anti-Gag HIV-specific CD8 T cell responses in the LTNPs with the protective alleles are greater in number, as may be expected, but also correlate inversely to the relative contribution of T_{CM} cells to the viral reservoir. Thus, this model proposes that anti-Gag CD8 T cell responses in those with protective alleles are central to sparing HIV infection in T_{CM} cells, resulting in a preserved self-renewal capacity of this subset.

Posttreatment Controllers and Limited T_{CM} Viral Reservoir

Posttreatment controllers (PTCs) are a group of individuals who initiate ART early in infection and who are able to control viral replication for years after cessation of ART (Saez-Cirion et al. 2013). The PTCs, unlike other HIV controllers, do not have the protective alleles nor demonstrate potent anti-Gag CD8 T cell responses as measured by functional suppression assays. PTCs demonstrate less immune activation of CD4 T cells than their controller counterparts. In PTC, T_{TM} contribute the most to the viral reservoir, whereas in controllers, T_{CM} and T_{TM} contributed equally to the pool of infected cells. Furthermore, there were lower levels of HIV DNA in naïve T cells (T_N) in PTC compared to controllers. It may be that it is the limited viral reservoir in long-lived cells such as T_{CM} in PTC that results in “the gradual shrinking of the reservoir in PTCs for whom T_{TM} is the main reservoir” (Saez-Cirion et al. 2013).

Taken together, these different models of control – LTNP or PTC – indicate that while many aspects of pathogenesis differ, preservation of T_{CM} and a limited T_{CM} reservoir are common to disparate models of viral control.

Resistance of T_{CM} to Apoptosis in Elite Controllers

Underscoring the importance of T_{CM} in HIV pathogenesis (van Grevenynghe et al. 2008), T_{CM} from elite controllers (ECs) compared to those from successfully treated (STs) individuals have different half-lives. Examination of the lifespan of these cells after *in vitro* nonspecific T cell receptor (TCR) engagement indicates that T_{CM} from ECs are maintained for longer periods than T_{CM} from STs or HIV-uninfected individuals. This different lifespan is due to decreased apoptosis and not altered differentiation or proliferation. This preservation of T_{CM} in ECs is controlled, at least, in part, by FOXO3a (forkhead box O3a), a transcription factor whose phosphorylation inhibits activation of apoptotic-related factors and which is increased in T_{CM} from EC. An increased T_{EM} survival is also observed in EC, although the T_{EM} half-life is still relatively shorter than that of T_{CM} . T_{CM} in ECs and STs do not have different levels of activation marker expression, and the antiapoptotic phenotype in ECs is not correlated with protective alleles. Thus, it is likely that this antiapoptotic quality in T_{CM} from ECs increases the integrity of T_{CM} homeostasis, and it is this quality that plays a protective role in limiting HIV disease progression in ECs.

T_{CM} and Immune Reconstitution Post-ART

CD4 T cell immune reconstitution post-ART is an area of considerable interest. The strongest positive correlate of CD4 T cell levels after long-term ART is the absolute T_{CM} counts after long-term ART. This is even a stronger correlation than CD4 T cell counts at earlier time points after the initiation of ART, again, reinforcing the role of T_{CM} cells in T cell reconstitution. However, of note, the T_{CM} subset is defined, in some analyses including this one, by pooled T_{CM} and T_{TM} populations (i.e., using CD27⁺ and CCR7^{-/+} expression as population-defining markers). This is in

agreement to a previous work (Chomont et al. 2009) that had found that T_{CM} were under-represented in virally suppressed subjects with low CD4 counts, suggestive of T_{CM} depletion and/or differentiation of T_{CM} into T_{TM} in these individuals.

Subjects who initiate ART early after primary HIV infection that reach viral suppression after less than 6 months of ART have a higher proportion of T_{CM} cells in the peripheral blood than those who do not, suggesting that T_{CM} levels in the blood may be a good predictor of HIV suppression during ART (Karris et al. 2014). However, to note, there is a continuous decline of T_{CM} over time in the GALT in the same subjects, suggesting a different kinetic of viral suppression or an intensive cell death mediated by higher activation of CD8 T cells in tissues such as the GALT compared to peripheral blood.

SIV Infection Models and T_{CM} Preservation in Natural Hosts

The SIV model in the natural host, the sooty mangabey (SM), and the nonnatural host, the rhesus macaque (RM), indicate differences in the T_{CM} compartment. T_{CM} cells are preserved and have a longer half-life – 17 years compared to 16 months – in the SM nonpathogenic infection, compared to the RM pathogenic infection, respectively (McGary et al. 2014). In RM, the greater the fraction of T_{CM} cells, the greater the CD4 T cell numbers and the lower the plasma viral load. However, the levels of proliferating T_{CM} correlate negatively with CD4 counts in RM, while in the natural host, the SM, proliferation of T_{CM} correlates positively with CD4 counts. This indicates that increased levels of homeostatic proliferation of T_{CM} cells, possibly as a result of their continuous activation and/or depletion, are deleterious during SIV infection in RM. Other researches examining the SIV nonpathogenic and pathogenic models have corroborated this finding.

Thus, in various models of HIV disease progression, in the presence or absence of ART, or protective alleles, preservation of the T_{CM} compartment is correlated to control of viral infection, and improved prognosis or outcome, during HIV infection. Thus, T_{CM} preservation defines a

critical parameter of HIV pathogenesis, and interventions that preserve or enhance the T_{CM} subset warrant further evaluation for therapeutic effects.

T_{CM} Cells as a Reservoir for HIV During ART

Despite the fact that ART has dramatically reduced the death rate from AIDS and improved the quality of life of many HIV-infected patients, it is now clear that ART does not eradicate HIV. The virus persists in long-lived latently infected CD4 T cells that are the source of viral rebound when ART is discontinued. This HIV reservoir constitutes the major barrier to achieving a cure, and it is the T_{CM} cells that have been identified as a main reservoir for HIV during ART (Chomont et al. 2009).

Due to the long-lived nature and low levels of proliferation of T_{CM} cells, as aforementioned, latently infected T_{CM} cells can persist in HIV-infected individuals for their lifetime. T_{CM} cells are the main reservoir for HIV during ART in subjects who have reconstituted their CD4 T cell compartment (Chomont et al. 2009). Conversely, the reservoir is mainly localized to CD4 T cell displaying a T_{TM} phenotype in subjects with low absolute CD4 T cell counts. Genetic analysis of the proviral populations in T_{CM} show that this subset constitutes a stable reservoir with minimal proliferation levels, in agreement with their enhanced survival capacity.

The identification of the T_{CM} subset as a major reservoir for the virus prompted the development of in vitro models of latency that recapitulate the homeostatic and pro-survival features of this subset (Bosque and Planelles 2009; Lassen et al. 2012). Using these models, unique characteristics of the T_{CM} reservoir have been evidenced. For example, cellular proliferation induced by IL-7 is not accompanied by HIV reactivation in T_{CM} ; thus, there is a dissociation of cell cycle activation and transcription from the viral promoter (Bosque et al. 2011). Interestingly, in this model, stimulation with synthetic TLR1/2 ligands disrupts HIV latency, highlighting the potential use of TLR ligands as novel anti-latency agents. When compared to T_{TM} , T_{CM} cells appear

to be less sensitive to a variety of reactivation strategies including TCR stimulation and protein kinase C (PKC) activation, suggesting that this subset may represent the most critical barrier to the success of eradication strategies (Lassen et al. 2012).

The half-life of the replication-competent T_{CM} reservoir has not been measured as yet; however, the slow decay of total HIV DNA levels in this subset during ART suggests that T_{CM} encompass a highly stable reservoir for HIV (Buzon et al. 2014). Of note, the recently described T_{SCM} cells, which represent the earliest and most long-lasting developmental stage of memory T cells and are likely to act as T_{CM} progenitors, may constitute the most long-lived reservoir for HIV in vivo (Buzon et al. 2014).

Conclusion

T_{CM} cells play a critical role in the pathogenesis of HIV and SIV infections. Preservation of T_{CM} cells is correlated to control of HIV infection, indicating that this subset plays a crucial role in the maintenance of T cell immunity. T_{CM} cells also represent the main reservoir for HIV during ART in subjects who have reconstituted their CD4 T cell compartment. This illustrates the dual role of this subset in HIV infection: T_{CM} are essential to control viral replication in ART-naïve subjects. However, in virally suppressed individuals receiving ART, these same cells are responsible for the persistence of a long-lived reservoir for the virus.

Cross-References

- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [T-Cell Homeostasis](#)
- ▶ [T Memory Stem Cells](#)

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decreased to one-third of pre-cervical cytology screening levels.

Cervical cancer was designated an AIDS-defining illness in 1993 (see “► [HIV-Associated Cancers](#)”). The widespread use of antiretroviral therapy (ART) has been associated with a substantial decrease in other AIDS-defining malignancies – Kaposi’s sarcoma and lymphoma; however, the incidence of cervical cancer has increased such that cervical cancer remains a significant cause of morbidity for HIV-positive women (Shiels et al. 2011) (see “► [Epidemiology of AIDS-Defining Malignancies](#)” and “► [Epidemiology of Non-AIDS-Defining Malignancies](#)”).

HPV is the causative agent for cervical cancer. Persistence of cervical infection with HPV is necessary but not sufficient for the development of cervical cancer. HPV prevention with prophylactic vaccination is likely to have a significant role in decreasing the future incidence of this and other HPV-associated malignancies.

Cervical Cancer and HIV

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Definition

Cervical cancer or cancer of the cervix uteri is an abnormal growth of squamous or glandular epithelial cells caused by persistent infection with the ► [human papillomavirus \(HPV\)](#). HPV infections of the cervix may cause cervical intraepithelial neoplasia (CIN) and subsequently develop into invasive cervical cancer (ICC). The successful introduction of cervical cancer screening programs with the cervical cytology, otherwise known as the Papanicolaou (Pap) test (which may detect treatable premalignant CIN lesions), is associated with a 70% decline in cervical cancer incidence and mortality.

Introduction

Cervical cancer is the second most common cancer among women worldwide; however, about 86% of the cases occur in low-resource countries. In countries where cervical cancer screening with cervical cytology is routinely performed, the incidence and mortality of cervical cancer have

Epidemiology

While in developed countries cervical cancer is the tenth most common type of cancer in women (9.0 per 100,000 women), in developing countries it is second only to breast cancer as the most common type of cancer (17.8 per 100,000) and cause of cancer deaths (9.8 per 100,000) among women. In Africa, Central America, and Southern Asia, cervical cancer is the number one cause of cancer-related mortality among women (WHO/ICO Information Centre on HPV and Cervical Cancer 2010). In Eastern and Western Africa, incidence and mortality rates of ICC have been estimated at 35 and 25 per 100,000 women, respectively (Forman et al. 2012).

The relative incidence of cervical cancer in HIV-positive compared with HIV-negative women is unclear – studies have estimated these rates at 1.5–8 times that of the general population (Einstein and Phaëton 2010) (see “► [HIV Cancers in Resource-Limited Regions](#)”). Unfortunately, cancer registries have not been established in lower-income countries; however, it has been estimated that in Uganda and South Africa, the

relative risk of cervical cancer in HIV-positive women is about twice that of the general population (Mbulaiteye et al. 2011). In Tanzania, 30% of women diagnosed with cervical cancer were HIV positive, and these women were significantly more likely to present with cervical cancer at a younger age and more advanced stage compared with women who were HIV negative.

Etiology

In the 1990s, HPV was identified as the causative agent of cervical cancer. HPVs are a large family of small double-stranded DNA viruses that infect squamous epithelia. The 30–40 HPV genotypes affecting the genitalia vary in their oncogenic potential; HPV types 16 and 18, accounting for approximately 65–70% of cervical cancers worldwide, are 2 of approximately 15 HPV types identified as oncogenic or high risk (HR). Certain low-risk HPV types, including types 6 and 11, are largely responsible for genital warts or condyloma.

Genital HPV infections are the most common sexually transmitted infections in women and have a peak prevalence between ages 18 and 25. Most of these HPV infections clear spontaneously (or at least become clinically undetectable), but in 10% of immunocompetent women, these infections persist (i.e., they are detected for greater than 1 year). It is the persistent HR HPV infections that may cause changes in the cervical epithelium leading to the development of cervical cancer precursors – high-grade squamous intraepithelial lesions (HSIL) also known as cervical intraepithelial neoplasia (CIN) 2 or 3 – and possible progression to ICC.

It is now understood that infection with HPV requires a micro-abrasion in the genital epithelium to the level of the basement membrane. In the cervix, the infection site is typically a group of cells in the transformation zone, where the squamous epithelium of the ectocervix transitions to the glandular epithelium of the endocervix. HPV is then able to infect a small group of basal keratinocytes. If the host immune system does not clear the infection, then the HPV infection

persists and may lead to cellular changes in the epithelium that are associated with the histologic findings consistent with an active HPV infection (koilocytosis, low-grade SIL and HSIL).

The timeline for these transformative changes in the cervix is best illustrated by the ages associated with peak prevalence: HPV infection is most commonly detected in women at ages 15–25, HSIL in women 25–35, and ICC in women over age 45. Thus, as a conservative estimate, HPV infection typically persists for at least 10–20 years before transformation to cervical cancer occurs.

HPV prevalence in women with normal cytology varies significantly with age and geographic location – in North America HR HPV prevalence in women under age 25 is 20+%, whereas the prevalence in women 35+ is under 10%. There does, however, seem to be significant regional variation – in the Caribbean and Eastern Africa, the regionally adjusted HPV prevalence is 35% (compared with 9% in Western Europe and 5% in North America) (Forman et al. 2012).

HIV-positive women have a far higher prevalence of HPV infections and a greater likelihood of persistent infection and are more likely to be infected with multiple HPV types compared with HIV-negative women (see “► [HIV-Associated Cancers](#)”). Some studies have found that HPV prevalence and persistence is higher in women with lower CD4 counts; however, results have not been consistent. In addition, the likelihood of prevalent HPV infections in HIV-positive women with normal cytology does not vary with age. Studies of HIV-positive women since the widespread availability of HAART do not consistently show lower HPV infection rates compared with studies from the pre-HAART era.

Studies in South Africa and Kenya of HIV-positive women with normal cervical pathology have revealed that HPV is detectable in 44–55% and that HPV 16 is detectable in 3–7% of subjects. Age has not been predictive of HPV infection. The relationship of low CD4 counts to HPV prevalence, incidence, and clearance has been inconsistent across studies. However, independent of CD4 count, HPV was more likely to persist in HIV-infected patients, with a South

African study finding that only 6% of women with HR HPV cleared the infection over the following 18 months (Denny et al. 2008; De Vuyst et al. 2012).

Approximately 70% of ICCs worldwide are associated with HPV 16 and/or 18. Although there is sparse data to date, multiple studies in Africa (including Kenya, South Africa, Mozambique, and Zambia) identifying HPV genotypes in ICC in HIV-positive women have found the combined prevalence of HPV 16 and/or 18 to be 54–86% (De Vuyst et al. 2012; Denny et al. 2012; Naucler 2011).

Screening and Early Detection

In countries where cervical cancer screening with Pap testing (cytology) is routinely performed, the incidence and mortality of ICC have decreased to one-third of pre-Pap screening levels. The standard of care based on cervical cytology screening is as follows: an abnormal cervical cytology will trigger a referral for colposcopic evaluation of the cervix with directed biopsy for histology. Colposcopy is evaluation of the cervix after application of acetic acid 5% (and sometimes Lugol's solution, a strong iodine stain) under magnification. Any abnormal areas are biopsied and then clinical follow-up is determined by the histologic findings. Untreated CIN3 has a 30% chance of progressing to invasive malignancy, whereas women who have undergone treatment for CIN3 are very unlikely to subsequently be diagnosed with ICC. Therefore biopsies showing HSIL or CIN2/3 are considered premalignant conditions. Treatment of HSIL involves ablation (e.g., cryotherapy or laser) or excision by cervical conization (loop electrical excisional procedure (LEEP) or "cold knife") of the entire cervical transformation zone. In HIV-negative women, recurrence rate of HSIL following treatment by ablation or excision of the transformation zone is under 10%. In addition, colposcopic directed biopsy or cervical conization may identify early, asymptomatic ICCs which can be treated by conization or simple hysterectomy. It is the treatment of premalignant lesions and diagnosis of earlier stage cervical

cancers that has contributed to the decrease in incidence and mortality of ICC.

Despite the apparent success of the Pap test, cases of cervical cancers still occur in countries where routine cervical screening is performed. This is likely due to missed Pap tests, inadequate follow-up, or inaccurate cytology results. While cervical cytology has been very effective in eradicating squamous cervical cancer, it has had minimal effect on lowering rates of glandular cervical cancers (which comprise 10–25% of cervical ICCs).

HIV-positive women are more likely to have abnormal cervical cytology, with prevalent rates of 25% and cumulative risk of abnormal cytology of 77% over 10 years (Massad 2008). Under current guidelines, a woman with an abnormal cytology would be referred for colposcopic evaluation of the cervix with directed biopsy. Similar to HIV-negative women, an HSIL cervical biopsy is an indication for treatment. However, the recurrence rate of HSIL in the HIV-positive patient is over 50% (Reimers et al. 2010) which is significantly higher than in HIV-negative women. Thus, in HIV-positive women, follow-up after treatment is critical, and the goal of treatment is cancer prevention, not HPV eradication.

As an abnormal cervical cytology may indicate abnormalities elsewhere in the lower genital tract, it is important to closely evaluate the vagina, vulvar, and peri-anus at the time of colposcopy, especially if the source of abnormal cells is not found in the cervix. In addition, CIN, especially in HIV-positive women, is strongly associated with intraepithelial lesions of the vagina, vulvar, peri-anus, and anus.

Commercial testing for HR HPV DNA (first commercially available in the 1990s) has been shown consistently to be superior to cytology in terms of its sensitivity and negative predictive value and is becoming a major tool in cervical cancer screening of women age 30 and older, at least in some developed countries. A single negative cervical HPV test with a normal cervical cytology in such women is associated with an exceedingly low rate of cervical cancer in the next 5 years, allowing screening intervals to be considerably lengthened.

Current recommendations for screening of HIV-infected women include more frequent surveillance and careful follow-up; this likely accounts for the minimal increase in cervical cancer incidence despite the higher burden of premalignant disease. The role of HPV testing for cervical cancer screening in HIV-positive women remains unclear as prevalence rates for oncogenic HPV are much higher in HIV-infected women 30 and over compared to the general population.

HPV testing has not been adopted as a standard cervical cancer screening strategy in HIV-positive women age 30 and older. However, there may be a role for this strategy in (at least) some populations of HIV-positive women. In a cohort of 420 HIV-infected and 279 HIV-uninfected women from the Women's Interagency HIV Study (WIHS) enrolled in 2001–2002, the 5-year risks of CIN2+ in women with baseline normal cervical Pap test and negative HR HPV testing were comparable to the risks in HIV-negative patients at 0.3–0.4%. Notably, within this WIHS cohort HPV prevalence was low; only 12% of the HIV-positive and 9% of the HPV-negative women had HR HPV detected at baseline with normal cervical cytology. For these women, the 5-year risks of CIN2+ were 5% regardless of HIV status. The risks of CIN2+ for HIV-infected women did not differ by baseline CD4 counts. No women in this cohort were diagnosed with cervical cancer at up to 9 years of follow-up (Keller et al. 2012). Thus, utilizing HPV testing as a component of cervical cancer screening strategies may be cost-effective, at least in populations of HIV-positive women with a low prevalence of HR HPV.

Cervical Cancer Screening in Developing Countries

Cervical cancer screening programs are being slowly introduced to sub-Saharan Africa where screening rates range from 2 to 20% in urban areas and 0.4–14% in rural areas (Brower 2011). Cytology specimens have to be transported to a central laboratory, processed, and read, and then the results need to be communicated to the patient;

if abnormal, follow-up evaluations then need to be conducted. Due to difficulty in accessing patients, especially in rural locations; inadequate transportation systems in which specimens may be lost, broken, or significantly delayed; central laboratories with slow processing; and poor communication systems, cervical cancer screening with cytology has proved especially difficult to successfully implement in low-resource settings.

Therefore, in low-resource settings, the emphasis has been on developing inexpensive techniques that can be implemented in a single visit by trained nurse providers (“see and treat”). Visual inspection with acetic acid (VIA) or visual inspection with Lugol's iodine (VILI) followed by treatment of abnormal findings with cryotherapy has been the most widely promoted, although with variable success.

A study of over 1,000 HIV-positive women from India with no prior history of cervical cancer screening compared VIA, VILI, HPV testing (HC2), and cytology to assess the test characteristics of the different screening options in a resource-limited setting. All women underwent colposcopy with directed biopsy regardless of findings from screening; 55 (5%) of the women were diagnosed with CIN2/3 and five with ICC. HPV testing detected all cases of CIN3+ and had 95% sensitivity for the detection of CIN2+ (95%); however, as 26% of the women tested positive for HPV, the specificity was only 77%. The likelihood of a positive test with VIA, VILI, and cytology (ASCUS+) was 15%, 15%, and 8%, respectively, whereas the sensitivity for CIN2+ was 84%, 89%, and 63%, respectively. Thus, in this setting, cytology was outperformed by all other modalities, and it was suggested that if HPV testing became affordable, then HPV testing followed by VIA would be the most effective means of screening for CIN2+ of the cervix (Joshi et al. 2013).

Prevention with Vaccination

Prophylactic HPV L1 virus-like particle vaccines are highly efficacious in preventing CIN2/3 lesions caused by HPV 16/18 for women without

HPV 16 and/or 18 at the time of vaccination. Data from clinical trials of these vaccines, together with post-vaccine surveillance, indicate that they have a good safety profile. As the current HPV vaccines provide protection against only two of the 15 oncogenic HPVs, they will not eliminate cervical cancer or other HPV-associated anogenital diseases but could reduce the incidence of cervical cancer by up to 65–70% if effectively delivered to adolescent females prior to the onset of sexual activity (Stanley 2012). Newer vaccines with activity against a wider range of HPV types are now under development.

Trials of HPV vaccination in HIV-positive men have shown that there is appropriate immunogenicity in this patient population. However, the efficacy of vaccination in HIV-positive women and men has not yet been demonstrated and is being evaluated in ongoing studies. HPV vaccination is recommended in HIV-positive women (and men) through age 26 and has an excellent safety profile.

Prophylactic HPV vaccination has not been shown to be effective in preventing persistent HPV infection and associated neoplasia in women with evidence of prior HPV infection of the genotypes within the vaccine.

As the prevalence of antibodies to HPV type 16 and/or 18 is very high (40%) in HIV-positive women from South Africa, Botswana, and Brazil, it is very important that vaccination occur prior to the onset of sexual activity to have maximal benefit (Firnhaber et al. 2011).

Invasive Cervical Cancer

In the general population, the majority (69%) of cervical cancers are squamous carcinomas (associated with HPV 16 (59%) and HPV 18 (13%)) and 25% are adenocarcinoma (HPV 16 (36%); HPV 18 (37%)). Data is limited on the histologic distribution of cervical cancers in HIV-positive populations. A retrospective analysis in South Africa found that squamous carcinomas represented more than 90% of invasive cancers detected among both HIV-positive and HIV-negative women (Simonds et al. 2012).

Early cervical cancer (stage IA–IB2) is typically asymptomatic and has an excellent prognosis. Cervical cancer can spread by direct extension (to the uterine corpus, vagina, parametria, bladder, or rectum) or by lymphatic or hematogenous dissemination. Advanced cervical cancer (stage 2+) may present with vaginal bleeding, pelvic or lower back pain, hematuria, or other bladder or bowel complaints.

The diagnosis of ICC is made by histologic examination of tissue. Early stage cervical cancers may be detected during the workup for abnormal results of cervical cancer screening. Abnormal cervical cytology or positive HPV testing is followed by colposcopic evaluation with directed biopsy. If a cervical cancer precursor (HSIL) is found on biopsy, removal of the transformation zone by conization may allow for the detection of an occult cancer. Women with clinical evidence of invasive cancer such as a gross lesion require a pelvic exam and biopsy for histologic verification.

A retrospective study from South Africa found that HIV-positive women were diagnosed with ICC at a younger age and more advanced stage than HIV-negative women. However, treatment outcomes were comparable when controlling for the stage and completion of radiation therapy (Simonds).

Recommended treatment options are based on cancer stage regardless of HIV status. Selected women with microscopic disease (stage IA1) may be treated with cone biopsy or simple hysterectomy (removal of the cervix and uterus). Women with a nonbulky (≤ 4 cm) tumor confined to the cervix, uterus, and upper third of the vagina (stage IA2, IB1, IIA1) without lymph node metastases are candidates for radical hysterectomy (removal of the uterus, cervix, and surrounding tissue including significant dissection around the ureters). However, chemoradiation is also a viable option for stage IB and IIA disease.

The standard of care for the treatment of locally advanced cervical cancer (LACC) is concurrent chemoradiotherapy using cisplatin as the chemotherapeutic radiosensitizing agent. The use of this regimen as standard of care is based on multiple randomized, controlled trials (RCTs) that showed improved efficacy when cisplatin was added to

radiation therapy, even in high-risk patients who had histologic evidence of metastatic disease in pelvic and/or para-aortic lymph nodes. There is little data available on the efficacy of standard chemoradiotherapy for the treatment of LACC in the HIV-positive woman.

Conclusion

Although cervical cancer treatments have been improving in recent years, there is no question the largest opportunity to decrease the burden of HIV-associated cervical cancer is in prevention. Prophylactic vaccination against HPV will likely have substantial clinical benefits in HIV-infected women, especially if the vaccine is administered prior to sexual debut. However, the clinical trials of vaccination in HIV are ongoing, the efficacy and duration of immunity are unknown, and likely only cancers attributed to HPV types 16 and 18 will be prevented with the current licensed vaccines.

Algorithms for screening in HIV-uninfected women might not apply to HIV, as the course of the disease from persistence to cancer appears to be different in the setting of HIV, particularly in those with poor immune status. Also, treatments for cervical precancerous lesions in HIV should focus on cancer control rather than cure, as clearance of HPV in the setting of HIV is unlikely.

Because most cervical cancer trials are conducted in developed countries and have typically excluded the enrollment of HIV-positive women, there is minimal data particular to cervical cancer treatment in the setting of HIV. The burden of disease of HIV-associated cervical cancer in developing and middle-income countries is large but only estimated at present. There is a pressing need to directly address potential drug–drug interactions of ART on standard and targeted cervical cancer treatments through pharmacokinetic trials, as well as finding ways to minimize dose delays and modifications due to marrow toxicities of both chemotherapy and pelvic radiation in the setting of HIV. Lastly, as locally advanced cervical cancer is more common among HIV-positive women, better-tolerated and

improved treatments need to be evaluated (Einstein and Phaëton 2010).

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Chagas Disease and HIV

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Definition

Coinfection *T. cruzi*/HIV occurs mainly in patients who acquired Chagas disease in endemic areas through vector transmission. The expansion of the HIV epidemic to periurban and rural areas and the urbanization of Chagas disease to non-endemic areas have impact on the prevalence of this coinfection. Reactivation of Chagas disease is considered an AIDS-defining condition in Brazil since 2004, and it is associated with severe

meningoencephalitis or myocarditis, increase of maternofetal transmission of Chagas disease, and a high mortality. Reactivation is immunodeficiency dependent and was observed in 10–20% of *T. cruzi*/HIV-coinfected patients.

Introduction

Chagas disease (American trypanosomiasis) was described by Carlos Justiniano Chagas in 1908, who characterized its etiological agent and the biological vector, host, animal reservoirs, and human disease. It is endemic in Latin América, from the South of the USA to the South of Argentina and affects approximately eight million infected people and causes 12,500 deaths per year (Moncayo and Silveira 2009). In endemic countries, the migration of the infected population from the rural areas to the urban centers associated with the control of the vector, and blood transfusion and derivatives transmissions led to the urbanization of Chagas disease in nonendemic regions (Schmuñis and Yadon 2010).

Chagas disease is now an emerging disease associated with congenital, blood, and organ transplant transmissions also in nonendemic countries (CDC 2006; Garcia et al. 2012; Salvador et al. 2015), in which approximately 1–26% of immigrants are infected, depending on the country and/or the immigrants' nationality. In the USA, approximately 300,000 individuals are estimated to be infected with *T. cruzi* (Schmuñis and Yadon 2010).

More recently, initiatives in nonendemic countries have been implemented to control the transmission of this disease by blood transfusion and transplants of organs from infected donors from endemic regions.

In the context of great morbidity of vector-transmitted Chagas disease due to the prevalence of millions of chronic cases in Brazil and Latin America, mainly in urban centers, the coexistence of *T. cruzi* infection in 1.3–2.6% of HIV-infected individuals (Dolcini et al. 2008; Almeida et al. 2010) allows the emergence of reactivation of Chagas disease in this population as well as in immunosuppressed patients under organ transplantation or treatment of autoimmune diseases.

In fact, many physicians are not aware of the occurrence of reactivation of chronic Chagas disease in patients under immunosuppression or with HIV infection neither about the occurrence of congenital infection.

Chagas Disease

Trypanosoma cruzi (*T. cruzi*), the etiologic agent of Chagas disease, is a protozoan characterized by flagellate forms in the peripheral blood and biological fluids and nonflagellate (amastigote) forms in the tissues of infected animals and humans. The biological vectors are domestic and sylvatic insects of the subfamily Triatominae (Hemiptera, Family Reduviidae); the most frequent vectors are *Triatoma infestans*, *Triatoma brasiliensis*, *Panstrongilus megistus*, *Rhodnius prolixus*, and *Rhodnius pallescens*. Triatominae are found from the United States (USA) and Mexico, the north to Argentina and Chile in the south, and a wide range of mammals and nonmammals (marsupials, Chiroptera, Rodenta, Edentata, native primates) can serve as reservoir hosts. Transmission occurs at the bite sites of blood-sucking triatomine bugs by the inoculation of the flagellate metacyclic forms of the feces through the bite wound and mucous membranes/conjunctiva. Endemic to Latin America, Chagas disease has a great impact on morbidity and mortality affecting vulnerable individuals. The main control strategy in endemic areas is controlling vector activity. In Brazil and Southern Cone, Andean, Central American, and Amazon countries, coordinated multicenter programs have led to a 70% reduction in the number of new infections in South America due to the interruption of vectorial and blood transfusion transmissions (Who, how, what and where? 2010; Dias et al. 2002). In this context, more recently outbreaks of Chagas disease transmitted by oral route emerged in Brazil, Colombia, Venezuela, and Ecuador, mainly in Amazon region.

Clinical Aspects

Clinical Phases of Chagas Disease

Two phases were described in human disease: acute and chronic (Umezawa et al. 2001).

In acute Chagas disease transmitted by vector, only one of 30 infected individual presents with apparent disease. The signs and symptoms started usually 1–3 weeks after the parasite inoculation and include: (a) a febrile syndrome (38–39 °C, myalgia, headache); (b) phagocytic mononuclear involvement represented by lymph node enlargement, splenomegalia, and hepatomegalia; (c) legs edema; and (d) unilateral swelling of the eyelids (Romaña's sign -Umezawa et al. 2001). Severe myocarditis and/or meningoencephalitis were reported in 5–10% of cases, mainly in newborns, breastfeeding infants, and older individuals with intense inflammatory reaction adjacent to ruptured nests of parasites and damaged muscle cells; pseudocysts are frequently seen. Parasitemia is high in the first 4 weeks, when trypomastigote were detected by direct microscopy of peripheral blood. Serological testing may reveal IgM antibodies to *T. cruzi*.

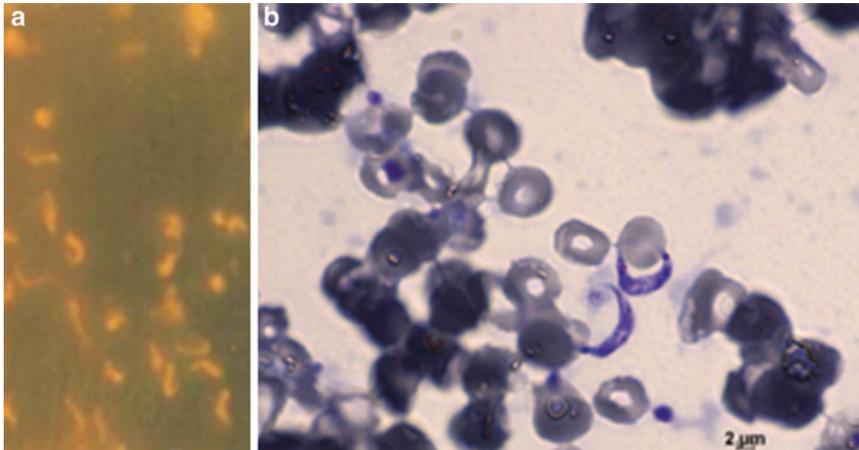
The chronic phase begins about 2–3 months after the initial infection and the parasitemia is low and intermittent.

In the chronic phase, the disease is classified in the following forms: (a) indeterminate form (without digestive or cardiac involvement) in 60–70% of the patients that lasts for the individual's entire life in the majority of the patients; however, 2–5% of the patients in this form change to cardiac or digestive forms each year; (b) cardiac form, observed in adults in 20–30% of the cases, presenting with disturbances of cardiac rhythm and conduction system, cardiac failure, and thromboembolism; and (c) digestive form, manifested by megaesophagus and/or megacolon or mixed form (cardiac plus digestive) in 5–15% of the cases.

Laboratory Diagnosis

Reactivation of Chagas disease is confirmed by the following criteria (gold standard):

1. Parasitological (the most reliable): the presence of trypomastigotes by direct microscopy upon fresh analysis of the peripheral blood (or other biological secretions, CSF) or smear stained with Giemsa or hematoxylin-eosin (Fig. 1a). Concentration methods such as



Chagas Disease and HIV, Fig. 1 *Trypanosoma cruzi* on direct microscopy of blood of a patient with Chagas disease reactivation: (a) stained smear from peripheral blood; (b) parasites seen on quantitative buffy coat (QBC),

preparation from Laboratory of Clinical Investigation on Parasitology LIM 46, Hospital das Clínicas da Faculdade de Medicina, University of São Paulo, Brazil

microhematocrit, Strout methods, buffy coat, and QBC (quantitative buffy coat + by orange acridine) – Fig. 1b – are much more sensitive than fresh preparation or stained smear of peripheral blood. For biological fluids, the precipitate of centrifuged material is examined. This method is much more sensitive in the site of infection, for example, CSF in meningoencephalitis, where motile trypomastigotes were observed and sometimes could be positive in the blood, particularly if patients present with fever or systemic signs.

2. Histopathological method – presence of amastigotes within acute inflammatory infiltrate. When peripheral blood did not reveal the parasite, biopsies can be performed in the site of the lesion.

When treatment had started and gold standard methods cannot not be applied, quantitative PCR might be useful for diagnosis of reactivation if the number of parasite DNA copies is very high, above the levels observed in coinfecting patients with high parasitemia (Freitas et al. 2011). However, the role of quantitative PCR as “unique tool” in the diagnosis of reactivation by a single sample is a matter of discussion since: (a) the reactivation occurs in the tissues and sometimes no changes are observed in the peripheral blood; (b) for mild

increase of parasitemia there is an overlap between higher parasitemia in *T. cruzi*/HIV coinfection without reactivation (Freitas et al. 2011) and the cutoff between coinfection and reactivation depends on the molecular type of parasite; (c) eventhough some less sensitive primers had been reported as indicative of reactivation. As PCR is positive in 45–95% of patients with chronic Chagas disease (Portela-Lindoso and Shikanai-Yasuda 2003), prospective monitoring with quantitative PCR is recommended to monitor reactivation by detection of increasing levels of parasitemia.

Coinfection *T. cruzi*/HIV

Chronic Chagas disease is confirmed by:

(1) Highly sensitive anti-trypomastigotes IgG (ELISA), which are the gold standard for diagnosis (>95% positivity) but may cross-react with *Leishmania*. (2) Immunoblotting (or other high-performance serological tests), such as radioimmunoprecipitation or recombinant assays using trypomastigote antigens) may be used as a confirmatory method (Umezawa et al. 1996). In immunosuppressed patients at least two different serological tests are recommended to confirm the diagnosis of Chagas disease.

In chronic phase, parasites can be found in the blood only by indirect parasitological enrichment

methods (in vitro xenodiagnosis and blood culture), presenting lower positivity (30–50%) than polymerase chain reaction (PCR, 45–95% positivity) (Portela-Lindoso and Shikanai-Yasuda 2003).

Coinfection *T. cruzi*/HIV

In addition to oral, congenital, and organ transplant transmission, *T. cruzi*/HIV coinfection has been found in urban centers of endemic and non-endemic regions, and HIV infection has spread to regions in which Chagas disease is endemic. As a consequence, the occurrence of Chagas disease globalization and the expansion of HIV epidemic might impact the prevalence of *T. cruzi*/HIV coinfection.

Considering the prevalence of two to three million people infected with *T. cruzi* and the estimated number of HIV-infected patients as well as the frequency of *T. cruzi* infection of 1.3% in HIV-infected patients (Almeida et al. 2010), a total of more than 16,000 *T. cruzi*/HIV-infected patients are under risk of Chagas disease reactivation in Brazil and more than 21,000 in Latin America on the basis of number of HIV/AIDS patients (Almeida et al. 2011). Further reports in Argentina (Dolcini et al. 2008) registered 2.6% of Chagas disease in HIV-infected patients and 8.2% in drug users infected with HIV. This frequency was 1.9% in Spain (Garcia et al. 2012), possibly increasing previous estimates for Latin America and emphasizing the importance of this coinfection in nonendemic regions.

In a systematic review only 291 cases of coinfection HIV/*T. cruzi* were reported up to March 2010 (Almeida et al. 2011), 76.2% from Brazil and 19.2% from Argentina, the remaining from Chile, USA, and Spain. Chronic form of Chagas disease is predominant (97.9%) in patients with coinfection, mainly in indeterminate form (50.8%) and cardiac form (37.3%). Digestive (5.1%) and cardiac + digestive forms were less frequent (6.8%). Since March 2010, more 15 cases of coinfection from Spain (Salvador et al. 2015) were described.

To provide health care for patients with this coinfection at basic and complex levels in non-endemic countries, a National Network for Attention and Studies on *T. cruzi*/HIV coinfection was created in Brazil in 2006, and later included centers in Spain and Argentina as an International Network, open for all countries (Ramos et al. 2010).

The main objectives of this network are: (1) to contribute for structuring a comprehensive care network for people living with Chagas disease under immunosuppression as well as to provide a continuing education network for health care professionals involved in management of these patients; (2) to contribute for the development of actions based on scientific evidence in accordance with ethical standards, including clinical, epidemiological, immunological, and laboratorial research; (3) to contribute toward identifying priorities for research in Brazil on this subject.

Reactivation of Chagas Disease

The occurrence of meningoencephalitis and myocarditis in patients under immunosuppression induced by oncohematological neoplasias have been reported since the late 1960s in Brazil and other endemic countries (França et al. 1969), usually without symptoms. Reactivation of trypanosomiasis induced by immunosuppressive therapy for graft rejection control in solid organ transplant patients and for treatment of autoimmune diseases was further reported. In experimental studies, reactivation of chronic trypanosomiasis was associated with increased parasitemia and mortality and severe myocarditis, confirming the role of *T. cruzi* as opportunistic parasite under immunosuppression (Brenner and Chiari 1971).

The first AIDS patient with reactivation of Chagas disease was described in 1990 (Del Castillo et al. 1990). In addition, in Spina França et al. (1988) had reported both anti-*T. cruzi* antibodies and parasites in the CSF of a patient with HIV and Chagas disease reactivation. The first case of reactivation of Chagas disease associated with AIDS was observed in the USA and described in a conference before 1990 but only published in 1992 by Gluckstein et al.

Most of the cases were further described in Brazil and Argentina (Ferreira et al. 1997; Sartori et al. 2002, 2007; Cordova et al. 2008), and three centers in Brazil and one in Argentina described about half of the reported cases. High morbidity and mortality and poor prognosis for severe cases even with early treatment were described as well as its association with decreased levels of CD4⁺T cells, commonly less than 200 cells/mm³ (Ferreira et al. 1997; Sartori et al. 2002, 2007; Cordova et al. 2008).

The reactivation of Chagas disease as an AIDS-defining condition was recognized in Brazil in 2004 and in 2005 by the Pan American Health Organization and World Health Organization (Ramos et al. 2010) since (a) HIV infection changes the natural history of Chagas disease and HIV infection, in terms of severe morbidity (meningoencephalitis and myocarditis) and high mortality of up to 100% in severe cases and depending on early antiparasitic treatment; (b) presents different response to the treatment with recurrence; (c) is associated with low levels of CD4⁺T cells: <200/mm³ in the majority of the cases (Rocha et al. 2006; Almeida et al. 2011); and increased rate of maternofetal transmission of Chagas disease (more than 15×).

From 291 cases of coinfection HIV/*T. cruzi* described up to March 2010, 41.2% represented reactivations of Chagas disease, 74.2% as meningoencephalitis, 16.7% as myocarditis, 3.3% in the duodenum, 0.8% in the pericardium, peritoneum, stomach, uterus cervix, and eye muscle (Almeida et al. 2011). In prospective studies, oligosymptomatic patients presenting as febrile syndromes or even without symptoms (mother of a newborn with Chagas disease) were found with positive microscopy on peripheral blood (Sartori et al. 2007).

Since March 2010, only five cases of meningoencephalitis in adults and three acute congenital Chagas disease were reported in Venezuela, Argentina, Chile, USA, and Brazil, possibly expressing the influence of highly active antiretroviral therapy (HAART) and the emphasis in special aspects of evolution of patients or maternofetal transmission.

Rate of Reactivation

The rate of reactivation is not well known and depends on many factors, such as deficiency of cellular immunity and lack of adherence to HAART. According to description in single centers, it could be as high as 15–20% but larger prospective multicenter studies are needed to establish this frequency in patients with follow-up longer than 6 months, where the estimation is lower (approximately 10%).

The proportion of immunosuppressed patients that will present reactivation of Chagas disease is not well known, and a prospective study employing xenodiagnosis indicates that 50% of those with higher parasitemia will reactivate in the next 5 years (Sartori et al. 2002). Prospective studies monitoring parasitemia and innate and acquired immune response in patients under immunosuppression could contribute to answer this question.

An imbalance of TH2 response in coinfection among patients with Chagas disease and HIV infection was shown by an increase of the IL4/IFN γ ratio in comparison to those without HIV infection. The association of Chagas disease reactivation in HIV-infected patients with <200 CD4⁺T cells/mm³ in 80% of the cases express the inability of macrophages to control parasite multiplication and to kill the parasites. In effect, experiments with SCID mice infected with *T. cruzi* showed decrease of IFN γ and increase of parasites in the myocardium, associated with lower tissue inflammation but early mortality (Silva et al. 1993). Additionally, in placental histocultures HIV decreases the production of inflammatory cytokines.

Bidirectional influences were observed in HIV/*T. cruzi* interaction: increased levels of parasite in the peripheral blood and tissues in human and mice are a cofactor for TH2 response and are associated with increased HIV replication in human and restricted systems such as placenta and human macrophage cultures.

An increased rate of reactivation and maternofetal transmission of Chagas disease were observed in HIV-infected patients in comparison with non-HIV-infected patients. Changes in the morbidity, mortality, and response to the

therapy were observed in comparison to acute disease without HIV infection. Changes in the natural history of both HIV and the parasitic infection are attributed to the reactivation since it causes death in severe cases. The natural history of Chagas disease may not change in coinfecting patients without increased parasitemia but the factors responsible for this are not well understood.

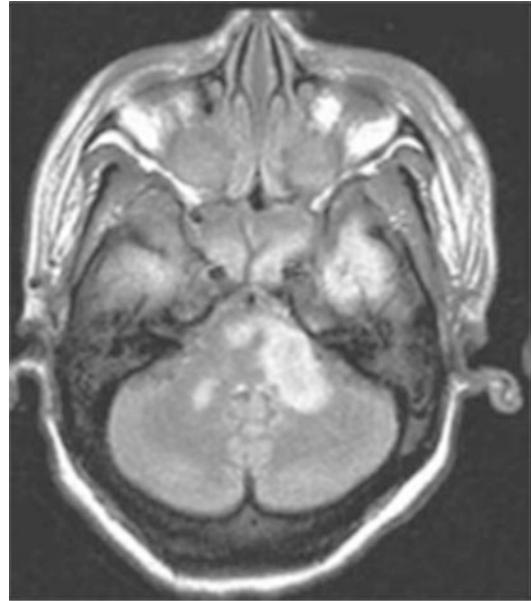
Clinical Syndromes

Meningoencephalitis

Central nervous system involvement is expressed by febrile syndrome associated with intracranial hypertension (headache, vomiting) and signs of cerebral involvement (seizures and focal signs). In the CSF, mild or moderate pleocytosis of more than 10 cells/mm³, mainly mononuclear cells, and mild or moderate increase of protein levels are usually observed. The gold standard for diagnosis is the presence of trypomastigotes on direct microscopic examination of the centrifuged CSF sediment (Sartori et al. 2007). This is found in about 74% of the tests performed. Less frequently this form was detected in the peripheral blood. From 44 patients with histopathological analyses, amastigote forms were identified in inflammatory lesions in the central nervous system (70.4%), myocardium (13.6%), duodenum (9%), skin (6.8%), and 2.3% in stomach and 2.3% in the uterus. In the brain, inflammatory infiltrates, hemorrhage, necrosis, and parasites were largely seen in the macrophages and glial cells.

The level of CD4⁺T cells was usually <200 cells/mm³, but some cases of reactivation could present with >350 cells/mm³. The average CD4 count in cases with reactivation was 98 cells/mm³ (range 1 cell/mm³ to 551 cells/mm³) versus an average of 562 cells/mm³ (range 44 cells/mm³ to 1,949 cells/mm³) in cases without reactivation.

Computed tomography or magnetic resonance imaging show in more than 90% of the cases hypodense lesions, predominantly in the subcortical region, with or without ring enhancement. But lesions can also be found in the thalamus, the cerebellum, or basal nuclei (Fig. 2) The lesions are single or multiple lesions simulating brain tumors



Chagas Disease and HIV, Fig. 2 CNS Magnetic Resonance Imaging of a patient with Meningoencephalitis (Courtesy of Division of Infectious Diseases, Hospital das Clínicas da Faculdade de Medicina, University of São Paulo, Brazil)

with mass effects due to hemorrhagic foci, preferentially involving the white matter.

Myocarditis

Heart involvement is described in about 20% of patients with reactivation. But this rate might be underestimated since it could be silent and not suspected in cases of meningoencephalitis. Additionally, it might be similar to the evolution of chronic cardiopathy. Based on necropsy series or in well-documented cases, the rate of simultaneous involvement of heart and CNS is supposed to be about 40–50%. Clinical signs are represented by arrhythmia (ventricular extrasystoles, supraventricular arrhythmia, atrial flutter, and fibrillation) and congestive heart failure (generalized edema, hepatomegaly, dyspnea, and tachycardia), and echocardiogram can show pericarditis or increased cardiac volume. Trypomastigotes can be detected in the peripheral blood and/or in the pericardial fluid. Amastigote nests can be identified in histopathological exams and/or immunohistochemical exams or electron

microscopy can confirm this diagnosis. Biopsy and/or autopsy studies not only confirm the diagnosis of Chagas disease reactivation but also exclude the presence of other protozoa such as *T. gondii* (Ferreira et al. 1997; Sartori et al. 2007).

Other Syndromes

Involvement of digestive tract, uterus cervix, peritoneum, and eye muscle were rarely described. In a prospective study (Sartori et al. 2007), mild oligosymptomatic cases with febrile disease (similar to infectious mononucleosis) and/or asymptomatic reactivation during pregnancy associated with maternofetal transmission were reported.

Maternofetal Transmission

A higher rate of transmission of the disease to newborn was described in pregnant women with coinfection HIV/*T. cruzi* (Sartori et al. 2007), in comparison with patients without HIV infection (1–13.8%). Although only one prospective study was published showing that maternofetal transmission occurred in three of four pregnancies (two with reactivation in the mother), other case reports of pregnant women with reactivation or no reactivation but high parasitemia detected by parasitological or molecular methods show more than 50% of transmission to the fetus. Transmission was associated with abortions, low weight for the gestational age, and even preterm newborn with meningoencephalitis or sepsis. Mortality is over 70%. There is not enough information about follow-up of newborns of mothers with this coinfection to better know the spectrum of signs and symptoms of congenital infection. In fact, higher parasitemia described in coinfection than in Chagas disease without HIV infection might be one of the explanations for the high rate of transmission of Chagas disease to the newborn.

Regarding the benefit of HAART in the pregnant coinfecting women, there are few reports of both noninfected or infected preterm baby in the follow-up.

Associated and Predictive Factors

Two variables were associated with Chagas disease reactivation in HIV-infected patients:

1. CD4⁺T cells <200/mm³ in the peripheral blood in more than 80% of the cases. However, this level is not predictive “per se” of this reactivation since some patients with this low level did not reactivate and additionally, a few cases of reactivation occurred in patients with >350 cells/mm³.
2. A high parasitemia in HIV-infected patients, expressed by >20% of nymphs positive in xenodiagnosis was shown to predict the reactivation in the next 5 years (Sartori et al. 2007) in 50% of these patients (Sartori et al. 2007). Quantitative PCR (Freitas et al. 2011) further confirmed the presence of two different groups in coinfecting patients: one with high levels of parasitemia and other with low levels, similar to those described in non-HIV-infected patients.

Some authors observed simultaneously high viral load in patients with Chagas disease reactivation, possibly associated with non-adherence to HAART and low levels of CD4⁺T cells, allowing the evasion of the parasite from the immune response. However, its importance as cofactor or reactivation is a matter of discussion.

Differential Diagnosis

Toxoplasmosis and tumoral lesions are important differential diagnosis since similar image findings are observed in these circumstances. The epidemiological data and the diagnosis of chronic Chagas disease by serology reinforce the clinical suspicion. Indirect parasitological methods such as blood culture or xenodiagnosis or PCR are not useful for diagnosis of reactivation, as they are also positive in chronic Chagas disease (Freitas et al. 2011). Therefore, the diagnosis of reactivation of Chagas diseases is only made by the finding of trypomastigotes in the CSF, peripheral blood or biological fluids, or amastigotes in histopathological lesion. These are very useful to confirm the diagnosis, since Chagas disease and toxoplasmosis may coexist.

Treatment

Benznidazole is the drug of choice and was used in 87% of treated patients. Antiparasitic treatment

should be introduced as soon as possible since the prognosis is poor in severe cases of meningoencephalitis and/or myocarditis especially if treatment is delayed. When patients received treatment for ≥ 30 days, the survival rate was approximately 80% versus 20% when they received the treatment for ≤ 20 days.

Nifurtimox was used for treatment for 14% of the patients in the literature and is recommended when benznidazole cannot be prescribed. Dosis for benznidazole was 5 mg/kg/d and for nifurtimox 7 mg/kg, 2–3 x/day for at least 60 days.

Although benznidazole is teratogenic, pregnant women should receive the treatment since reactivation of Chagas disease is a life-threatening disease for her and her baby.

Prescription of fluconazole was reported in 3% of the patients and itraconazole or ketoconazole in 2% of patients. These are not first-line therapies for Chagas disease reactivation but may be used as adjunctive therapy if drugs of choice cannot be prescribed because of uncontrolled adverse effects.

If therapy is successful, parasite will not be detected 2 weeks posttherapy and signs and symptoms decrease in the same period.

Adverse effects within the first weeks of treatment include exanthema, pancytopenia particularly granulocytopenia. Later on, peripheral neuropathy may occur. There is no consensus about the indication and duration of maintenance therapy, but benznidazole (5 mg/kg/d, three times a week) can be prescribed as long as $CD4^+$ T cells are below 350 cells/mm³ in patients on HAART.

Preemptive Therapy

There is data of preemptive benznidazole treatment of patients without reactivation but presenting high parasitemia (defined by persistent positive blood culture and/or xenodiagnosis or uncontrolled arrhythmia). These treatments resulted in negativation of PCR and or indirect parasitological enrichment methods in a percentage higher than observed in chronic disease. Severe adverse events were not registered but careful monitoring of adverse effects is highly recommended during the period of treatment (Sartori et al. 2007). There are no data on

treatment of patients with coinfection with very low/negative parasitemia.

Prognosis

Overall mortality in prospective studies was 73.3% in cases of reactivation and depended on the severity of involvement: 88.9% in meningoencephalitis and 0% in mild or oligosymptomatic cases reported (Sartori et al. 2007). In another series of *T. cruzi*/HIV coinfecting patients including the patients without Chagas disease reactivation, the overall mortality observed was 29.6% and average of survival time-period was three times longer in patients without reactivation (34 months) than in patients with reactivation (11 months) (Almeida et al. 2010).

The influence of HAART on the evolution of coinfection is not well. But HAART seems to have decreased the occurrence of reactivation in the last decade and maternofetal transmission was rarely described in women under HAART (Sartori et al. 2007). The control of viral replication and the increase in $CD4^+$ T cells possibly allow the recovery of cellular immunity and macrophage activity and the control of parasite multiplication and dissemination.

Regarding immune reconstitution syndrome (IRIS), there is concern but very limited data that introduction of HAART early during the course of antiparasitic therapy could induce a paradoxical inflammatory response (paradoxical or unmasking IRIS). Therefore, some experts recommend that HAART should be introduced some weeks after the start of antiparasitic therapy. Prospective studies did not find an influence of HAART on *T. cruzi* parasitemia detected by classical parasitological methods (Sartori et al. 2007).

Conclusion

Estimates of prevalence of *T. cruzi*/HIV coinfection in Brazil, Argentina, and Spain suggest that this coinfection is worldwide underestimated. Reactivation of Chagas disease in HIV-infected patients is considered as an AIDS-defining illness and should be diagnosed and treated as early as possible since the mortality of the untreated

reactivation is very high, but the survival increases to 80% if antiparasitic treatment of more than 30 days can be achieved. Monitoring patients with *T. cruzi*/HIV coinfection is useful to detect oligosymptomatic cases or to detect an increase of parasite levels which is an indication of therapy or preemptive therapy (Sartori et al. 2002; Sartori et al. 2007; Freitas et al. 2011).

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Children, Care and Treatment

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Definition

Care and treatment of children affected by HIV refers to the bundle of services aimed at improving the lives of this vulnerable population. Affected children are those known to be infected with HIV or those at high risk of infection due to “exposure” from their HIV-infected mother during pregnancy. These services include prevention strategies, screening and diagnostic testing, provision of antiretroviral (ARV) medications, clinical and laboratory monitoring for treatment response and medication toxicities, management of co-occurring conditions, and an array of counseling services for patients and caregivers.

Overview

Globally, an estimated 3.3 million children (0–15 years of age) are infected with HIV (UNAIDS 2013). Most of these children were infected through vertical transmission – during pregnancy,

the birthing process, or through breast milk. The vast majority of children infected with HIV live in low- and middle-income countries where the HIV burden in adults is also high. More specifically, about 90% of children infected with HIV live in sub-Saharan Africa, while less than 0.2% live in North America and Western Europe combined (UNAIDS 2013). These differences are primarily a reflection of social and economic circumstances that impact access to and development of health services and healthcare infrastructure. Despite these differences, the principles underlying care and treatment of children affected by HIV are universal; however, guidelines on how to provide these services are context specific and vary from country to country. This essay will focus on international care and treatment standards as outlined by the World Health Organization (WHO) while highlighting key differences in the US guidelines proposed by the National Institutes for Health (NIH) and the Department of Health and Human Services (HHS).

Ideally, the continuum of care and treatment of children affected by HIV begins with prevention of mother-to-child transmission (PMTCT) services and continues as children transition through adolescence into adulthood. PMTCT will be discussed in detail in a separate chapter; however, the approach to care and treatment of the HIV-exposed infant and early infant diagnosis (EID) will be discussed herein. Similarly, adolescent care will be discussed elsewhere, but the topic of disclosure of one’s HIV status warrants review in this chapter as well. Other topics relevant to the care and treatment of children affected by HIV will also be reviewed and include HIV testing and counseling, antiretroviral therapy (ART), clinical and laboratory monitoring, and prevention of common coinfections.

HIV Testing and Counseling

HIV testing and counseling can be either patient or provider initiated. Historically HIV testing was primarily provider initiated and offered when patients accessed healthcare services for a variety of reasons. However, this resulted in many new diagnoses being made in persons who were

accessing care relatively late after acquisition of HIV and often with advanced disease. Now, in addition to this strategy, new emphasis is being placed on empowering patients to seek testing both in traditional healthcare settings and in their communities. Community-based testing and counseling with linkage to care and treatment services has been emphasized by the WHO as an important component of public health campaigns aimed at identifying and treating persons infected with HIV. Furthermore, targeting these interventions toward key populations such as adolescents and young adults engaged in high-risk behaviors may have added benefit and maximize resource utilization. In the case of infants and children, who are too young to understand the need for or importance of HIV testing and counseling, provision of these services requires the participation of their caregivers who may be parents, adult siblings, extended family, or orphanage attendants and as such necessitates unique strategies and approaches.

Available tests include antibody tests and nucleic acid tests. Antibody tests detect HIV-specific antibodies produced by the immune system as part of the normal host response to HIV infection. These tests are very sensitive and as such are the preferred tests for screening for HIV. However, antibody tests do not directly detect the HIV virus and thus have limited utility in those less than 18 months of age who have both endogenous and circulating maternal antibodies. Nucleic acid tests utilize polymerase chain reaction (PCR) technology to directly test for HIV infection by amplifying either HIV RNA (i.e., viral load) or HIV DNA (only present after reverse transcription has occurred in an infected cell). These tests are very specific for HIV and traditionally have been used for confirmatory testing and for monitoring response to ART. However, due to limitations of antibody testing in infants, PCR is an important part of the screening algorithm for HIV-exposed infants.

Early Infant Diagnosis

HIV testing and counseling services should be offered to all pregnant women during pregnancy. Identifying pregnant women with HIV and

connecting them with PMTCT services is integrally important to preventing vertical transmission. It also helps to identify HIV-exposed and potentially infected infants, and it can provide an opportunity for offering testing and counseling services to siblings and other family members.

HIV-exposed infants are those born to HIV-infected mothers and who have not yet had definitive HIV testing. Since vertical transmission of HIV can occur in utero, during the birthing process, or postpartum through breast milk, HIV testing should begin soon after delivery and continued until there is no longer exposure to HIV (i.e., cessation of breast feeding). If PCR is available, early infant diagnosis (EID) testing should be obtained soon after birth then repeated at specified intervals. NIH/HHS recommends PCR at 2–3 weeks of life, age 1–2 months, and at age 4–6 months (NIH/HHS 2014c). In the US, this testing is routinely available and results are usually reported within 1 week. However, due to expense and some technical aspects, PCR is not as readily available in resource-limited settings, though this is changing with recent global efforts to scale up HIV care and treatment services in countries with the highest HIV prevalences. WHO recommends that HIV-exposed infants receive PCR testing at 4–6 weeks of life. In many resource-limited countries, repeat PCR is recommended at age 3–4 months. Any positive PCR result should be confirmed with repeat testing. ART should not be delayed while awaiting confirmatory test results (WHO 2013a, 2014b).

HIV antibody testing is nondiagnostic in HIV-exposed infants less than 18 months of age and as such is not the preferred diagnostic test in this age group. It is normal for antibodies of all sorts, including antibodies formed against HIV, to be transferred from mother to fetus through the placenta. This in utero transfer peaks in the third trimester of pregnancy, and maternal antibodies can persist in the infant up to 18 months of age. However, during the first year of life, these circulating maternal antibodies start to wane while the infant begins to increase production of their own antibodies. Thus, if there is no ongoing exposure to HIV, then a negative antibody test can provide evidence that the infant is not infected with HIV,

but this is not definitive until at least 18 months of age. Any positive antibody test prior to 18 months should be followed up with PCR. After 18 months of age, antibody testing is considered definitive.

Antiretroviral Prophylaxis for HIV-Exposed Infants

Testing of the HIV-exposed infant should occur in conjunction with the provision of ARV prophylaxis aimed at preventing infection that may occur during the birthing process or due to ongoing exposure through breast milk. WHO recommendations on which ARV to use and for how long depend on whether there is ongoing exposure through breast milk. If replacement feeding is provided to the infant (i.e., no breast milk), then either once daily nevirapine (NVP) or twice daily zidovudine (AZT) can be administered to the child for the first 4–6 weeks of life. If the child is breastfeeding, then once daily NVP is recommended for the first 6 weeks of life. However, the latter recommendation assumes that the mother who is providing breast milk is also on ART per the most recent PMTCT guidelines. If the mother is not on ART, infant ARV prophylaxis should be continued until the mother is stable on ART or until cessation of breastfeeding (WHO 2013a, 2014b). NIH/HHS recommendations deviate slightly from WHO recommendations insofar as they explicitly discourage HIV-exposed infants from breastfeeding. NIH/HHS recommends twice daily AZT for the first 4–6 weeks of life (NIH/HHS 2014c).

If at any point a positive test result is obtained and a diagnosis of HIV infection is made, or if a presumptive diagnosis of HIV is made based on clinical findings in the absence of testing (i.e., if testing is unavailable), then ARV prophylaxis should be transitioned to ART.

Breastfeeding

The potential benefits of breastfeeding are numerous and beyond the scope of this essay. However, breastfeeding as it pertains to the care and

treatment of children affected by HIV remains a controversial topic. As such, recommendations on breastfeeding remain context specific and vary from country to country. That said, most low-income countries with high HIV prevalence promote breastfeeding with concomitant maternal ART and infant ARV prophylaxis as the preferred strategy to give infants born to mothers known to be infected with HIV the greatest chance of survival. There is evidence from these settings that early cessation of breastfeeding results in excess infant mortality, mostly due to diarrheal illnesses and malnutrition. Furthermore, exclusive breastfeeding for the first 6 months of life is associated with significantly lower risk of HIV transmission as compared to mixed feeding where both breast milk and any other food or liquid is given to the infant. Food insecurity, availability of potable water for mixing formula, and cost of replacement feeding all factor into these findings and ultimately influence context specific recommendations (Black et al. 2008).

Antiretroviral Therapy

Antiretroviral therapy (ART) refers to the use of a combination of ARV drugs to control HIV infection. ART is sometimes termed highly active antiretroviral therapy (HAART). The overall goal of ART is to improve the lives of those infected with HIV. ART accomplishes this goal by suppressing replication of the HIV virus, thereby preserving host immune function, avoiding opportunistic infections, and allowing for a long and productive life. For those who are able to access ART, HIV infection has been transformed from a progressive and eventually fatal disease to a chronic illness. That said, ART is not a cure and patients should be prepared to continue it lifelong.

There are several classes of ARV drugs, each attacking HIV at different points in its replication cycle. Nucleoside reverse transcriptase inhibitors (NRTIs) such as abacavir (ABC), zidovudine (AZT), lamivudine (3TC), and tenofovir (TDF) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine (NVP) and efavirenz (EFV) target the enzymatic conversion

of single-stranded HIV viral RNA into double-stranded DNA (i.e., reverse transcription). Protease inhibitors (PIs) such as lopinavir (“boosted” with ritonavir, LPV/r) block the enzyme that cleaves HIV viral transcripts into functional proteins. Fusion inhibitors and integrase inhibitors block the HIV virus from docking on host cells and from integrating into the host cell genome, respectively. Three or more ARV drugs from a variety of classes are used in combination with two main objectives: to achieve potent suppression of the HIV virus and to prevent the development of HIV drug resistance mutations that may limit the efficacy of one or more ARV. More recently, ART has also been considered an important form of prevention. HIV-infected persons who have achieved viral suppression on ART have fewer viral particles in body fluids that might transmit the HIV virus (blood, semen, breast milk, etc.), and thus are less likely to infect others who are exposed to these fluids.

Eligibility for ART Initiation

Untreated HIV infection results in high levels of mortality among infants in low-income and high HIV prevalence areas with approximately 50% mortality by 2 years of age (Newell et al. 2004), but by 5 years of age, mortality and disease progression without ART is similar to rates in young adults (Dunn et al. 2008; 3Cs4kids 2008). As such, the WHO recommends that ART should be initiated in all HIV-infected children under the age of 5 years, including those with a presumptive diagnosis of HIV infection. Priority should be given to children less than 2 years of age, those with advanced HIV disease as indicated by having WHO clinical stage 3 or 4 defining illnesses (Table 1) and those with CD4-positive T-cell count (“CD4 count”) less than 750 cells/mm³ or percentage of CD4-positive T-cells relative to total white blood cell counts (CD4%) less than 25%, whichever is lower (WHO 2013a, 2014b).

NIH/HHS recommendations for ART initiation are similar in that they recommend ART initiation for all HIV-infected children less than 12 months, but for children older than 12 months of age, ART eligibility is dependent on meeting clinical and laboratory criteria. Any child older than 12 months

Children, Care and Treatment, Table 1 A selection of WHO stage 3 and 4 defining illnesses for children 0–15 years of age

Stage 3	Stage 4
Unexplained moderate malnutrition	Unexplained severe malnutrition
Unexplained persistent diarrhea	Chronic diarrhea due to cryptosporidiosis or isosporiasis
Unexplained persistent fever	<i>Pneumocystis (jirovecii)</i> pneumonia
Persistent oral candidiasis (after first 6 weeks of life)	Esophageal candidiasis
Lymph node or pulmonary tuberculosis	Extrapulmonary tuberculosis
Severe recurrent bacterial pneumonia	Recurrent severe bacterial infections (not pneumonia)
Unexplained anemia, thrombocytopenia, or neutropenia	HIV-associated malignancy (e.g., Kaposi sarcoma)
Lymphoid interstitial pneumonitis	Central nervous system toxoplasmosis
Chronic lung disease, including bronchiectasis	Extrapulmonary cryptococcosis

of age with an HIV viral load greater than 100,000 copies/mL should be initiated on ART. ART should also be initiated in any child 1–3 years of age with CD4 count less than 1000 cells/mm³ or CD4% less than 25%. For children 3–5 years of age, they recommend ART initiation when CD4 count is less than 750 cells/mm³ or CD4% is less than 25%. Regardless of age, any child with a United States Centers for Disease Control and Prevention (CDC) classification system clinical category B or C condition (similar to WHO clinical stage 3 and 4 defining illnesses) should be initiated on ART (NIH/HHS 2014b).

Both the WHO and NIH/HHS use a CD4 count of 500 cells/mm³ as the cutoff for ART initiation in the general population including children older than 5 years of age, adolescents, and adults. ART should also be initiated in anyone in special populations including those with active tuberculosis (TB), hepatitis B virus infection and chronic liver disease, pregnant and breastfeeding women, and HIV-positive partners in serodiscordant couples, regardless of CD4 count (NIH/HHS 2014b; WHO 2013a; WHO 2014b).

ART Regimens

There are several classes of ARV drugs with multiple drugs in each class. Deciding which combination of ARV drugs to prescribe for a particular patient can be challenging. Considerations include the patient's age and weight, formulation and route of administration (e.g., tablet vs. liquid suspension), medication side effect profiles, prior ARV exposure (in utero as part of PMTCT, as infant ARV prophylaxis, or as part of a previously interrupted ART regimen), and interactions with other medications the patient is taking. When availability of ARV drugs and cost is not a limiting factor, the approach to deciding the most appropriate ART regimen also includes HIV genotyping to determine resistance patterns (NIH/HHS 2014b). That said, ARV availability as reflected by national formularies is much more limited in the low-income countries most affected by HIV.

With these factors in mind, current WHO recommendations for first-line ART in infants and children less than 3 years of age are for two NRTIs and a PI (e.g., ABC+3TC+LPV/r). The NRTI "backbone" should always include 3TC. ABC is often preferred over AZT, since AZT can induce anemia which is already often a problem in this age group and is potentially more problematic in environments where malnutrition and malaria are common (WHO 2013a, 2014b).

For children older than 3 years of age (including adolescents), the WHO recommends a first-line regimen composed of two NRTIs and an NNRTI (e.g., ABC+3TC+EFV). EFV is the preferred NNRTI. Again, the NRTI backbone should always include 3TC. TDF should be avoided in those less than 10 years of age or weighing less than 35 kg as to avoid potential adverse effects on bone development, though, in those older than 10 years of age and more than 35 kg, TDF is preferred as to align with the first-line recommendations for adults (WHO 2013a, b, 2014b).

If a patient develops treatment failure while on first-line ART, they can be switched to second-line ART. Ideally, development of this new regimen would be tailored to the individual and to results of HIV drug resistance testing. Resistance testing should be obtained, while the patient remains under the selective pressure of failing ART regimen as to

avoid the emergence of a subpopulation of nonresistant wild-type virus if ART were suspended. However, resistance testing is not routinely available in low-resource settings, so instead the WHO has proposed empiric second-line regimens, and country-specific guidelines further vary depending on national formularies. That said, the WHO recommends that after failing a NNRTI-based first-line regimen, second line should include a PI. If already on a PI-based first-line regimen, then the PI should be switched to an NNRTI. 3TC should remain a part of all second-line regimens while other NRTIs can be exchanged for one another. Maintaining 3TC is recommended because the most common mutation against 3TC (M184V) results in a virus that is less virulent than wild-type HIV virus (WHO 2013a, 2014b).

Weight should be checked at every clinical encounter and doses of medications adjusted, if necessary. This should be continued until weighing more than 35 kg when adult dosing can be prescribed. Similarly, liquids formulations should be switched to pill form once able. Furthermore, any changes that can be made to minimize the number of pills (pill burden) and the frequency of medication administration should be made. Simplified regimens are easier for children and caregivers and can potentially improve adherence to prescribed therapies.

Monitoring

Clinical and laboratory monitoring should start at the time of diagnosis of HIV infection or from birth in the case of HIV-exposed infants. Initial evaluation should include WHO clinical staging, screening for TB, CD4 count, and viral load testing if available. Additionally, screening for hepatitis B, hepatitis C, and sexually transmitted infections should also be considered.

After diagnosis of HIV but prior to ART initiation, serial evaluations occurring at least every 6–12 months should be performed to determine ART eligibility and should include repeat CD4 count, interval history, and a physical exam. Monitoring should be more frequent if there are active issues or comorbid conditions that require

Children, Care and Treatment, Table 2 Common ARVs and selected associated side effects and toxicities to guide monitoring

Antiretroviral drug	Potential side effects and toxicities
Abacavir (ABC)	HLA-B*5701-associated hypersensitivity reaction
Zidovudine (AZT)	Anemia, neutropenia, myopathy, lipodystrophy
Efavirenz (EFV)	CNS toxicity (confusion, depression, seizures), gynecomastia
Lopinavir/ritonavir (LPV/r)	QT interval prolongation, dyslipidemia, diarrhea
Nevirapine (NVP)	Hepatotoxicity, severe rash (Stevens-Johnson syndrome)
Tenofovir (TDF)	Tubular renal dysfunction, Fanconi syndrome, osteopenia

treatment and that may influence decisions regarding ART initiation.

Monitoring should also be more frequent in the weeks and months just after ART initiation. During this critical time period, it is important to ensure optimal adherence and to assess for potential drug toxicities. Once stable on ART, periodic CD4 count and viral load testing should continue to be obtained every 6–12 months to monitor treatment response, and additional laboratory monitoring should also be considered depending on the ART regimen and the potential short- and long-term effects attributable to its component ARV drugs (Table 2).

ART Response

Prior to the introduction of viral load testing, clinical and immunological criteria were used to define treatment failure. However, viral load testing allows for earlier and more sensitive detection of treatment failure and as such has become the gold standard for monitoring ART response. In high-resource settings, viral load testing is readily available, and NIH/HHS recommendations for monitoring rely on viral load testing as the main indicator of ART treatment success and failure. Treatment success is essentially synonymous with viral suppression which is defined as a viral load below the level of detection of available assays, generally less than 50 copies/mL. Treatment failure is defined as a viral load exceeding 1000

copies/ml on at least two consecutive measurements within a 3-month interval and after a minimum of 6 months of optimal dosing of and adherence to ART (NIH/HHS 2014b).

The WHO also recommends viral load testing as the preferred approach to monitoring ART success and treatment failure, but due to limited availability in resource-limited settings, it is considered complementary to clinical and immunologic measures of ART response. Clinical signs of treatment failure include new or recurrent clinical events suggestive of advanced or severe immunodeficiency (WHO clinical stage 3 or 4 illness) after 6 months of ART. Immunologic criteria for treatment failure in children under 5 years of age is persistent CD4 count less than 200 cells/mm³ or CD4% persistently less than 10%. For children older than 5 years of age, immunologic failure is defined by a CD4 count persistently less than 100 cells/mm³. Persistently declining or lack of improvement in CD4 count or CD4%, while not meeting values outlined in the definition of immunologic failure, might also be suggestive of treatment failure. Furthermore, to meet immunologic criteria for treatment failure, concomitant infection that might cause a drop in CD4 count or CD4% must also be excluded (WHO 2013a, 2014b).

Adherence

It should be noted that nonadherence to ART is the most common cause of treatment failure. Nonadherence is also the main risk factor for development of HIV drug resistance mutations. However, treatment failure due to nonadherence does not always translate to resistant virus necessitating a change in ART regimen. As such, evaluation of adherence and ongoing adherence education and counseling when indicated should be a part of all routine clinical encounters. When treatment failure is suspected, intensive adherence support should also be provided. Only after optimizing dosing, assuring optimal adherence, and/or confirming HIV drug resistance via genotyping should an alternative ART regimen be considered.

Adherence to ART prescribed for infants and children poses additional challenges including: the need for caregiver support for medication administration, use of liquid suspensions that

require precise measurement, and low literacy. When poor adherence is documented or suspected, caregivers and other treatment supporters should be involved in adherence education and counseling, and they should be encouraged to participate in creative and individualized solutions for improving adherence.

Prevention of Common Coinfections

HIV infection, if untreated, eventually results in severe immunocompromise and opens the door for a cadre of opportunistic infections that in the pre-ART era characterized AIDS. Now, these infections are preventable with maintenance of immune function via viral suppression with ART. However, many people who are eligible for ART still do not access care and treatment, and even when they initiate ART, they remain at increased risk for a variety of coinfections.

Co-trimoxazole Preventative Therapy

Co-trimoxazole is a sulfa-containing broad-spectrum antibiotic with activity against some Gram-positive and Gram-negative bacteria, fungi including *Pneumocystis jirovecii*, and protozoa including toxoplasmosis and malaria. Co-trimoxazole preventive therapy (CPT) refers to the prophylactic use of this antibiotic to prevent coinfection with the aforementioned microbes in susceptible hosts.

The WHO recommends CPT for all infants, children, and adolescents infected with HIV. In areas where malaria and/or severe bacterial infections are highly prevalent, they recommend continuing CPT until adulthood, regardless of ART status, WHO clinical stage, or CD4 count. In settings where there is low prevalence of malaria and bacterial infections, CPT may be discontinued for HIV-infected children older than 5 years of age, on ART for at least 6 months, who are clinically stable or virally suppressed, and with a CD4 count greater than 350 cells/mm³ (WHO 2014a).

NIH/HHS recommends CPT for all children under 12 months of age, for children 1–6 years of age when CD4 count is less than 500 cells/mm³ or CD4% is less than 15%, and for children older

than 6 years when CD4 count is less than 200 mm³. Discontinuation of CPT can be considered in children 1–6 years of age after receiving ART for at least 6 months, and CD4 count is greater than 500 cells/mm³ or CD4% is greater than 15%. For children older than 6 years, the cutoff is a CD4 count of more than 200 cells/mm³ (NIH/HHS 2014a).

For HIV-exposed infants, CPT can be started at 4–6 weeks of age and continued until HIV infection has been definitively excluded.

Tuberculosis

Globally, TB is among the most common opportunistic infections and a leading cause of mortality in people infected with HIV. TB can cause active or latent infection, but primary active TB and reactivated latent TB are more common with HIV infection. Any form of active TB in a child infected with HIV is an indication for ART initiation. As such, screening for TB should be a part of the every clinical encounter for HIV-infected children in TB-endemic areas. If active TB is identified prior to ART initiation and the patient is stable, the intensive phase of antitubercular treatment (ATT) should be started prior to ART to minimize the risk of developing TB immune reconstitution inflammatory syndrome (IRIS). Regardless of the timing of ATT, care should be taken to avoid drug interactions between the components of ATT and ART.

The WHO also recommends 6 months of isoniazid preventive therapy (IPT) for HIV-infected children who do not have active TB but who are at risk for acquiring TB or having reactivation of latent TB. IPT is recommended for HIV-infected children in the following situations: (1) when there is a known exposure to someone with active TB, regardless of age, (2) when living in a TB-endemic area and older than 1 year of age, and (3) after completing treatment for active TB disease, an additional 6 months of IPT should be administered.

Mycobacterium Avium Complex

Non-TB mycobacteria such as *Mycobacterium avium* complex (MAC) are ubiquitous in the environment, and in the pre-ART era, MAC was the second most common opportunistic infection in

HIV-infected children in the USA. Similar to TB, MAC can cause of spectrum of disease ranging from isolated lymphadenitis to primary pulmonary infection to disseminated disease. That said, severe disseminated disease almost exclusively occurs in the setting of severe immunocompromise. As such, recommendations for MAC prophylaxis are dependent of CD4 count. MAC prophylaxis with clarithromycin or azithromycin is recommended for HIV-infected children less than 1 year of age when CD4 count is less than 750 cells/mm³, for children 1–2 years of age when CD4 count is less than 500 cells/mm³, for children 2–6 years of age when CD4 count is less than 75 cells/mm³, and in children age 6 and older when CD4 count is less than 50 cells/mm³ (NIH/HHS 2014a).

Vaccines

HIV-infected and HIV-exposed children stand to greatly benefit from protection against vaccine-preventable diseases. Availability of vaccines and immunization schedules vary widely from country to country, but in general HIV-infected children should receive all of the recommended vaccines available to the general population. The only exception is that children with severe immunosuppression due to advanced HIV should not receive live-attenuated vaccines.

All inactivated vaccines can be safely administered to children with severe immunosuppression; however, due to impaired cellular immunity, they may not be as effective at eliciting a lasting immune response as in children with normal immune systems.

Live-attenuated vaccines include the varicella, measles, mumps, and rubella vaccines. These vaccines can be considered for HIV-infected children who are not severely immunocompromised, but should be avoided in children 6 months to 5 years of age with CD4% less than 15% or those 5 years and older with CD4 counts less than 200 cells/mm³.

Disclosure

Disclosure of HIV status to children affected by HIV is a crucial component to the long-term care

and treatment of this vulnerable population, and it involves both the child and their caregivers. This is a subject that must be broached gradually and with sensitivity to an individual's circumstances, family dynamics, and caregiver concerns.

Disclosure to children of their own HIV status is limited by maturity and understanding that develop with time. Thus, disclosure should be a gradual process that begins at school age with simple concepts of disease and is completed during adolescence with full disclosure of the implications of HIV infection and chronic disease management. Along with full disclosure, there should also be a transition of responsibility with a shift from caregiver-dependent care to self-directed care. Healthcare providers and caregivers sometimes worry that this process will be psychologically or emotionally traumatizing for children, but there is little evidence to support these concerns. To the contrary, disclosure is actually associated with improved health outcomes. Furthermore, risks to the affected child's well-being can be minimized by ensuring that the person(s) disclosing information have a strong and positive relationship with the child.

Concurrent with disclosure to children of their own HIV status, affected children should also be informed of the HIV status of their caregivers. Similarly, this should be an incremental process. This element of disclosure is also associated with improved health outcomes.

Disclosure should also be accompanied by counseling about the potential risks and benefits of disclosing their own or their caregivers' HIV status to others. Education about the importance of privacy and realities of stigma should be components of this counseling. That said, there are certainly situations where disclosure to others is necessary, so counseling should also assist them in how, to whom, and under which circumstances to disclose this information (WHO 2011).

Conclusion

Currently there is no cure for HIV. However when ART is administered as early as possible in the course of infection, children with HIV can lead

longer, healthier lives. Coordinated efforts are needed between health workers responsible for the care of children and those responsible for the care of pregnant mothers. Early infant diagnosis and the identification and management of opportunistic infections are critical. Psychosocial support is equally important, and the failure to provide such services can result in a lack of compliance with medications and appointments. Despite these ongoing challenges, progress in the field to date has provided “achievable targets” in our mission to eliminate pediatric AIDS and the hope for a healthy future for children and families living with HIV throughout the world.

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Children, Epidemiology of HIV/AIDS

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Definition

Pediatric HIV disease is due to infection with human immunodeficiency virus-1. The first pediatric cases were identified in the early 1980s

(Lindegren et al. 2000). Almost all transmission of HIV-1 to infants and young children occur through mother to child transmission of HIV with a smaller number of cases related to blood product transfusion, injections with contaminated needles, or sexual abuse (Lindegren et al. 2000). Among newly infected adolescents, transmission most commonly occurs through sexual transmission (Idele et al. 2014).

Introduction

The global HIV/AIDS epidemic has left a heavy toll since the HIV-1 virus was first identified in the early 1980s with over 39 million deaths reported due to HIV/AIDS-related causes. Important segment of this intergenerational pandemic are HIV-infected infants and children less than 15 years of age and young persons between 15 and 24 years of age. As of 2013, there were an estimated 35 million persons living infected with HIV/AIDS, which included 3.2 million children less than 15 years of age and close to 11 million young persons between the ages 15 and 24 years of age. It is estimated that 24.7 million (71%) of these HIV-infected adults and children live in Sub-Saharan Africa (UNAIDS 2014a; Gillespie et al. 2015).

Incidence and Mortality: In terms of incident HIV infections reported worldwide in 2013, an estimated 240,000 were children <15 years of age. In addition, among the 2.1 million newly infected adults in 2013, about one third or close to 700,000 were young persons between the ages of 15 and 24 years (UNAIDS 2014a; Gillespie et al. 2015). In the United States, the rates of new pediatric infections declined dramatically from 1994 when it was estimated that there were 1650 new pediatric infections each year to less than 200 new infections annually in most recent years (CDC Surveillance 2011). In 2013, new infections among US adolescents ages 13–19 years of age were reported at 1,931 of whom 81% were males and 67% were African American, 18% Hispanic, and 11% Caucasian. In the age group 13–24 years, 40,634 young persons were living with HIV/AIDS in 2013 including

30,222 males and 10,412 females (CDC-DHAP 2013). Worldwide, in 2013 there were 1.3 million adults and 190,000 children less than 15 years of age who died of HIV-related causes (UNAIDS 2014a; Gillespie et al. 2015).

Modes of Transmission: Most children living with HIV have become infected from their mothers either during pregnancy, labor and delivery, or postnatally during breastfeeding in settings where breastfeeding is essential to infant survival. In resource-limited international sites, up to a third of infections occur during breastfeeding (DeCock et al. 2000). In contrast, the majority of young persons between the ages of 15 and 24 who are living with HIV usually became infected from sexual transmission, either heterosexual transmission or among young men who have sex with men (MSM). Internationally, incident infections among young persons have been primarily due to heterosexual transmission whereas in the United States, MSM incident infections represented 92% of new infections among adolescents and young adults aged 13–24.

Trends: The worldwide statistics, while grim, show progress in reduction of incident pediatric cases of HIV with greater than a 50% reduction in the numbers of new infant infections when compared to the mid 2000s where over 500,000 infants were newly infected each year (Gillespie et al. 2015). Most of these advances have built on findings from clinical trials demonstrating effective combination antiretroviral interventions for prevention of mother to child transmission of HIV (PMTCT) and which have subsequently been rolled out under international donor and Ministry of Health programs.

UNAIDS estimates that between 2005 and 2013, 1.1 million new pediatric infections were averted by PMTCT programs (UNICEF 2014d). Overall coverage of PMTCT has increased with 67% of pregnant women in low and middle-income countries receiving antiretrovirals (ARVs) for PMTCT in 2013, compared to 47% in 2010 (UNICEF 2014; UNAIDS 2014b). In the United States, HIV screening coverage is universal during pregnancy and with rapid testing for those who present late or at labor/delivery.

In contrast, new infections among adolescents and young adults have continued to fuel the HIV pandemic. These new infections in young persons are occurring despite proven effective preventative interventions such as condom use, circumcision, and pre-exposure prophylaxis. In 2013, WHO launched new treatment guidelines (WHO 2013) which emphasize early treatment of adults including pregnant HIV-infected women in order to decrease the risk of HIV transmission to sexual partners and mother to child transmission of HIV.

In this entry, we will discuss approaches to early diagnosis and treatment of young children infected with HIV including recommended first-line treatment. We will also review the unique challenges of diagnosis and treatment of HIV-infected adolescents along with strategies to improve long-term adherence to antiretroviral treatment and highlight some innovative research approaches toward achieving long-term remission of pediatric HIV infection.

Young Children Living with HIV – United States and internationally: Strategies in the First Decade of Life (Birth to Age 9 Years) to Ensure Early Diagnosis and Prompt Initiation of Treatment

As noted above, great progress has been made in PMTCT over the past decade. In high-income countries such as the United States, the MTCT rate is less than 1% through PMTCT interventions including use of triple antiretrovirals during pregnancy, at labor/delivery, and with provision of formula and avoidance of breastfeeding (Whitmore et al. 2012). However, in low and middle-income countries, national PMTCT program coverage remains low. Thus, transmission rates remain high despite known effective antiretroviral interventions: Nearly 700 children are newly infected with HIV each day (UNAIDS 2014b). To ensure their health and survival, it is crucial that the HIV-exposed infants receive early infant diagnosis (EID) and initiate ART as early as possible after confirmation of their HIV infection.

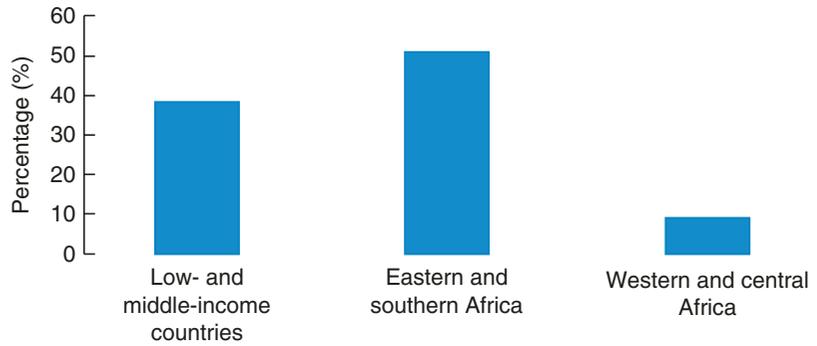
Strategies for Early Infant Diagnosis: WHO 2013 guidelines for low and middle-income

countries recommend initial testing of all HIV-exposed infants within 4–6 week of birth (WHO 2013). However, guidelines in higher income countries recommend HIV testing within 48 h of birth (Panel ART 2015). Diagnosis of HIV in infants >18 months and older children can be done using serologic antibody testing. However, serologic testing among children under 18 months is complicated by the presence of maternal anti-HIV antibodies and only determines HIV exposure. Therefore, nucleic acid testing (NAT) using HIV DNA, RNA, or total nucleic acid or viral p24 antigen is required to confirm HIV infection among infants up 18 months of age (WHO 2013; Panel ART 2015). This creates challenges in that nucleic acid testing is more expensive than serologic rapid antibody testing which be done with older children and adults. In addition NAT assays are technically demanding, require high technology laboratories, and are not easily accessible or locally available in most rural areas. To overcome these challenges, blood samples from HIV-exposed infants are often collected at local health centers on dried blood spots (DBS) which can then be sent to central laboratories for NAT.

While EID coverage has expanded over the last 5 years, there remain significant gaps in testing: only 6 out of 22 priority countries (South Africa, Swaziland, Botswana, Namibia, Zambia, and Zimbabwe) reported coverage above 50% (WHO 2013; Prendergast et al. 2015; UNAIDS 2014c). In addition, only 40–50% of all HIV-exposed infants received a test before 2 months of age as noted in Fig. 1 (UNAIDS 2014a; WHO 2013). In high-income countries, NAT testing of HIV-exposed infants is recommended at birth (for high risk perinatally exposed infants), 14–21 days, 1–2 months, and then again at 4–6 months of age (Panel ART 2015), at which point NAT negative infants can be considered HIV uninfected since they are formula fed and not HIV exposed through breast milk. In comparison, repeat NAT testing of HIV-exposed infants in low and middle-income countries who are mostly breastfed is recommended at 4–6 weeks and then 9 months of age with either serologic or viral testing to assess ongoing exposure through breastfeeding

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Fig. 1 Percentage of children born to HIV infected women who had HIV testing done within 2 months of birth in low and mid income settings; eastern and southern Africa, western and central Africa (Source: Global AIDS Response Progress Reporting 2013)



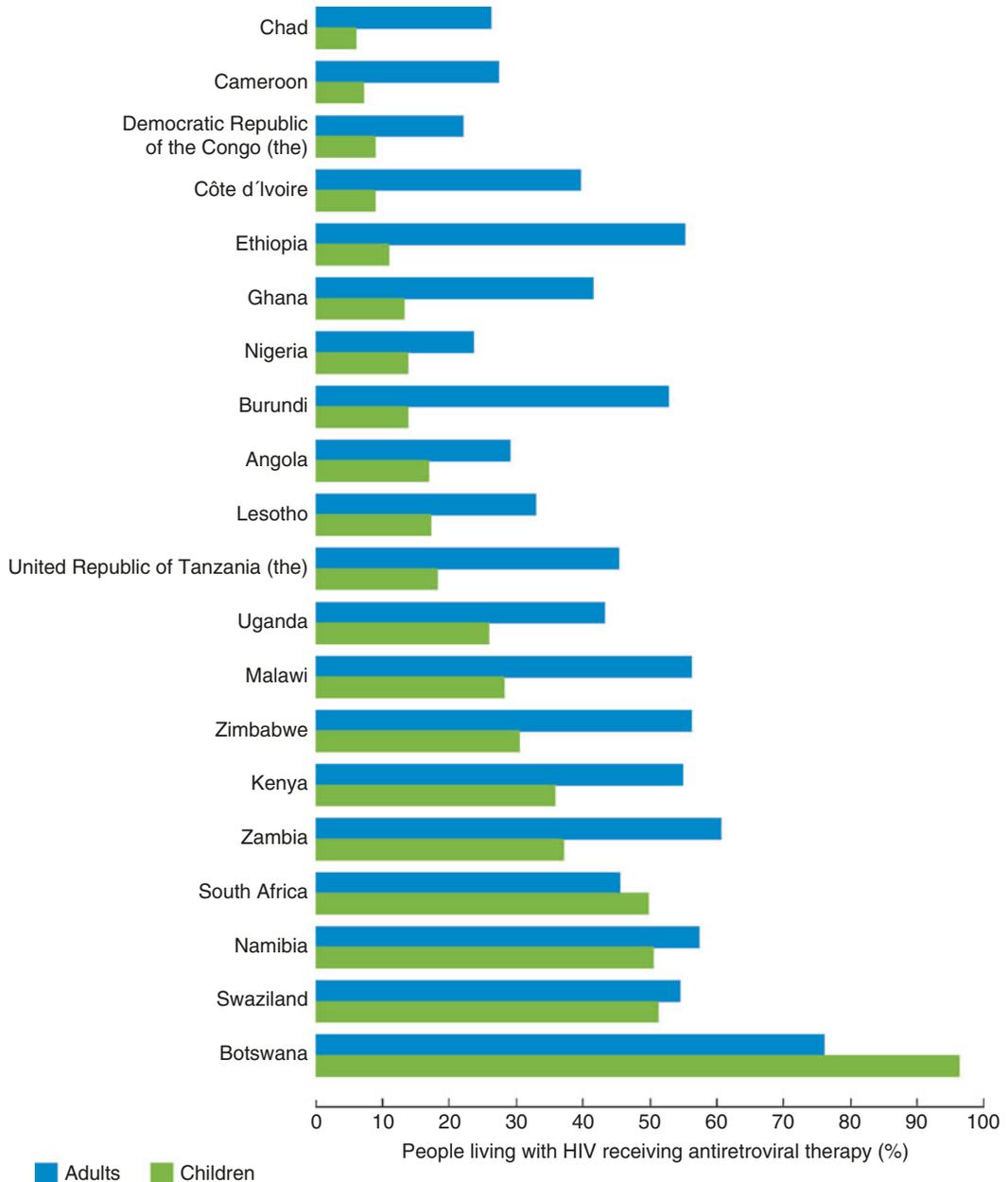
and confirm diagnosis respectively. A final serologic assessment of status can only be made at >18 months if there is no ongoing exposure or at 4–6 weeks after the breastfeeding period ends.

EID has increased the number of HIV-infected infants identified; however, challenges remain with linkage to care and treatment. There are often long turnaround times for receiving infant HIV results from central laboratories and high rates of loss to follow-up are an issue in many PMTCT programs (Chatterjee et al. 2011; Ciaranello et al. 2011). To overcome these problems, point-of-care (POC) virologic testing is being piloted which can give results on the same day of the clinic visit. It is hoped this may improve retention in care if mothers are able to receive results in real time. However, the majority of POC instruments are still in the development stage and not ready for clinical use (UNAIDS 2014a; Jani et al. 2014). Another challenge is that postpartum retention within PMTCT programs is low, with some studies reporting up to 80% of women lost to follow up by 6 months postpartum (Marcos et al. 2012; Ghadrshenas et al. 2013) and only 30–40% of HIV-infected infants initiated on ART (UNAIDS 2014a; Chatterjee et al. 2011). Integrating HIV testing into expanded program on immunization, pediatric wards, nutrition clinics, under-five and community based health programs can reach more mothers and infants who may have missed opportunities for testing, linking to care and treatment, and improve earlier identification of HIV-exposed children (UNICEF 2013a). This approach aims to strengthen systems and provide more comprehensive, effective, and efficient care.

When to Start ART

Before ART was available, 50% of HIV-infected infants died before their 2nd birthday (Newell et al. 2004). However, the CHER study demonstrated that early initiation of HIV-infected infants with ART before 12 weeks of age reduced the probability of early mortality by 75%, compared to delaying treatment until the infants met clinical or immunologic criteria (Violari et al. 2008). Likewise, studies in the United States and Europe have shown that infants initiated early on ART infants are less likely to progress to AIDS or death compared to those who are initiated later (Chiappini et al. 2009; Goetghebuer et al. 2012). Because the risk of rapid disease progression and death is high in young infants (Panel ART 2015; Violari et al. 2008; Palumbo et al. 2010) recommendations for high, middle, and low-income countries are similar with expedited initiation of ART for infants <12 months of age. Despite these recommendations, most children living with HIV in resource-limited international settings do not receive ART: In 2014 WHO reported only 23% of children (0–14 years) who are eligible are receiving ART worldwide (WHO 2014b). As noted in Fig. 2, ART coverage among children lags significantly behind HIV adults in the 22 priority countries (UNAIDS Global Report 2013).

The decision of when to initiate ART in HIV-infected children and adolescents varies by region and age and continues to generate controversy among HIV experts. The median age of ART initiation for children living with HIV in many countries is 4 years of age (Fenner et al. 2010); and countries vary on the criteria used to make the decision for treatment initiation



Children, Epidemiology of HIV/AIDS, Fig. 2 Percent-age of adults (aged 15+) and children (aged 0–15 years) living with HIV who were receiving antiretroviral therapy

in 2013, in 21 high priority countries (Source: 2013 esti-mates from UNAIDS, WHO and UNICEF)

for children 12 months and above. A comparison of United States (Panel ART 2015) and WHO ART guidelines (WHO 2013) is shown in Table 1. In the United States, the factors to consider in making the decision when to initiate therapy in children >12 months of age include:

- Increasing HIV RNA level (>100,000 copies/ml)
- CD4 count/percentage values approaching the age-related threshold for treatment
- Development of clinical symptoms
- The ability of caregiver and child to adhere to the prescribed treatment

Children, Epidemiology of HIV/AIDS, Table 1 Comparison of United States and WHO treatment guidelines for HIV-infected infants and children

Age	Criteria	Recommendation
US guidelines		
<12 months	Regardless of clinical symptoms, immune status, or viral load	Urgent treatment
1 to < 6 years	CDC stage 3 – defining opportunistic illnesses	Urgent treatment
	CDC stage 3 immunodeficiency, CD4 < 500cell/mm ³	Urgent treatment
	Moderate HIV-related symptoms	Treat all
	Plasma HIV RNA level >100,000 copies/ml	Treat all
	Age 1–<6 years, CD4 500–999 cell/mm ³	Treat all
	Mild HIV-related symptoms or asymptomatic and CD4 ≥ 1000 cell/mm ³	Consider treatment
>6–15 years	CDC stage 3 – defining opportunistic illnesses	Urgent treatment
	CDC stage 3 immunodeficiency, CD4 < 500cell/mm ³	Urgent treatment
	Moderate HIV-related symptoms	Treat all
	Plasma HIV RNA level >100,000 copies/ml	Treat all
	CD4 count 200–499 cell/mm ³	Treat all
	Mild HIV-related symptoms or asymptomatic and CD4 count ≥500cell/mm ³	Consider treatment
WHO 2013 guidelines		
<12 months	Irrespective of CD4 count and WHO stage	Treat all
1–5 years	Irrespective of CD4 count and WHO stage	Treat all
>5–15 years	CD4 ≤ 500 cells/mm ³ irrespective of WHO stage	Treat all
	WHO clinical stage 3 or 4	Treat all

According to the US guidelines, ART does not need to be started urgently for healthy HIV-infected children >1 year of age; and caregivers may postpone therapy on a case-by-case basis or providers may elect to defer therapy based on clinical and or psychosocial factors (Panel ART 2015). Close monitoring of virologic, immunologic, and clinical status is provided every 3–4 months (Panel ART 2015).

Most low and middle-income countries use the WHO guidelines to inform health policy. In 2013, WHO HIV consolidated treatment guidelines were revised based on review of the evidence, operational considerations, and preferences expressed by care providers to simplify and expand treatment in children (WHO 2013). These modifications included initiating ART for all HIV infants and children under 5 years of age regardless of CD4 cell count and to increase the threshold for ART initiation to ≤500 cell/mm³ in children 5 years and older in alignment with adult treatment guidelines. The guidelines development group made these changes despite the lower risk of disease progression in children 2–5 years compared to those <2 years and limited clinical trial

data evidence. However, programmatic data suggest that retention is better among children on ART than those in care but not yet started on ART (WHO 2013; Massavon et al. 2014; Penazzato et al. 2012). Increased mortality has also been reported among the children and adolescent enrolled in care programs but not yet started on ART compared to those on ART (Massavon et al. 2014). Access to immunologic testing required for decision-making is still limited in countries with a high burden of pediatric disease and low pediatric coverage (UNAIDS 2014a; Barker and Mate 2012). The need for access to CD4 cell testing to initiate treatment is avoided with the new guidelines. Therefore, simplification of the eligibility criteria may significantly improve the overall health outcomes for children with HIV.

HIV-Infected Children Under 3 Years of Age

Optimizing treatment of young HIV-infected children is important to promote viral suppression, survival, and growth. Other considerations include the fact that young HIV-infected children are exposed to various antiretroviral drugs, used

Children, Epidemiology of HIV/AIDS, Table 2 Comparison of US based and WHO (2013) recommended first-line antiretroviral treatment regimens for children

Preferred regimen (US guidelines UNAIDS 2014b)	
Children aged 14 day to <3 years	Two NRTI plus LPV/r
Children aged >3 years to <6 years	Two NRTI plus EFV
	Two NRTI plus LPV/r
Children aged ≥6 years	Two NRTI plus ATV plus low-dose RTV
	Two NRTI plus EFV
	Two NRTI plus LPV/r
Alternative regimens(US guidelines UNAIDS 2014b)	
Children aged ≥14 days	Two NRTI plus NVP
Children aged ≥3 month to <6 years and weighing >10 kg	Two NRTI plus ATV plus low-dose RTV
Children aged ≥2 years	Two NRTI plus RAL
Children aged ≥3 years to <12 years	Two NRTI plus twice daily DRV plus low-dose RTV
Children aged ≥12 years and weighing >40 kg	Two NRTI plus once daily DRV plus low-dose RTV
	Two NRTI plus DTG
Preferred NRTI (US guidelines UNAIDS 2014b)	
Children from birth to <3 months	ZDV plus (3TC or FTC)
Children aged ≥3 months and ≤12 years	ABC plus (3TC or FTC)
	ZDV plus (3TC or FTC)
Adolescents 13 years at Tanner 3	ABC plus (3TC or FTC)
WHO 2013 preferred regimen UNAIDS 2014a	
Children < 3 years	ABC or AZT + 3TC + LPV/r
Children 3 years to <10 years and adolescents <35 kg	ABC + 3TC + EFV
Adolescents (10–19 years) and ≥35 kg	TDF + 3TC (or FTC) + EFV

for PMTCT, especially NNRTIs. In addition, young children have high viral replication, thus require a potent first-line regimen to achieve viral suppression. The 2013 WHO guidelines recommended lopinavir/ritonavir (LPV/r) based ART for all infants regardless of exposure to PMTCT. A systematic review of two randomized trials showed that children younger than 3 years had a reduced risk of virologic failure or death and discontinuation of treatment on LPV/r based ART compared to NVP (Palumbo et al. 2010; Violari et al. 2012). In addition, there is evidence of emerging NNRTI resistance among children under 18 months without exposure to ARV drugs or PMTCT (Paredes et al. 2013; Chakanyuka-Musanhu et al. 2013). While LPV/r is the preferred first-line regimen, it is still not readily available in resource-limited countries; therefore, NVP-based regimens are an effective alternative.

WHO promotes the use of non-thymidine analogues (ABC) in first-line regimen to preserve the

response to zidovudine (ZDV) or d4T for second-line ART. ABC is available in pediatric fixed-dose combination and can be dosed once daily. Harmonization of the preferred regimen with older children and adults may also increase availability in resource-limited settings. Therefore, ABC and lamivudine (3TC) are the chosen preferred NRTI backbone. In the United States, first-line treatment regimens are similar for children under 3 years; however, alternative regimens are available based on age (Table 2).

Children 3 Years and Older

Internationally, Efavirenz (EFV) is the WHO preferred NNRTI for first-line treatment of children 3 years and older. Observational data suggest that EFV has a better short-term toxicity profile and better virologic suppression than NVP (Shubber et al. 2013). ABC and 3TC are the preferred NRTI to enable once a day dosing as well as harmonization with adolescents and adults regimens. Tenofovir (TDF) is approved by the US Food

and Drug Administration and European Medicines Agency for children 2 years and older. However, TDF is known to reduce bone mineral density (Hazra et al. 2005; Purdy et al. 2008). In addition, there is limited experience with TDF in young children and knowledge of its effect on growth and future fracture risk is unknown. While TDF is a recommended alternative to ABC, its use for children between 2 and 15 is very limited in low and middle-income countries.

New pediatric formulations (PIs and integrase inhibitors) are available in high-income countries and included in the first-line treatment regimen (Table 2) (Panel ART 2015). However, these formulations are not yet readily available in low and middle income countries. Therefore, second line is limited to protease inhibitor based ART with LPV/r or Atazanavir. Low and middle income countries need expanded access to new formulations and also need to plan for options for third-line ART, as more HIV-infected children initiated on ART are surviving to adolescence and adulthood.

Adolescent and HIV Second Decade of Life (10–19 Years)

WHO defines adolescents living with HIV (ALHIV) as children between the age of 10–19 years and were perinatally HIV-infected or acquired HIV through sexual activity or exposure through injection drug abuse or unsafe blood products (UNAIDS 2014b). By the end of 2013, UNAIDS estimated 2.1 million adolescents living with HIV worldwide of which 56% were girls, with 80% living in Sub-Saharan Africa and unaware of their HIV status (UNAIDS 2014b; UNICEF 2013b). Approximately 300,000 new infections worldwide occurred among adolescents aged 15–19 years in 2012, which accounted for about 13% of the 2.3 million new infections globally in 2012 (UNICEF 2013b). In contrast, the Centers for Disease Control and Prevention (CDC), estimates that by the end of 2012, approximately 7,300 adolescents aged 13–19 years were living with HIV in the United States. HIV/AIDS is the number one cause of mortality among adolescents in Africa and second worldwide (WHO 2014b). Globally AIDS-related deaths fell by

40% between 2005 and 2013 except for adolescents. There is limited data to explain why deaths among adolescents with HIV are not decreasing. However, the low level of testing among adolescents could potentially contribute to the increase in AIDS-related mortality (Idele et al. 2014). Those who do not know their HIV status are unlikely to seek care and treatment with antiretroviral therapy, which results in a delay in treatment and advancement of HIV disease (Idele et al. 2014). In addition, mortality among adolescents in pre-ART HIV care has been reported to be significantly higher when compared to adults (Massavon et al. 2014; Okomo et al. 2012).

Modes of Acquisition

The modes of transmission for adolescents include perinatally acquired transmission with increasing numbers of long-term survivors, infection acquired through blood products or contaminated needles, and those who acquired HIV behaviorally through unprotected sex or by drug abuse and sharing of needles.

The high rate of undiagnosed HIV infection, poor rates of linkage to care because of poor health seeking behavior among adolescents, and low antiretroviral adherence rates with resultant poor viral suppression may be a factor that will fuel the future of the epidemic. Adolescents living with HIV are a heterogeneous group and global aggregate data on the epidemic among them can mask significant regional and population variances. Although data is limited studies available from low and high burden countries suggest that HIV prevalence is disproportionately high among adolescents belonging to “key populations,” especially sexually exploited adolescents, adolescents who inject drugs, and adolescent MSM, (UNAIDS Global report 2013; Thailand Bureau of Epidemiology 2012; Busza et al. 2013). Heterosexual transmission is the predominant mode of HIV acquisition in adolescent females. In addition, HIV prevalence among adolescent females tends to be higher than among adolescent males in countries with generalized epidemic (UNAIDS Global Report 2013; UBOS 2011).

The reasons for this gender disparity are thought to be related to the early sexual debut

before the age of 15 in some females and increased risk related to having sex with older male sexual partners (Kelly et al. 2003).

ART and Mortality Among Adolescents

Despite the progress made in the AIDS response in recent years, emerging evidence suggests that (UNAIDS 2014d) adolescents are falling behind as a result of not receiving the attention and services they require (UNAIDS 2013). Currently there is insufficient data to accurately determine the number of adolescents who need and receive ART. In addition, AIDS-related mortality among adolescents has increased by 50% over the past 7 years but fell for all other age groups, according to UNAIDS estimates (UNAIDS 2014d). Adolescents (10–19 years) are the only age group in which AIDS-related deaths increased from 2001 to 2012. From 2005 to 2012, the annual number of AIDS-related deaths among adolescents almost doubled. The inability of adolescents to benefit equally from treatment underscores the need for more HIV testing and counseling among this age group and for adolescent friendly health services (UNAIDS 2015).

Adherence

HIV-infected adolescents encounter adherence challenges including pill burden and dealing with their sexuality in the face of HIV. In addition, adolescents commonly face issues of denial and anxiety related to their HIV infection. This is especially common among the youth who have been recently diagnosed, and this may result in refusal to start or continue combined ART. Misinformation about HIV, lack of trust in health care workers, and lack of knowledge about the availability and effectiveness of ARV treatments can also be barriers to retaining adolescents in care and maintaining them on ART.

Perinatally infected youth often have longstanding histories of inadequate adherence. Adolescents in southern Africa are less adherent to ART and have lower rates of virologic suppression and immunologic recovery compared to adults (Nachega et al. 2009). Regimen fatigue

also has been identified as a barrier to adherence in adolescents. Studies have shown that depression and anxiety are associated with poorer adherence, especially to complex ARV regimens. HIV-infected adolescents often have concomitant mental illnesses low self-esteem, chaotic lifestyles, and do not cope well with their illness. Alcohol or substance abuse, poor school attendance, psychiatric disorders, and advanced HIV disease are associated with poor adherence. Additional challenges include nondisclosure of HIV status to parents or partners (Lowenthal et al. 2014).

Secondary Prevention Strategies for HIV-Infected Adolescents

High adherence to taking ART can play an important role in preventing HIV transmission. To have effective prevention of HIV transmission requires: (1) ART capable to achieve continuous viral suppression, (2) high adherence to an effective ART, and (3) an absence of a concomitant STD. In spite of the cultural difference among adolescents around the world, they have two common characteristics, they want respect and to be sure that their confidentiality is protected. With the growing number of adolescents, WHO promotes implementation of adolescent friendly services. To be considered adolescent friendly, health services should be accessible, acceptable, equitable, appropriate, and effective (WHO 2006). Interventions that focus on adolescents, youth, and the three elements for preventive transmission are needed for secondary prevention and reduction of new infections.

Future Directions

The Search for a Cure

Since the first cases of HIV were reported in the early 1980s, the research and public health communities have sought both an effective preventive vaccine as well as a cure or long-term clinical remission for those already infected with HIV. Both efforts have proved daunting over the past three decades. For preventative HIV vaccine

development, these challenges relate to several factors: the virus mutates rapidly and the correlates of immunity are still not clearly understood. For already infected individuals, challenges for development of an effective long-term remission or cure include that the virus incorporates into the DNA of host immune cells and the presence of long lived reservoirs containing HIV-infected memory cells which make it extremely difficult to eradicate the virus. However, the case report of the adult Berlin patient who appeared cured after receiving a bone marrow transplant from a donor who was homozygous for the delta 32 deletion of the CCR5 gene do provide a proof of concept for development of strategies that could prevent attachment of the virus onto host cells (Yuki et al. 2013). Likewise, documented “elite controllers” whose virus is nondetectable in plasma despite no ongoing treatment suggest that it may be possible to at least achieve long-term remission from HIV disease progression among chronically infected adults. In pediatrics, there is also encouragement related to the “Mississippi baby” case where a newborn with acute infection was begun on treatment in the first days of life and who was subsequently lost to follow-up and stopped therapy around 18 months. The infant was relocated and found to be without detectable virus in plasma for close to 2 years without treatment, before the virus then became detectable again (Persaud et al. 2013).

In response to the “Mississippi baby” case, several pediatric protocols are underway to see if newborns infected around the time of birth can be treated soon after birth, with a goal of achieving long-term remission. Among those infected children treated from birth who remain undetectable for plasma viral load for a long period, treatment interruptions will be attempted with close monitoring among those children where no virus has been detectable for several years. Other approaches toward a pediatric cure will be use of stem cell transplants when bone marrow transplants are already scheduled for other indications.

In addition to the research focused on achieving long-term remission and prevention of HIV disease progression, programmatic efforts need to continue to focus on a comprehensive approach to

treating children with pediatric HIV as a chronic illness that addressing developmental and psychosocial needs of infected children as they age into adolescence and adulthood.

HIV Chronic Care

There are limited data on long-term outcomes of ART adherence, retention within programs in resource-limited settings. Although there is progress in the availability of child friendly ART formulations, there is still a need to develop more fixed-dose combinations and granules especially for protease inhibitor based regimens. Improvements of supply chain of pediatric ART and diagnostics will help ensure adequate care and monitoring response to treatment. Over the years, attempts have been made to simplify the guidelines for children because of the differences in initiation criteria. However, there are still barriers in access to CD4 cell testing which is necessary to determine eligibility for ART in low and middle-income countries. Some countries like Uganda have already simplified their treatment guidelines further by implementing test and treat for all children under 15 years, regardless of CD4 count and WHO stage.

Conclusion

The pediatric HIV epidemic worldwide is showing major progress in reduction of new cases of mother to child transmission, but adolescent infections continue to fuel the epidemic. In addition, treatment coverage continues to lag behind for both children and adolescents compared to adults. Further research is needed to address better adherence support, age appropriate disclosure, and chronic complications of HIV infection and treatment among children and adolescents.

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Chronic Immune Activation in HIV

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Definition

The term immune activation describes the activation of the cellular components of the immune system, which may in turn lead to systemic inflammation. It is usually detected by the expression of cellular or soluble markers derived from innate or adaptive immune responses. Immune activation is associated with HIV disease progression as well as the increased morbidity and mortality in HIV-infected patients despite antiretroviral therapy. A number of interventions that target immune activation have been or are being investigated. Currently, there is no conclusive evidence that interventions other than antiretroviral therapy (ART) lead to clinical benefit.

Introduction

HIV infection is characterized by a global activation of the immune system. Cellular activation is evident in T cells with higher CD38 and HLA-DR expression, in NK cells with higher CD69 and HLA-DR expression, in monocytes with higher expression of CD16 and tissue factor (TF), and in

B cells manifesting as expansion of immature/transitional B cells expressing CD10. Soluble markers of monocyte and macrophage activation including neopterin, soluble (s)CD14 (a marker of LPS-induced monocyte activation), and sCD163 (a monocyte- and macrophage-specific hemoglobin scavenger receptor) are also increased. In addition, serum or plasma levels of pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF), C-reactive protein (CRP), and beta-2 microglobulin (β 2M) are also elevated (Nixon and Landay 2010).

In untreated HIV infection, immune activation is associated with the decline in CD4 T cell count and progression to AIDS. The use of combination ART has revolutionized the management of HIV-infected patients, resulting in a dramatic reduction in morbidity and mortality. Despite ART, however, patients may still have greater mortality when compared to the general population, with serious non-AIDS events (SNAEs) such as cardiovascular, liver, and end-stage renal disease and non-AIDS-defining malignancies being the predominant causes of mortality.

Immune activation is an important contributor to the elevated morbidity and mortality of HIV-infected patients despite ART. A number of interventions to reduce immune activation in patients on ART are currently under investigation. To date, there is little conclusive evidence on which strategy will lead to clinical benefits. Given that the majority of patients respond well to ART and enjoy a good quality of life, interventions to reduce residual immune activation must have low adverse effect profiles and robust evidence for clinical benefit before they can be implemented.

Immune Activation in Chronic HIV Infection

Immune Activation and HIV Disease Progression

Immune activation is a critical factor in HIV and simian immunodeficiency virus (SIV) disease progression. Natural hosts of SIV such as sooty

mangabeys, African green monkeys (AGM), and mandrills rarely develop immunodeficiency or pathogenic SIV infection. On the other hand, non-natural hosts such as rhesus macaques (RM) develop AIDS after infection with SIV. Though both groups have high viremia, natural hosts are able to dampen immune activation after acute infection and have low levels of immune activation in chronic infection (Chahroudi et al. 2012).

Immune activation is detrimental in HIV infection (reviewed by Paiardini and Muller-Trutwin 2013). The levels of T cell activation and plasma cytokines (such as IP-10 and IL-6) during acute HIV infection predict CD4 T cell decline and HIV disease progression. Immune activation can lead to activation-induced cell death or apoptosis, CD4 T cell depletion, and progressive restriction in T cell repertoire in both CD4 and CD8 T cells. It may also promote HIV infectivity whereby a vicious cycle is set up as HIV replication promotes immune activation which leads to increased targets for HIV to infect (as HIV preferentially infects activated CD4 +CCR5+ T cells), thereby leading to greater viral production. Furthermore, ongoing immune activation is associated with increased numbers of regulatory T cells (Tregs) in the lymphoid tissues. Tregs secrete transforming growth factor- β (TGF- β), triggering collagen production that results in structural damage and fibrosis of the lymph node. Fibrosis of lymph nodes is associated with apoptosis of naïve CD4 T cells by inhibiting their access to IL-7 (Zeng et al. 2012). Hence, HIV-infected patients with higher levels of immune activation have greater CD4 T cell depletion, faster progression to AIDS, and shorter survival independent of HIV viral load (Giorgi et al. 1999).

Factors Contributing to Immune Activation in Chronic HIV Infection

A number of factors contribute to the stimulation of immune responses in chronic HIV infection. These include the presence of HIV and its antigens, microbial translocation, the presence of coinfections, and homeostatic proliferation in

response to CD4 T cell depletion (Paiardini and Muller-Trutwin 2013).

HIV and Its Antigens

HIV RNA can activate plasmacytoid dendritic cells through binding to pattern recognition receptors (PRR) such as TLR 7, leading to the secretion of type I interferon (IFN). HIV can also cause immune activation through antigenic stimulation of HIV-specific CD8 and CD4 T cells. Though the level of HIV viremia correlates with T cell activation (Deeks et al. 2004), it is not the sole cause of immune activation. Markers of immune activation can remain elevated even when plasma HIV viremia is undetectable by conventional assays in patients on ART or elite controllers with spontaneous control of HIV viremia.

Microbial Translocation

In HIV infection, mucosal immune responses in the gastrointestinal tract are impaired. CD4 T cells in gut-associated lymphoid tissue (GALT) are targets for HIV infection as high proportions of these cells express CCR5 and are activated. This leads to substantial depletion of CD4 T cells in GALT. In addition, TH17 cells are preferentially lost in the gut in HIV infection. TH17 cells secrete IL-17 and IL-22, promote neutrophil recruitment, and are important in the defense against bacterial and fungal infections and the maintenance of epithelial barrier function (Brenchley 2013). The loss of TH17 cells is associated with systemic T cell activation in SIV-infected macaques, and the reduction of TH17/ Tregs ratio is related to SIV disease progression.

Furthermore, gut mucosal barrier integrity is also impaired in HIV infection. It is postulated that exposure to HIV envelope glycoprotein gp120 leads to disruption of tight junctions, enhanced mucosal permeability, as well as induction of pro-inflammatory cytokines, especially TNF (also known to disrupt epithelial cell tight junctions). Increased mucosal permeability then allows the translocation of bacterial products, eliciting further inflammatory responses through binding to pattern recognition receptors on monocytes, macrophages, and plasmacytoid dendritic cells, and

may also indirectly cause bystander polyclonal T cell activation (Brenchley 2013).

AGM (natural hosts of SIV) maintain mucosal barrier integrity and display little immune activation in chronic SIV infection, while RM have disruptions of the epithelial barrier of the colon and increased lipopolysaccharide (LPS) staining. The levels of LPS staining in the colon also correlated with the levels of LPS in the draining lymph nodes and remote peripheral lymph nodes. LPS can bind to membrane or sCD14 and TLR4 complex leading to the production of IL-6, IL-1, and TNF by monocytes and macrophages. The injection of LPS into SIV-infected AGM was associated with increases in T cell activation, sCD14, and SIV viremia. These data suggest that microbial translocation due to impaired mucosal barrier integrity is a potential contributor to immune activation in chronic HIV infection.

Coinfections

Immunodeficiency associated with HIV infection also leads to the reactivation and replication of highly prevalent coinfection pathogens such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV), leading to stimulation of pathogen-specific immune responses. HIV viremia has been associated with the activation and expansion of CMV and EBV-Sp-CD4 and CD8 T cells. Thus, sustained CMV-mediated immune activation may contribute to overall immune activation in HIV-infected individuals.

Homeostatic Proliferation

CD4 T cell depletion in HIV infection may stimulate physiologic homeostatic mechanisms, leading to persistent lymphocyte proliferation in response to homeostatic cytokines (IL-2, IL-7, and IL-15). This may also lead to the differentiation and the generation of effector T cells that are proinflammatory, thereby contributing to immune activation. Evidence for decreased expression of the IL-7 receptor α chain (CD127) with blunted responses to IL-7 in T cells as well as defective IL-7 utilization by naive T cells and lower IL-7 production by reticuloendothelial cells due to lymph node fibrosis suggests possible defects in

compensatory responses to lymphopenia in HIV infection.

Immune Activation in Patients on ART

ART leads to virologic suppression and reduction in immune activation. However, levels of markers of immune activation do not fully normalize in all patients. When compared with the general population, the levels of CRP, IL-6, and D-dimer, markers of monocyte activation such as sCD14 and sCD163, and markers of T cell activation, particularly CD38+HLA-DR⁺ co-expression, remain elevated in HIV-infected patients despite ART.

Though the use of ART has resulted in a dramatic reduction in morbidity and mortality, patients on ART still have greater mortality when compared to the general population (reviewed by (Nakagawa et al. 2013), with SNAEs including cardiovascular, liver, and end-stage renal disease and non-AIDS-defining malignancies being the predominant causes of mortality (Hsu et al. 2013).

Immune Activation and Clinical Associations in Patients on ART

Inflammation is central to the process of atherosclerosis, tumor progression, and liver fibrosis. High levels of biomarkers associated with inflammation (CRP and IL-6) and coagulopathy (D-dimer and fibrinogen) are associated with increased risk of cardiovascular disease, malignancies, and mortality in the general population.

Higher levels of CRP, IL-6, D-dimer, sCD14, and lymphocyte activation have all been associated with increased mortality in HIV-infected patients despite ART. Furthermore, higher baseline levels of CRP, IL-6, and D-dimer are also associated with increased risk of non-AIDS malignancies and cardiovascular disease (CVD), independent of other CVD risk factors. Higher levels of IL-6 and CD8 T cell activation have been associated with functional impairment and frailty and higher levels of TNF with SNAEs in patients on ART (reviewed by Ledenman et al. 2013). Therefore, immune activation is

likely a key contributor to the elevated morbidity and mortality in HIV-infected patients despite ART. Other factors that may also play a role include the insults sustained secondary to the direct effect of HIV and associated immunodeficiency prior to ART initiation, underlying co-morbidities and coinfections, as well as ART toxicities.

Factors Contributing to Persistent Immune Activation in Patients on ART

The drivers of persistent immune activation in patients on ART are diverse. Intermittent HIV viremia can occur in 20–30% of ART-treated patients and is associated with higher IL-6, D-dimer, and sCD14 levels and SNAEs. The presence of persistent coinfections also contributes to continual stimulation and activation of the immune system. Asymptomatic CMV infection has been associated with CD8 T cell activation in patients on ART. CMV-specific CD4 T cells can cause a systemic inflammatory response that is sustained even during latent infection and is associated with atherogenesis. Hepatitis C coinfection is also associated with increased CD8 T cell activation when compared with HIV mono-infected patients despite ART. Furthermore, impairment in mucosal barrier integrity may not be fully reversible, and markers of microbial translocation, e.g., LPS, sCD14, and bacterial 16s rDNA, do not always normalize with ART. LPS can induce tissue factor (initiator of the coagulation cascade) expression on monocytes and may contribute to atherogenesis and increased CVD in patients on ART.

Strategies to Reduce Persistent Immune Activation Despite ART

As mentioned before, ART results in a reduction in immune activation. However, levels of immune activation do not normalize in a substantial number of patients. Furthermore, persistent immune activation despite ART is associated with increased SNAEs and mortality.

The 2013 World Health Organization guidelines recommended the initiation of ART in

asymptomatic HIV-infected patients at CD4 T cell count of ≤ 500 cells/ μL . The use of ART within 6 months of HIV infection has been associated with reduced reservoir size and reduced T cell activation when compared to ART initiation at ≥ 2 year after HIV infection. Whether earlier ART initiation can reduce the levels of immune activation to that of uninfected persons is not clear (reviewed by Sandler and Sereti 2014). Results from the START study (ClinicalTrials.gov identifier: NCT00867048), a multicenter international trial designed to assess the risks and benefits of initiating ART at CD4 T cell count of >500 or <350 cells/ μL , will shed further light on the clinical benefits of earlier ART initiation.

Given that mean CD4 T cell count at the time of presentation to care is <400 cells/ μL in a number of cohorts, the majority of newly diagnosed patients will meet the 2013 WHO recommended threshold for ART initiation soon after presentation to care. Therefore, the remaining discussion will focus on the strategies that reduce persistent immune activation in patients on ART.

The interpretation of currently available data on strategies to reduce immune activation in patients on ART is often difficult. Firstly, a significant number of studies targeted untreated patients. Although these studies may assist in understanding the underlying pathophysiology, HIV viremia is a strong stimulator of immune activation, and data from untreated cohorts may not be generalizable to patients on ART. Secondly, though many studies used biomarkers of immune activation as endpoints, few used clinical outcome measures. Therefore, it is difficult to determine whether reduction in a particular marker of immune activation will translate into clinical benefit. Thirdly, studies were often of short duration and involved only small number of patients and may not have adequate power to detect clinical responses or long-term adverse effects associated with the intervention.

A number of strategies to reduce persistent immune activation despite ART have been under investigation. These include reducing chronic antigen stimulation from residual HIV viremia or from coinfection pathogens, dampening global inflammation, improving immune recovery, modulating

lymphoid tissue fibrosis, and reducing microbial translocation. Given that this manuscript is an overview, it will focus on interventions that have robust data and studies that involved patients on ART.

Suppressing Chronic Antigen Stimulation

Reducing Residual Viremia

Intensification studies have been performed to assess the impact of adding antiretroviral agents to a suppressive ART regimen (as measured by conventional assays). None of the raltegravir (an integrase inhibitor) intensification studies performed to date were able to demonstrate reduction in ultra-sensitive plasma HIV RNA levels. In addition, the majority of studies also found no reduction in markers of T cell or monocyte activation. However, a few studies have noted a reduction in T cell activation and D-dimer levels as well as an early transient increase in 2-LTR circles after raltegravir intensification, suggesting that residual viremia was occurring prior to raltegravir intensification and was contributing to immune activation in some patients.

Maraviroc (a CCR5 antagonist) intensification studies have also been performed and yielded conflicting data. Some found reduction in T cell activation while others found an increase in CD4 and CD8 T cell activation both in the peripheral blood and in the rectal mucosa as well as an increase in sCD14 after maraviroc intensification (Hunt et al. 2013). The binding of maraviroc to CCR5 might have prevented the interaction between CCR5 and its natural ligands. Excess CCR5 ligands might then bind to other chemokine receptor such as CCR3 and CCR4 on T cells, leading to T cell activation (Hunt et al. 2013). Therefore, the beneficial effect of adding antiretroviral agents to an already suppressive ART regimen is uncertain based on currently available data.

Treatment of Coinfections

HCV treatment and suppression of HCV viremia are associated with a reduction in CD4 and CD8 T cell activation. Sustained virologic response is also associated with reduced liver-related complications as well as both liver-related and non-liver-

related mortality in coinfecting patients. Unfortunately, HCV treatment may be limited by contraindications, adverse events, high costs, and drug interactions. Next-generation agents with higher efficacy and better side-effect profiles may revolutionize the management of HIV/HCV coinfecting patients and may reduce the incidence of non-AIDS malignancies, specifically hepatocellular carcinoma.

Treatment of other persistent viral infection has also been investigated (reviewed by Hsu et al. 2013). Eight weeks of valganciclovir in CMV seropositive patients on ART led to a reduction of CMV viremia as well as a reduction in CD8 T cell activation. The study was too short and small to observe for effects on CD4 T cell count or clinical outcome measures. However, the results suggest that residual CMV replication is a contributor to CD8 T cell activation in patients on ART.

In a study targeting HSV coinfection using 12 weeks of valacyclovir in HSV-1 and HSV-2 seropositive patients on ART, no change in T cell activation and CRP or IL-6 levels was demonstrated. The authors postulated that the negative findings may be because the dose of valacyclovir used was insufficient to suppress HSV shedding or that HSV-2 reactivations are too localized to cause systemic immune activation.

Anti-inflammatory Agents

Statins

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors. Not only do statins reduce serum cholesterol; they may also have anti-inflammatory properties. Statin use has been associated with reduced monocyte activation, a decline in CRP, and reduced T cell activation in patients on ART as well as in untreated, HIV-infected patients. A retrospective observational study of patients on ART showed that statin use is associated with a threefold reduction in mortality (Moore et al. 2011). Though not statistically significant, a trend for reduction in SNAEs and mortality has also been seen in other retrospective observational studies. Therefore, statins hold promise as an agent

that reduces immune activation in patients on ART and warrant further clinical investigation.

Hydroxychloroquine

Hydroxychloroquine (HCQ) and its analogue chloroquine (CQ) have immunomodulatory, anti-inflammatory, and anti-HIV properties. These drugs have been investigated in patients with uncontrolled HIV replication as well as in patients on ART. However, the findings are inconclusive at this stage.

COX-2 Inhibitors

COX-2 inhibitors inhibit cyclooxygenase type 2, reducing prostaglandin E2 production, thereby reducing activation of T cells through the cyclic adenosine monophosphate (cAMP) pathway. Studies on COX-2 inhibitors have been small and reduction in T cell activation tended to occur in viremic patients. However, it is important to bear in mind that COX-2 inhibitors are associated with increased cardiovascular risk, via a direct pharmacologic consequence of inhibition of COX-2. Therefore, assessing the utility of COX-2 inhibitors without using clinical outcome measures may be insufficient.

Aspirin

Aspirin is a cornerstone in the secondary prevention of vascular disease. In a pilot study, aspirin use was associated with reduced platelet activation, a decrease in sCD14, and reductions in CD38 and HLA-DR on CD4 and CD8 T cells. However, there was no change in IL-6, D-dimer, and CRP levels. An aspirin study with larger number of participants is in development with the AIDS Clinical Trials Group and should provide further data.

Prednisone

Conflicting data exist regarding the effect of prednisone on immune activation in patients on ART. Long-term prednisone use, especially at doses >7.5 mg/day, is associated with significant adverse effects such as osteoporosis, impaired glucose tolerance, dyslipidemia, weight gain, cataract formation, and increased risk of infections. Even short courses have been associated with

increased risk of osteonecrosis in HIV-infected patients. Therefore, the adverse effects of prednisone may outweigh any potential benefits.

Cytokine Inhibitors

Several inhibitors of proinflammatory cytokines such as TNF, IL-6, and IFN inhibitors are currently being evaluated in nonhuman primate/SIV models especially in regard to their efficacy in reducing persistent immune activation and their safety profiles.

Improving Immune Recovery

Given that homeostatic proliferation, depletion of TH17 cells, and imbalance in TH17/Tregs ratio are possible sources of persistent immune activation, a number of studies have thus been done to assess interventions that optimize immune recovery.

Cytokine Therapies

Subcutaneous administration of IL-2 in concert with ART resulted in a reduction in CD4 T cell proliferation and CD38+/HLR-DR+ expression on CD4 and CD8 T cells as well as a sustained increase in CD4 T cell count with improved CD4 T cell survival. However, this did not translate into measurable clinical benefits as shown in the largest to date immune intervention randomized clinical trial in ART-treated patients (Abrams et al. 2009).

Subcutaneous administration of IL-7 also led to increase in CD4 and CD8 T cells in the peripheral blood and increased CD4 T cells in the colonic mucosa. There were also concomitant reductions in colonic and systemic inflammation with decreased neutrophil infiltration and TNF expression in the colon and decreased plasma sCD14 and D-dimer (Sereti et al. 2014). These results are of interest but it remains unclear if the effects are persistent.

Modulating Lymphoid Fibrosis

As previously discussed, fibrosis of the lymphoid tissues impairs T cell survival and impedes their homeostatic proliferation by limiting both utilization and local IL-7 production. Binding of angiotensin II to the angiotensin II receptor on cardiac

fibroblast, hepatic stellate cells, or mesangial cells leads to proliferation as well as collagen and TGF- β synthesis. In a small pilot study, the use of lisinopril was associated with a small but statistically significant reduction in CRP and TNF levels when compared with placebo. A number of trials on the effect of angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists in modulating lymphoid tissue fibrosis are currently underway.

Both TNF blockade with adalimumab and pirfenidone administration were associated with attenuated TGF- β expression, reduced lymph node fibrosis, and preservation of lymph node CD4 T cells in rhesus macaques with SIV infection. Data on patients with HIV infection are not yet available.

Targeting Microbial Translocation and Impaired Mucosal Barrier Integrity

Given that HIV infection has been associated with depressed levels of beneficial gut microbiota and elevated levels of pathogenic microbiota in a few studies, a range of prebiotics (selectively fermented ingredients that change the growth and/ or activity of certain gut microflora, resulting in health benefits), probiotics (live microorganisms that when consumed confer a health benefit), and synbiotics (a combination of pre- and probiotics) are under investigation. Data from patients with untreated HIV infection suggest that pre- and probiotic agents may be associated with improvement in microbiota composition and reduction in microbial translocation. Data on patients on ART are inconclusive at this stage.

A number of novel agents that have shown promise in nonhuman primates with SIV infection are currently under investigation in patients with HIV infection: rifaximin is a minimally absorbed oral rifamycin antibiotic that has activity against both gram-positive and gram-negative enteric bacteria; sevelamer is a phosphate binder that is used in patients with end-stage renal failure but can also bind to endotoxins; lubiprostone is a chloride channel activator that has the potential to enhance recovery of mucosal barrier function; mesalamine (5-aminosalicylic acid) is an anti-

inflammatory agent used in the management of inflammatory bowel disease; and IL-21 is essential in the maintenance of TH17 cells and mucosal function. Data on the efficacy of these agents in reducing immune activation in patients on ART are pending.

Therefore, though a number of studies have been performed to evaluate strategies to reduce persistent immune activation on ART, there is little conclusive evidence on which strategy will lead to clinical benefit. The use of statins seems to show the most promise at this stage. Given that the majority of patients respond well to ART with, for the most part, a good quality of life, interventions to reduce persistent immune activation must be tolerable, have low adverse effect profiles, and have robust evidence for clinical benefits before they can be implemented. Furthermore, it is also possible that earlier ART initiation may negate the need for adjuvant therapies.

Conclusions

Immune activation is detrimental in chronic HIV infection, causing progressive depletion in CD4 T cells and immunodeficiency. Despite the use of ART, the levels of immune activation do not fully normalize. Persistent immune activation despite ART is associated with increased risk of SNAEs and morbidity. Data on interventions to reduce persistent immune activation while on ART are increasing. At this stage, however, no conclusive evidence exists regarding which intervention results in clinical benefit. Further studies investigating better the biology of pathways involved in residual immune activation in HIV infection will be instrumental in designing targeted interventions. Currently, early diagnosis with prompt initiation of ART remains the most successful intervention in HIV infection.

Cross-References

- ▶ [Cardiovascular Complications](#)
- ▶ [Lymphocyte Apoptosis](#)
- ▶ [Microbial Translocation](#)

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Circumcision and AIDS

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Definition

Male circumcision is an ancient ritual that is now being used in a medical context to prevent HIV transmission to men. This is based on three randomized clinical trials showing that medical male circumcision (MMC), where there is complete surgical removal of the foreskin (FS), in east and southern Africa significantly reduces HIV infections by up to 60%. Voluntary MMC is now a public health HIV prevention strategy in many African countries, although the mechanisms of

reducing viral acquisition by removal of the foreskin are uncertain.

Introduction

Male circumcision is one of the oldest cultural rituals and dates back to 2300 BC in ancient Egypt and is also known to have been practiced by South Sea Islanders, Australian Aborigines, Sumatrans, Incas, Aztecs, and Mayans. Today it is still practiced by Jews, Muslims, and ethnic groups in east and southern Africa, where in the latter it is termed traditional male circumcision. At least three types of ritualistic circumcision procedures have been identified: (1) the complete removal of prepuce tissue to expose the whole glans of a flaccid penis (the most common), (2) snipping the frenum and leaving the foreskin (FS) being left intact, and (3) partial removal of the FS and cutting the remnant remaining as lateral flaps of loose skin. Traditional male circumcision in South Africa is practiced in males between the ages of 17 and 22 years, and 10–27% of men have partial circumcision with some or all of the FS remaining. Importantly, in the context of high rates of HIV transmission, partially circumcised men have a 7% greater risk of being HIV infected than fully circumcised men and an equal risk compared to uncircumcised men. The risk of becoming HIV infected increases if circumcision is delayed until males are older. A seminal epidemiological study that established the relation between lack of male circumcision and HIV susceptibility found that 43% of uncircumcised men in Kenya who acquired a genital ulcer also seroconverted to HIV-1 after a single sexual exposure (Cameron et al. 1989). This association was further supported by the inverse association between low (<1%) HIV prevalence in countries where more than 90% of the male population practiced circumcision versus higher prevalence (>15%) where only a quarter of the male population practice circumcision (Bongaarts et al. 1989). These studies led to the implementation of three randomized controlled trials showing that surgical complete removal of the FS by MMC provided up to 60% protection against HIV

acquisition (Gray et al. 2007; Bailey et al. 2007; Auvert et al. 2005) and an uncircumcised man had a 1.8- to 8.2-fold increased chance of acquiring HIV compared to a circumcised man. While there is very strong evidence that MMC shows preventative efficacy, there still exist communities in the world in which the practice is unacceptable due to cultural or social reasons. However, the prevailing thoughts are that acceptability of male circumcision in areas of HIV prevalence is critical for a reduction of HIV transmission.

The Role of the Foreskin

The foreskin is predominantly responsible for protecting the underlying glans epithelium and acts as a chemical, physical, and immune barrier against foreign assault by microorganisms and environmental damage. Its architectural structure varies depending on localization ranging from the single columnar epithelia of the urethra to the stratified squamous epithelium of the outer foreskin. The barrier function of the skin is maintained on three levels:

- (1) Physical barrier. The outermost skin layer, the stratum corneum, composed of anucleated, protein, and lipid-rich terminally differentiated keratinocytes is the most involved skin layer in maintaining the physical barrier.
- (2) Biochemical/chemical barrier. Antimicrobial peptides and amino acids such as the defensins form a chemical response to microbes. Melanin produced by melanocytes prevents UV-associated DNA cell damage.

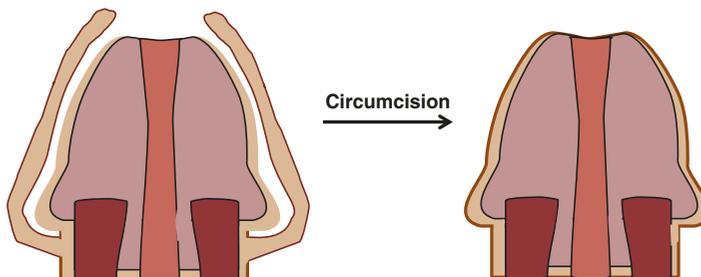
- (3) Immune barrier. Disruption of the physical barrier of skin induces immune responses, such as T-cell activation and increased numbers of Langerhans' cell (LC) density and proliferation (Ganor et al. 2010).

The foreskin physical barrier is maintained by various proteins. Intracellular proteins such as the intermediate filaments, keratin and filaggrin, have been shown to be integral to epithelial barrier function. Tight junctions such as the desmosomes and their proteins such as the cadherins also maintain barrier function by keeping the epidermal layer intact.

MMC involves the removal of the inner foreskin that is in direct contact with uncircumcised glans penis and the attached outer foreskin. Therefore, anatomically, it removes the inner foreskin and some of the outer foreskin resulting in the exposure of the head of the glans penis, as shown in Fig. 1.

Evidence of Potential Mechanisms of MMC

The foreskin has a higher density of HIV target cells and may be susceptible to epithelial disruptions during sexual intercourse, providing additional portals of entry for HIV-1 (Weiss et al. 2006). The removal of the foreskin would therefore reduce the number of target cells for HIV (McCoombe and Short 2006; Fischetti et al. 2009). There is yet no definitive evidence for the mechanism of MMC, but removal of LCs and CD4+ T cells, along with epidermal dendritic



Circumcision and AIDS, Fig. 1 Circumcision involves the removal of the inner foreskin that is in direct contact with uncircumcised glans penis and the attached outer foreskin (Courtesy of Rachel Esra, Division of Immunology, UCT)

cells that are known to be rich in the FS epithelial tissue, would lower the potential for cellular viral infection. While HIV transmission has been extensively studied in the FGT, very little is known about the actual HIV acquisition in males. Keratin is considered by some investigators to form an impermeable barrier to HIV, and as a result heavily keratinized sites such as the glans penis are protected against invasion by the virus. Conversely, the poorly keratinized inner foreskin and the penile urethra which are lined by non-keratinized squamous epithelium are thought to be the sites of HIV invasion during penile-vaginal or penile-anal intercourse (Hladik and McElrath 2008). However, immunohistochemical studies on the differences of the inner and outer foreskin with regard to keratin thickness have been inconclusive. Keratin thickness (stratum corneum layer of the skin) of the inner foreskin has been reported to be significantly thinner, thicker, and the same in different studies. Thus, the precise mechanism of MMC remains unclear, and further studies are required to pin-point how the surgical removal of this epithelial flap can profoundly influence the manner by which HIV is acquired.

Benefits of MMC

MMC has also been shown to reduce STI acquisition. The randomized circumcision trials in Uganda and Kenya were associated with the decreased frequency of genital ulceration (Mehta et al. 2012; Gray et al. 2007). Prior to these circumcision trials, other observational studies showed that male circumcision may play a role in the decrease of infection with high-risk human papillomavirus (HPV) and 35% reduction of HPV incidence in general. Additionally, MMC decreases the chance of herpes simplex virus-2 (HSV-2) infection by 25%. Male circumcision was also evaluated on the risk of bacterial STI acquisition among men. A South African trial found that male circumcision decreased *Chlamydia trachomatis* (CT) but had no impact on *Neisseria gonorrhoeae* (NG) (Auvert et al. 2009) and a Kenyan trial found no protective effect for *Neisseria gonorrhoeae*, CT, or *Trichomonas*

vaginalis (TV) but only found a protective effect for *Mycoplasma genitalium*. Although male circumcision has been shown to reduce the prevalence of STIs in heterosexual contact, the effects among men who have sex with men (MSM) is not clear. MMC may also confer protection for female partners. Two studies have shown that MMC reduces the risk of cervical cancer in females, while only one study has shown no protective effect. Furthermore, circumcision has also been shown to decrease the risk of HPV infection, bacterial vaginosis (BV), and TV in female partners.

Although MMC can reduce the risk of HIV infection by 60%, and has motivated implementation of voluntary circumcision in multiple countries, there remains approximately 40% of circumcised men vulnerable to viral acquisition after engaging in risky sexual practices. This raises the likelihood that HIV acquisition in the male genital tract (MGT) can also occur independently of the FS and is likely through the epithelium of glans and the urethral opening in the penis. These possibilities are at the cutting edge of our current knowledge, and it will be important to understand immunological events in the MGT so that alternatives to MMC can be developed.

Penile Immunity

The first line of defense in the MGT is the epithelia barrier reinforced with the chemical barrier as discussed above. Pathogens that are exposed to the host through the urethra/foreskin will be recognized by pattern recognition receptors on keratinocytes which are the most abundant cell type along with other specialized immune cells. These PRRs include the Toll-like receptors TLRs and the nucleotide oligomerization domain (NOD)-like receptors (NLRs) which sense what pathogen is present in order to elicit an immune response (Nguyen et al. 2014). TLRs were found to be widely distributed in the male genital tract with higher expression in the urethra (Anderson et al. 2011) and correlates with the finding that sexually transmitted infections affect the urethra. The other components of the innate immune

system involved in penile immunity are the natural killer (NK) cells and have the same function of killing virally infected cells as class I HLA-restricted cytotoxic T cells. HIV susceptible cells have been found dispersed within the genital tract and are a natural phenomena associated with cellular immunity in the MGT which HIV appears to exploit for successful infection. Macrophages are the predominant immune cell type in the male genital tract (Nguyen et al. 2014), and dendritic and Langerhans cells have been identified as the most abundant HIV target cells in stratified squamous epithelia and to efficiently transmit HIV to T cells (Ganor et al. 2010). These cells are CD1A⁺ DC-SIGN⁻ and are abundant in the FS. LCs are thought to play a dual role in HIV infection depending on their activation state. These cells are thought to act as carriers of the virus to establish systemic infection during specific states of activation, and once in the mature state, they are capable of degrading virus particles in their Birbeck granules (de Witte et al. 2007). While LCs are distributed widely in the penis, the other HIV target cell macrophages and $\alpha\text{E}\beta 7^+\text{CD}4^+$ T cells are preferentially resident in the urethra including CCR5⁺ and CXCR4⁺ cells on the urethral opening.

Penile Microbiome and Immunity Interplay

In addition to anatomical and immunological changes, MMC leads to microbiological changes in the penis, which are believed to be one of the mechanisms by which MMC reduces the risk of HIV acquisition by circumcised men. Furthermore, these microbial changes confer STI and BV protection to the female partners of circumcised men (Gray et al. 2007; Bailey et al. 2007; Avert et al. 2005), thus, in turn, reducing the susceptibility of female partners to HIV infection. Prior to circumcision, the anoxic microenvironment of the inner foreskin may support the survival of anaerobic genital microbes. For example, the most common coronal sulcus microbes pre-MMC are *Prevotellaceae*, *Veillonellaceae*, *Clostridiales*, *Actinomycetaceae*,

and *Porphyromonadaceae*, the most abundant being *Prevotella* spp. (Liu et al. 2013). Anaerobic microbes are associated with increased inflammation, and penile inflammation may lead to recruitment of HIV target cells to various regions of the penis, thus increasing the risk of HIV acquisition. During inflammation, cytokines and chemokines are responsible for the migration of T cells from the lower vascularized dermis to the epithelium of the inner foreskin. Furthermore, a higher density of CD4⁺ T cells and Langerhans' cells (LCs) has been reported in the inner foreskin compared to the outer foreskin, particularly in the presence of inflammation, thus, making the inner foreskin a potent site of HIV acquisition.

MMC has been shown to decrease the prevalence and abundance of anaerobic bacteria at penile mucosal surfaces and thereby remove the inflammation potential. In addition, bacterial biodiversity and heterogeneity decrease after MMC, while aerobic and skin bacterial taxa increase (Nelson et al. 2012). The reduction in anaerobic bacteria may lead to decreased inflammation and thus decreased HIV target cell recruitment. Apart from physical removal of the vulnerable inner foreskin, this is one of the proposed mechanisms by which MMC reduces the risk of HIV acquisition (Nelson et al. 2012).

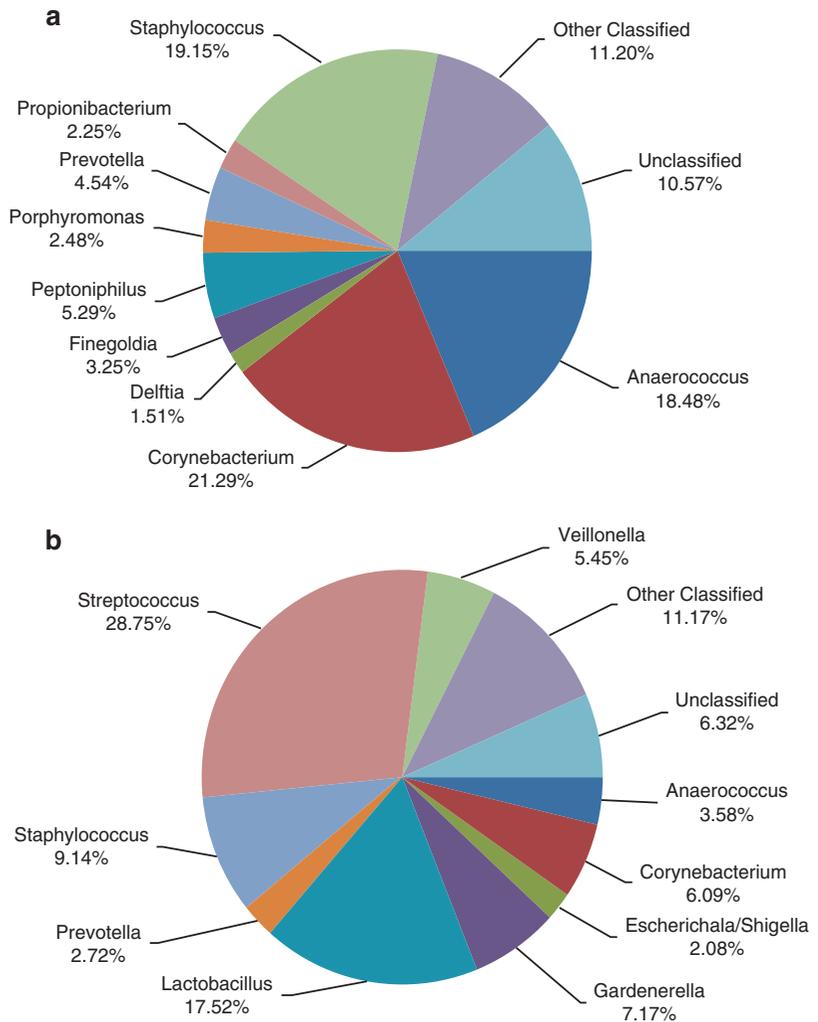
Urethral Microbiome

As discussed, HIV infections still occur in a proportion of circumcised men, suggesting that the foreskin proximal to the glans is not the only important penile HIV entry point. This has led to the exploration of the possibility of the penile urethra being another important HIV entry point in both circumcised and uncircumcised men. Similar to the foreskin microbiome, the urethral microbiome may also be a key determinant of the risk of HIV acquisition through exposure of the urethral orifice to the inner foreskin microbiome.

The penis does not support a single characteristic microbiota; it is therefore important to note that different regions of the penis exhibit slightly different microbiota (Nelson et al. 2012), as

Circumcision and AIDS,

Fig. 2 Distribution of major taxa in the coronal sulcus (a) and urine/urethra (b) (Nelson et al. 2012)



shown in Fig. 2. Therefore, in light of the relationship between the microbiome, inflammation, and the risk of HIV acquisition, different regions of the penile anatomy could be impacted differently subsequent to MMC. The most abundant taxa in penile urethra are rare or absent in the coronal sulcus microbiome. For example, *Veillonella*, *Aerococcus*, *Ureaplasma*, *Gardnerella*, and *Mycoplasma* have been reported to be found only, or almost exclusively, in the penile urethra prior to circumcision, Fig. 2a. There are however some similarities between the coronal sulcus and penile urethra microbiota. BV-associated taxa including *Mycoplasma*, *Ureaplasma*, and *Sneathia* were detected only in

sexually experienced participants. Therefore, similar to the coronal sulcus microbiome, the composition of the penile urethral microbiome is also influenced by partnered sexual behavior. In addition, the urethral microbiome is also comprised mostly of obligate/facultative anaerobes, e.g., *Streptococcus*, *Lactobacillus*, *Gardnerella*, and *Veillonella*, Fig. 2a. This may partly be due to the fact that in most uncircumcised men, the foreskin covers the urethral orifice of the non-erect penis, thus exposing the urethral orifice to the anoxic microenvironment of the inner foreskin which has been shown to support the survival of anaerobic microbes. Similar to what occurs in the coronal sulcus, these anaerobes possibly enhance

HIV acquisition at the urethral orifice via increased inflammation and HIV target cell recruitment. The urethra may therefore be a very fertile site leading to HIV infection upon viral exposure. Furthermore, the risk of acquiring HIV via urethral opening may differ between circumcised and uncircumcised men. In uncircumcised men, the microbiome around the urethra and under the foreskin may modulate the risk of HIV acquisition through the urethra. However, in circumcised men, the lower diversity of anaerobic microflora likely also impacts on the risk of HIV acquisition, and the urethral orifice may be the primary site of infection (Anderson et al. 2011).

Limitations of Current Knowledge and Research

MMC as a primary HIV prevention strategy may not be feasible on a worldwide scale due to cultural, social belief/acceptability, logistical, and financial barriers. It is therefore important to know and build on the current knowledge of the mechanisms by which MMC reduces the risk of HIV infection as this may lead to the development of novel, nonsurgical HIV prevention strategies (Price et al. 2010). Limitations in current knowledge/research of MMC however impede novel HIV prevention strategy development. It has been reported that although the reductions in the prevalence of anaerobic bacteria are often substantial, MMC does not significantly reduce all anaerobes showing no significant decrease after MMC (Liu et al. 2013). It is however not known why these anaerobes persist after MMC, and the magnitude of the contribution these anaerobes make toward the HIV incidence observed in men who have undergone MMC is also not known. This knowledge may lead to the development of novel ways of removing these anaerobes from penile mucosa in order to further reduce the risk of HIV acquisition among men who have undergone MMC.

As already discussed, the urethra may be a fertile HIV entry point in both circumcised and uncircumcised men. However, there is limited knowledge on the impact of MMC on the

reduction of HIV acquisition via the urethra. Although the urethral microbiome has been determined, an understanding of the impact (or lack thereof) of MMC on the urethral microbiome and inflammatory profile in the context of HIV infection may provide insight for the observed 40% of circumcised men who remain at risk of infection. Here, novel ways of altering the urethral microbiome and inflammatory profile may then need to be developed in order to reduce the risk of HIV acquisition in these remaining “unprotected” men.

Current MMC research is mainly based on identification of factors that modulate the risk of HIV acquisition from vaginal sexual intercourse. However, a substantial number of men engage in anal sex with women and/or other men. Knowledge on the utility of MMC in reducing the risk of HIV acquisition during anal sex is limited. It is not known whether or not the mechanisms of protection conferred by MMC are as effective in reducing the risk of HIV acquisition during anal sex as they are during vaginal sex. HIV viral shedding in the rectum is higher than that in the blood, semen, and in vaginal and cervical secretions. Circumcision may reduce HIV target cells in the penile mucosa of circumcised men, but if rectal mucosal secretions contain higher HIV concentrations than vaginal secretions, any potential protective effect of circumcision for the insertive partner may be overwhelmed by excess virus. Therefore, the risk of acquiring HIV from anal sex may remain high regardless of circumcision. There is therefore a need to study the effectiveness of MMC in reducing the risk of HIV infection specifically in men who engage in anal sex (and are the insertive partners in the case of men who have sex with men).

Conclusion

Evidence for the effectiveness of MMC in reducing the risk of HIV needs to continuously be expanded for all groups of sexually active men to further substantiate the recommendation of MMC as an effective HIV prevention strategy. Identity of changes in the urethra after MMC can provide insight into HIV acquisition in

circumcised men, but also allow the potential development of alternative preventive tools that lowers the inflammatory profile of penile epithelial tissue that may not have to rely on an ancient surgical intervention. Understanding these immune events and how HIV is acquired in males is key to this.

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Clinical Ethics in HIV/AIDS Prevention, Care, and Research

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Definition

Ethics refers to standards of right and wrong behavior, as well as to the systematic study of the norms, values, and principles that underlie those standards. *Morals* and *ethics* are often used as synonyms, although *ethics* typically refers to a set of social or professional norms while *morals* or *morality* are more personal. Ethics and law are also closely related; law is the codification of ethical standards and legal requirements often provide a practical framework for ethical decision-making. In contemporary health care, ethics is multidisciplinary, drawing on the professional standards and experiences of medicine,

public health, nursing, the behavioral sciences, social work, chaplaincy, health education, and law, as well as ethical theories of philosophy and theology. The ethics of clinical care and biomedical research are typically grounded in the principles of (1) *beneficence*, acting in the best interest of one's patient or research participant, and its corollary, *nonmaleficence*, the duty to avoid doing harm; (2) *respect for persons*, including both the right of *autonomous* individuals to make their own decisions and the duty to protect *vulnerable* individuals from exploitation and related harms; and (3) *justice* or *fairness*, the duty to treat similar cases similarly and distribute resources, benefits, and burdens equitably (Beauchamp and Childress 2012).

Introduction

The surprising emergence of HIV/AIDS in the early 1980s came at a time when many physicians believed that medical science had all but conquered the threat of infectious disease. Its appearance in the United States and Europe coincided with marked social changes, especially in sexual mores and drug use; in developing countries the pandemic followed the social and economic disruption that accompanied decolonization. These forces both fostered the spread of the virus among already marginalized populations and stigmatized those affected by it (Harden 2012).

HIV/AIDS also challenged the young field of bioethics and tested the limits of established ethical norms in health-care and biomedical research (Pinching et al. 2000). Many of the ethical issues and related standards in HIV/AIDS care, prevention, and research have differed between industrialized and low-income nations and have evolved markedly with the availability of antiretroviral (ARV) medication (Heitman and Ross 2002). Even in its current contexts, however, HIV is characterized by unique biological, social, and geographic factors that shape the experience of individuals and communities affected by the disease and who participate in its prevention, care, and/or research. After over three decades, there

are still unsettled ethical questions about the treatment, research, and prevention of HIV/AIDS for which policy is uneven and the law is silent.

Clinical Care

Key ethical issues in clinical care for HIV/AIDS include confidentiality and disclosure of HIV status, access to health services and disparities in care, and informed consent to testing and treatment.

Confidentiality, Disclosure, and Stigma

Since the initial identification of HIV infection as "gay-related immunodeficiency (GRID)" (Harden 2012), HIV has carried powerful stigma and confidentiality has been a fundamental ethical concern in its diagnosis and treatment. Physicians have known since Hippocratic times that unless patients trust their caregiver's promise of confidentiality, they will not disclose potentially shameful information that might be essential to their diagnosis and treatment. The stigma associated with the sexual practices and illicit drug use through which HIV is commonly transmitted highlighted this ethical issue early in the pandemic (Pinching et al. 2000). Clinicians worldwide recognized an essential link between maintaining confidentiality and controlling the spread of infection: at-risk individuals were unlikely to seek diagnostic testing or counseling on preventing transmission if they feared disclosure of their infection or personal behaviors (Bayer 2003; Brewster 2011). Because confidentiality was particularly central to the effective delivery of HIV services before ARV treatment became available, early AIDS-related law in the United States prohibited caregivers from disclosing a patient's HIV status without his or her consent.

However, certain conditions have always limited health-care providers' ethical obligation to maintain their patients' confidentiality, particularly the professional duty to warn identifiable others of known danger. HIV infection created ethical tension between caregivers' commitment to their patients' privacy and the duty to warn, particularly in regard to the public health practice

known as *contact tracing*. Since the early 1900s, public health officials have attempted to control and prevent certain contagious diseases, particularly sexually transmitted infections (STIs), through structured systems of testing, reporting, identifying, warning, and treating those exposed (Bayer 2003). Such programs require physicians to report the name of an infected patient to their local health department; health department officials then communicate with the patient to identify everyone he or she may have exposed; they then locate and offer testing and treatment to each contact, repeating the process until no unidentified/unwarned/untreated contacts remain.

Prior to the availability of effective treatment for HIV, health authorities encouraged at-risk individuals to be tested and to inform their partners if they tested positive. Although many who tested positive did not reveal their status, medical and public health professionals argued that mandatory reporting and contact tracing were not warranted because, without real options for treatment, it was not ethical to sacrifice known patients' confidentiality to alert unknown others to take preventive action (Bayer 2003). In this context, however, many health professionals recognized a professional duty to warn their HIV-positive patients' *known* sexual partners, especially spouses, and many states enacted laws permitting such disclosure even without the patient's consent. By the late 1990s, the development of ARV treatment changed this policy, and US public health officials instituted mandatory reporting of persons testing positive for HIV so that their contacts could be tested and treated as quickly as possible (Pinching et al. 2000; Bayer 2003). Today, HIV is one of several infectious diseases, along with tuberculosis (TB) and syphilis, for which most states maintain such a reporting and tracing system.

Globally, guidelines from the World Health Organization (WHO) and the United Nations Programme on HIV/AIDS (UNAIDS) recommend that public health legislation authorize, but not require, health-care professionals to notify their patient's sexual partners of the patient's HIV

infection (UNAIDS 2006). Where the person testing positive does not consent to partner notification, UNAIDS stipulates the following conditions for involuntary disclosure:

- (a) The HIV-positive individual has been counseled about the risks of transmission.
- (b) Counseling has not achieved behavioral changes.
- (c) The HIV-positive person refuses to notify or permit notification of his/her partner(s).
- (d) There is a real risk of HIV transmission to his/her partner(s).
- (e) The HIV-positive person is given reasonable advance notice of the planned notification.
- (f) The HIV-positive person is not identified to his/her partner(s), if this is possible in practice.
- (g) The health-care provider ensures follow-up and support of the patient.

Another exception to the general rule of confidentiality in the United States concerns HIV-infected health-care workers. In response to reports of possible transmission of HIV from caregivers to their patients, the CDC published guidance in 1991 on preventing transmission of HIV from health-care workers to their patients. States were required either to adopt this recommendation or develop their own policies. In general, state laws reflect that the risk of HIV transmission is low when health-care workers follow universal precautions and that the mandatory testing of caregivers would be stigmatizing with no medical benefit. However, most policies have recommended that HIV-infected health-care workers who perform exposure-prone, invasive procedures have their circumstances reviewed by an expert panel and that they inform patients of their serologic status before undertaking exposure-prone procedures (Center for HIV Law and Policy 2008). In 2011, the American Medical Association (AMA) released parallel recommendations emphasizing that "when the scientific basis for patient protection policy decisions are unclear, HIV-infected physicians or other health care workers must err on the side of protecting patients" (AMA 2011, p. 18).

Access to Medical Care

When AIDS was first identified as a form of acquired immunodeficiency, effective treatment was unknown and the diagnosis was almost always fatal. Patients with health insurance or other financial means had some access to medical care for symptom management and sometimes to clinical trials that offered experimental interventions. But even well-insured patients soon exhausted their coverage. Moreover, insurance companies often sought to limit their liability by requiring HIV testing for new applicants and refusing to provide health and life insurance coverage for those who tested positive. Poor and uninsured patients, who often learned of their diagnosis only when they became gravely ill, typically received care from residents in public teaching hospitals, as many senior physicians and those in the private sector questioned their legal and ethical obligation to treat people with HIV/AIDS (Harden 2012). AIDS activists, particularly in gay communities across the United States and Europe, advocated for increased access to care, increased research funding targeting AIDS, and improved education about its causes and prevention (Pinching et al. 2000). Many also organized their own information clearing houses and sources of alternative treatments.

The first drug approved by the US Food and Drug Administration for treatment of HIV, zidovudine (AZT), became available in 1987. At a cost of \$8,000 per year, many people simply could not afford treatment. Soon thereafter, the federal Health Resources Services Administration (HRSA) started the AZT Drug Reimbursement Program, which provided grants to the states to cover drug treatment for those without the means to pay for it themselves (Harden 2012). In 1990, Congress passed the Ryan White Comprehensive AIDS Resources Emergency (CARE) Act, which established funding for an array of services for people with HIV/AIDS (Harden 2012). Over the past 25 years, as improved drug treatment for HIV infection dramatically increased survival, programs funded through the Ryan White Act grew to provide necessary medication, primary care, mental health care, addiction treatment, case management, and related ancillary services. The

Affordable Care Act has also improved access to HIV/AIDS care by prohibiting lifetime caps on health insurance payments and denial of coverage for preexisting conditions. However, expansion of AIDS care services, successful treatment, and high survival rates have come at significant financial cost, as federal spending on AIDS care and research reached over \$24 billion in 2015 (AIDS.gov 2015).

From the outset, HIV has disproportionately affected marginalized populations in the United States. Members of historically disenfranchised groups, including men who have sex with men, sex workers, IV drug users, and members of racial and ethnic minority groups, continue to be at higher risk for HIV infection than the general population. Even with publically funded prevention, testing, and treatment programs in place, stigma, low health literacy, homelessness, incarceration, and instability related to drug abuse are significant barriers to access. And despite efforts to improve equity in access and health outcomes, racial and ethnic disparities remain significant: African Americans account for almost half of new infections each year, and over 60% of Americans living with HIV are African Americans or Hispanics (AIDS.gov 2015).

Internationally, access to treatment for AIDS has also posed significant ethical challenges. In the countries most affected by HIV in the first two decades of the pandemic, the lack of effective treatment for HIV was exacerbated by the lack of even basic medical care, particularly in Africa, Central and South Asia, and the Caribbean. Far more than in the United States or Europe, in developing countries, the cost of AZT and more advanced drugs kept treatment out of reach of most patients.

In 2003, President George W. Bush announced the President's Emergency Plan for AIDS Relief (PEPFAR), through which the United States would commit \$15 billion to combat HIV/AIDS internationally (AIDS.gov 2015). PEPFAR's initial goals centered on providing testing, counseling and ARV treatment, developing and strengthening the health services infrastructure needed to provide them, and developing systems for care and support of children orphaned after their parents' deaths from AIDS. PEPFAR's

specific focus on treating of pregnant women had the important benefit of preventing maternal-fetal transmission. Together with the Global Fund to Fight AIDS, Tuberculosis, and Malaria, PEPFAR has dramatically changed the landscape of HIV care and treatment in those countries most affected by the disease. There is still significant unmet need for services, however, especially for AIDS-related mental health services, family planning, and palliative care.

Informed Consent in Clinical Care

Informed consent is a cornerstone of ethical health care, even in an epidemic. Successful informed consent practices promote trust between health professionals and patients, enhance patients' understanding of treatment regimens, and improve outcomes by fostering their adherence. Basic informed consent requires the caregiver to tell the patient about the nature, expected benefits, risks of harm, and alternatives to a recommended intervention so that the patient has a sound basis on which to accept or refuse it. In the United States, the standard for informed consent is the disclosure and discussion of information that a "reasonable person" would want to know or find useful in making specific treatment choices.

Early ethical concerns around informed consent in AIDS care focused on the need for formal consent to HIV testing and screening. Because of the stigma associated with a diagnosis of HIV/AIDS, even an offer of testing carried the implication of immoral sexual activity or drug use, and for many years patients were typically asked to sign a specific consent form before HIV testing was conducted. However, practices changed after the introduction of ARV medication, especially once AZT was found to prevent maternal-fetal transmission of HIV. Many states soon required pregnant women to be offered HIV testing in prenatal care and institutions implemented screening programs (Heitman and Ross 2002). Documentation of informed consent to HIV testing was typically incorporated into the more general consent for diagnostic blood work across clinical contexts.

One situation in which informed consent is often not required for HIV testing is in response

to accidental sharps injuries and occupational exposures to patients' blood or potentially infectious bodily fluid. Prompt testing of both the person exposed and the source of exposure lets the caregiver and employer begin appropriate prophylaxis as soon as possible. In many states the patient may be tested for HIV without being asked for consent (US Department of Veterans Affairs 2015).

In the early years of AIDS, informed consent to treatment was complicated by patients' desperate hope that the disease was treatable and uncritical acceptance of treatment offered. However, once ARV treatment was introduced, the complex dosing regimens and common side effects made adherence difficult. Clinicians and public health practitioners worried that drug-resistant strains of HIV would develop if patients did not adhere to treatment regimens and debated how soon after diagnosis patients could truly understand the demands of treatment well enough to give meaningful consent to treatment (Heitman and Ross 2002). For high risk-populations affected by mental illness, homelessness, or substance abuse, and especially for preventing maternal-fetal transmission, many public health programs advocated for directly observed therapy (DOT), in which a case manager ensures that the patient takes prescribed medication on schedule. Development of single-dose combination therapy simplified adherence for most patients and limited the call for DOT to individuals whose difficulty providing self-care poses identifiable health risks for the larger community.

Prevention

In the early era of HIV/AIDS, when effective treatment was not available, behavioral strategies were essential to the prevention of transmission, and the ethical goals of harm reduction and countering stigma featured prominently in most AIDS prevention plans. Today, multifaceted prevention strategies include a central role for effective treatment, placing new ethical pressures on patients to adhere to their medication regimens. The promise of a vaccine remains an international research goal.

Behavioral Interventions and Harm Reduction

Early in the pandemic, recognition that HIV/AIDS is transmitted through contact with certain bodily fluids led to a number of preventive strategies focused on avoiding or limiting such contact. For example, early prevention campaigns in both the United States and abroad stressed the traditional morality of abstinence, monogamy, sobriety, and avoidance of intravenous drug use. However, public health experts soon acknowledged that effective prevention of HIV required finding ways to make stigmatized sexual practices and drug use “safe” because the prevalence of high-risk activities themselves was not easily reduced. Thus “safe-sex” programs promoted consistent use of condoms in all sexual encounters and needle decontamination and exchange practices for injection drug use on the grounds that they reduced the harms of HIV transmission, even if they appeared to promote behavior otherwise considered immoral or harmful (Harden 2012).

The goal of preventing transmission of HIV in clinical contexts similarly led to new standard behaviors in the late 1980s regarding physical contact with both infected and uninfected people. “Universal precautions” were instituted for clinical care in which all human blood and bodily fluids were treated as if they were infectious and all patients were treated as if they were HIV-positive. Not only did universal precautions improve caregivers’ overall infection control practices, the widespread use of gloves and other barrier protection in general patient care reduced their associated stigma for patients with HIV/AIDS (Harden 2012).

Treatment as Prevention (TasP)

The effectiveness of ARVs in reducing treated individuals’ viral load has given universal treatment ethical importance as a preventive strategy. Because ARVs can reduce viral load to virtually zero, the early treatment of HIV infection not only has life-saving benefits for the individual being treated but also directly reduces the risk of transmission, irrespective of other behaviors. TasP offers significant public health advantages in settings with limited resources. However, whereas traditionally the uninfected partner was the focus

of preventive efforts, TasP places a disproportionate emphasis on HIV-infected individuals to adhere to medication regimes over other behavioral forms of prevention (Haire and Kaldor 2013). Not only must the at-risk person trust in the adherence of their HIV-infected partner, an undetectable viral load may lead infected individuals to withhold information or lie about their seropositive status altogether (Heitman and Ross 2002).

Pre-exposure Prophylaxis (PrEP)

PrEP, the use of oral ARV drugs by members of high-risk groups prior to potential exposure to HIV, is another new prevention strategy that raises questions of relative benefit and harm from administering medications to uninfected individuals. In addition to the risk of side effects from the drug, as with TasP, the prophylactic use of ARVs shifts prevention strategies away from other effective and less inexpensive behavioral measures (Haire and Kaldor 2013). In low-resource settings where the supply of ARV drugs is limited, their use for prophylaxis may also make them unavailable to infected persons who will likely suffer significant harm from ineffective or non-treatment (Sugarman 2014).

Research

The fundamental ethical challenge in all clinical and public health research is determining when and how an individual may be exposed to potential harms in pursuit of generalizable knowledge of uncertain benefit to participants or future others. The AIDS pandemic not only illustrated the ethical imperative of well-designed research into the causes, prevention, and effective treatment of disease, it also forced the reassessment of ethical standards for infectious disease research, particularly in low-resource environments. Ethical consideration of HIV/AIDS research has emphasized the complexity of informed consent, the definition of standard of care for control groups, the meaning of “vulnerable population,” and the ethical tensions inherent in international collaboration.

Informed Consent in Research

Just as informed consent for treatment is intended to ensure that patients understand and freely agree to the interventions that they receive, the informed consent process in research aims to ensure that individuals understand that they are being recruited into a study, the nature of the study intervention, and its intended benefits, risks of harm, and alternatives. Early in the pandemic, the informed consent process in HIV was confounded by the lack of intervention outside of research settings and a general misunderstanding that the health professional's commitments in research are to answering the study question, not to advancing the patient's individual interests (Harden 2012). Controversy over informed consent in international AIDS drug trials in the late 1990s led to revision of the World Medical Association's Declaration of Helsinki and the Council for International Organizations of Medical Sciences' (CIOMS) International Ethical Guidelines for Biomedical Research (Macklin 2008). Both documents stress that physicians who involve their patients in research must point out this double role, identify which aspects of care are related to research, and have another person from the research team carry out the research informed consent process.

In international research, the informed consent process may also be complicated by cultural and linguistic differences and its significance may vary with local customs and norms. International standards call for the recruitment and informed consent process to be conducted with the would-be participant as an individual, in his or her own language, and for their individual consent to be documented in writing, if possible. However, in many lower-income countries, especially where there are low rates of literacy, a community leader's acceptance of a study is often essential to community members' willingness to participate. Similarly, the male head of household in some societies may be responsible for making decisions on behalf of female family members, while US and international standards of informed consent hold that each participant must consent as an individual (Macklin 2008). Nonetheless, family, community, and self are closely linked in

many societies where HIV is prevalent, and AIDS research may indirectly affect sexual partners, needle sharers, transfusion recipients, and health-care workers, from whom no one gets consent.

Research and the Standard of Care

Even with enhanced informed consent processes, AIDS research continues to present ethical tensions in low-resource settings where treatment options remain more limited than in industrialized nations. The definition of "standard of care" in international treatment and prevention research first became a prominent ethical issue in the late 1990s, in randomized controlled trials (RCT) of AZT for prevention of maternal-child transmission of HIV. RCTs are the "gold standard" in clinical research, evaluating two or more interventions presumed to provide equal benefit against each other. Promising new interventions are typically tested against the standard of care, but by definition one or more groups in an RCT will receive less beneficial or more harmful treatment than that which is ultimately shown to be the most effective.

Because the standard of care in low-resource settings where HIV/AIDS is most prevalent is generally much less effective than in industrialized settings, research there is more likely to subject participants to the risks of research with none of the benefits. There is ongoing debate among international ethicists about whether a "double standard" in the calculation of risks and benefits in research improves health care in low-income countries or perpetuates injustice (Macklin 2008). The National Institutes of Health's (NIH) ethical priorities for prevention trials call for researchers to engage multilevel stakeholders in ethical study design, including provisions to ensure that the research is responsive to the host country's needs and that potential barriers to future access to the study intervention are minimized (Garner et al. 2014).

Vaccine Research and Development

The development of an HIV vaccine has been a central goal of HIV prevention research since the inception of the pandemic. In the late 1990s,

UNAIDS sought to address the ethical tension between the urgency for vaccine development and the protection of research participants created by the high rate of HIV infection in many low-income countries with little infrastructure. Rather than imposing protections against phase I and II trials, UNAIDS recommended that countries define their own readiness to participate in early clinical trials and gain the direct experience and infrastructure needed for more complex phase III studies (Guenter et al. 2000).

To be considered successful, vaccine efficacy studies must show a difference in incidence between the intervention and control groups, meaning that there is an inherent need for new infections to occur among trial participants. In addition to this apparent conflict of interest between researchers and study participants, it is essential to determine in advance what provisions will be made for participants who become infected during the trial. Not only may the projected costs of lifelong treatment be prohibitive, offering treatment may be an unfair inducement in regions where access to treatment remains subpar (Guenter et al. 2000).

Vulnerable Populations

As evidenced in the complexity of informed consent in HIV/AIDS research, certain groups may be more subject than others to coercion or undue influence to take part in research that is not in their interests. Such vulnerability occurs typically as a result of social circumstances, health status, or other limitations on members' understanding or freedom to choose. US regulations classify children, pregnant women, and prisoners as de facto vulnerable populations whose recruitment into studies requires special attention to informed consent processes and protection from coercion. In the case of HIV/AIDS research, other recognized categories of vulnerable populations include such stigmatized groups as men who have sex with men, transgendered persons, injection drug users, and sex workers, as well as children, the homeless, the poor, and persons from resource-limited settings (Presidential Commission 2014).

In May 2001, the US National Bioethics Commission (NBAC) recommended an alternative to

the categorical definition of vulnerability, highlighting how individuals or groups might be vulnerable in multiple ways in different contexts. *Contextual vulnerability* includes six areas: cognitive, institutional, social, economic, deferential, and medical vulnerability. NBAC concluded that, when vulnerability is due to external circumstances, consideration of these contextual areas may identify potential for coercion or involuntariness better than categorical definitions. In research on HIV/AIDS, the same factors that put the potential research participants at risk for HIV also place them at risk for other vulnerabilities such as cultural exclusion, social and economic inequality, limitations to health-care access, and political oppression. NBAC cautioned that ethical review should analyze "how to identify and avoid situations that render some participants or groups vulnerable to harm or coercion" (Presidential Commission 2014, p. 6).

International Collaborative Research

Many of the countries where HIV/AIDS remains an enormous public health threat have relied upon international collaborations and expertise for the scale-up and expansion of HIV/AIDS care services and related research. Emanuel et al. (2004) recommend an 8-point framework for researchers and ethics review committees to use in determining whether international research is ethical. These principles are (1) collaborative partnerships, (2) social value, (3) scientific validity, (4) fair selection of study participants, (5) favorable risk-benefit ratios, (6) independent review, (7) informed consent, and (8) respect for study participants and their communities.

"Collaborative partnerships" are fundamental to ensure that all studies are responsive to the host country's identified needs and that exploitation is minimized. Collaborative partnerships require mutual respect, specifically related to the incorporation of cultural norms, values, and social practices within the study design. Host countries and communities should receive equal or a fair allotment of benefits from the conduct and results of the study, and the tangible and intangible rewards of each study (such as authorship, intellectual property rights, etc.) should be shared. Whenever

possible, disparities between countries should be minimized, such that the research study incorporates a component of capacity building or infrastructure development.

Conclusion

Over the 35 years since HIV/AIDS was identified as a new disease, the ethical questions raised by its treatment, prevention, and research have been as complex as its epidemiology. The development of ARV therapy for HIV has seen ethical issues raised by an untreatable and quickly fatal infection in a young population replaced by more difficult questions about stigma and skyrocketing costs of chronic care. Internationally, ethical issues raised by HIV/AIDS illustrate how social, cultural, and economic differences affect the health of different populations and emphasize the importance of international collaboration in efforts to reduce and ultimately eliminate the disease worldwide.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Behavioral Aspects of HIV Treatment as Prevention](#)
- ▶ [Children, Care and Treatment](#)
- ▶ [Children, Epidemiology of HIV/AIDS](#)
- ▶ [Female, Male and Transgender Sex Workers, Epidemiology of HIV/AIDS](#)
- ▶ [Health Care Workers, Epidemiology of HIV/AIDS](#)
- ▶ [HIV Counseling and Testing, Prevention of HIV](#)
- ▶ [HIV Testing and Counseling](#)
- ▶ [HIV-1 Prevention Using Live-attenuated Vaccines](#)
- ▶ [Prevention Clinical Trials: Highlights of Evidence and Research](#)
- ▶ [Prevention for People Living with HIV](#)
- ▶ [Women, Epidemiology of HIV/AIDS](#)

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Cofilin, Trafficking

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Definition

Cofilin is an actin-binding protein, one of the members of the ADF/cofilin family of proteins ubiquitously present among eukaryotes. ADF/cofilin proteins bind and depolymerize filamentous F-actin in a pH-dependent manner and are responsible for the high turnover rates of actin filaments. Currently, there are three highly conserved proteins in the ADF/cofilin family: ADF (actin depolymerizing factor) or destrin, cofilin 1 (non-muscle cofilin or cofilin), and cofilin 2 (muscle cofilin). In cells, cofilin and the Arp2/3 complex work together to regulate actin treadmilling, a process in which G-actin is preferentially incorporated into the filaments at the (+) end and then dissociated from the (−) end. The actin treadmilling process has been suggested to facilitate HIV trafficking across the cortical actin layer.

Introduction

The first member of the ADF/cofilin family proteins was identified in extracts of embryonic chick brain. Cofilin was later purified from porcine brain and named cofilamentous structures with actin, or cofilin for short. Cloned human cofilin has 166 amino acids and a deduced molecular weight of 18 kD (Bamburg 1999). Structurally, human cofilin possesses a core

consisting of a five-stranded mixed β -sheet ($\beta 1$ – $\beta 5$). The first four strands ($\beta 1$ – $\beta 4$) are antiparallel, and $\beta 5$ runs parallel to $\beta 4$ and antiparallel to $\beta 3$. In addition, the residues 159–161 at the C-terminus form a short strand, $\beta 6$. Five helices ($\alpha 1$ – $\alpha 5$) surround the central β -sheet, and a salt bridge is formed between His¹³³ and Asp⁹⁸ that may influence the pH sensitivity of cofilin in actin binding and depolymerization. Cofilin binds to G-actin, and the binding site is located in a region centered around Tyr¹¹⁷, which includes several residues in $\alpha 4$ (such as Lys¹¹², Lys¹¹⁴, Met¹¹⁵, and Ile¹²⁴) and $\beta 5$. In addition, a few other residues such as Ile¹², Pro¹¹⁰, and Leu¹²⁸ are also suggested in G-actin binding. Cofilin also binds to F-actin, and this requires two sites on cofilin, a G site that interacts with actin subdomains 1 and 3 and an F site that interacts with actin subdomains 1 and 2. Residues important for F-actin binding on cofilin have been identified through mutagenesis and include several residues in $\beta 4$ (such as Lys⁹⁶ and Asp⁹⁸) and $\alpha 5$ (such as Glu¹⁵¹, Lys¹⁵², and Gly¹⁵⁵). Other regions including $\beta 3$ and $\beta 5$ may additionally be involved in F-actin binding (Pope et al. 2004).

Cofilin depolymerizes actin filaments through two mechanisms: direct severing and increasing the off-rate of actin subunits from the (−) end. Cofilin has a higher affinity for ADP-actin than for ATP-actin, and cofilin binding to actin filaments facilitates phosphate dissociation from ATP-actin. Hydrolysis of ATP increases cofilin-binding affinity, changing the twist of the actin helix and severing actin filaments to shorter segments. These severed actin filaments can increase the speed of actin polymerization by providing more free (+) ends for nucleation by the Arp2/3 complex (Pollard and Borisy 2003).

The binding of cofilin to ADP-actin present at the (−) end also promotes dissociation of cofilin-ADP-actin. Cofilin in the released ADP-actin complex is competitively replaced by profilin, which then converts ADP-actin monomers to ATP-actin monomers to recycle them for new actin polymerization at the (+) end. Thus, it is suggested that cofilin and the Arp2/3 complex work together to regulate actin treadmilling, in which ATP-actin is preferentially incorporated into the filaments at the (+) end and then hydrolyzed into “older”

ADP-actin and dissociated from the (–) end (Carlier et al. 1997).

In cells, the activity of cofilin is mainly regulated by phosphorylation of serine 3 at the N-terminus, which inhibits cofilin binding to G-actin and F-actin. The kinases responsible for cofilin serine 3 phosphorylation are the LIM domain kinases (LIMK) and Tes kinases, which are targets of the Rho family GTPases such as Rho, Rac, and Cdc42. Rho family GTPases activate PAK1, PAK2, PAK4, ROCK, or MRCK α (myotonic dystrophy kinase-related Cdc42-binding protein kinase) which then activate LIMK through direct phosphorylation. Cofilin is activated through dephosphorylation of serine 3 by phosphatases such as PP1, PP2A, slingshot IL (SSH1L), and chronophin, which couple cofilin activity to different Signaling pathways. Recent studies have suggested that tyrosine 68 (Y68) can also be phosphorylated, and this phosphorylation appears to increase cofilin ubiquitination and proteasome degradation. In chemotactic cells, cycles of cofilin phosphorylation and dephosphorylation are required to sustain actin dynamics essential for driving directional cell migration (Bernstein and Bamburg 2010).

The ability of cofilin to modulate actin polymerization or depolymerization may depend on the local concentration of cofilin. Low concentrations of cofilin may favor severing, whereas high concentrations may favor actin nucleation. Nevertheless, it is unknown how this concentration-dependent mode of action demonstrated *in vitro* plays out in cells (Bernstein and Bamburg 2010). In general, decreasing cofilin expression through siRNA increases the amounts of cellular actin filaments, whereas overexpression of cofilin induces the formation of cofilin-actin bundles (Yoder et al. 2008). *In vitro*, cofilin also competitively interacts with the Arp2/3 complex by inducing structural changes in the actin filaments; these changes reduce the affinity for Arp2/3 and cause a loss of actin filament branches. This debranching process may play a role in modulating Arp2/3-induced actin branch growth in the leading edge of migrating cells (Chan et al. 2009).

In addition to regulating actin dynamics in cells, cofilin has also been shown to mediate actin nuclear

localization, which may be involved in the regulation of gene expression. Actin and actin-related proteins such as Arp7, Arp9, and Baf53 are parts of the chromatin-remodeling complex RSC and SWI/SNF (Cairns et al. 1998). Actin is also part of the pre-initiation complexes and is necessary for transcription by RNA polymerase II. Cofilin-mediated actin nuclear localization may serve to connect the cytoskeletal processes to chromatin remodeling and the initiation of transcription.

Inhibition of cofilin expression through siRNA knockdown in human T cells triggers apoptosis (Yoder et al. 2008). Cofilin is found to be translocated to mitochondria in apoptotic cells. Cofilin mitochondrial translocation seems to be necessary for the opening of the permeability transition pore and the release of cytochrome *c*. The relationship between cofilin-mediated actin dynamics and cell apoptosis is not completely understood. It is suggested that reduction of actin dynamics may promote a loss of mitochondrial membrane potential and an increase in oxidative stress. In addition, actin dynamics are implicated in the movement of mitochondria and proper mitochondrial partitioning during cell division (Bernstein and Bamburg 2010).

Cofilin serine 3 phosphorylation inactivates the actin-binding ability of cofilin. However, phospho-cofilin is recently shown to activate phospholipase D1 (PLD1). Cofilin directly and specifically interacts with PLD1 upon phosphorylation by LIMK1. Phospho-cofilin also stimulates PLD1 activity, suggesting that phospho-cofilin may control a variety of cellular functions by its stimulatory effect on PLD1 (Bernstein and Bamburg 2010).

In the human immune system, cofilin plays important roles in regulating T-cell migration, chemotaxis, and T-cell activation. Within the immunological synapse, cofilin is required for the formation of the supramolecular activation clusters critical for sustaining signaling and T-cell activation. Cell-permeable peptides that block cofilin interaction with F-actin impair receptor capping and immunological synapse formation, resulting in inhibition of T-cell activation. In transformed or pre-activated T cells, cofilin activation and dephosphorylation occur spontaneously, whereas in human resting T cells, in the absence of T cell activation or chemotactic stimulation, cofilin exists

largely as the serine 3 phosphorylated form. T-cell activation or chemotactic stimulation leads to cofilin activation by dephosphorylation, and the signaling cascade is mainly transduced through costimulatory receptors such as CD2 and CD28 and through chemokine receptors such as CXCR4. While TCR/CD3 stimulation activates the Arp2/3 complex for actin polymerization, CD28-mediated costimulation triggers cofilin activation, which is required for dynamic actin reorganization and sustaining T-cell signaling (Samstag et al. 2003). The GTPase Ras and PI3K (phosphatidylinositol-3-kinase) signaling cascade is suggested to mainly regulate dephosphorylation of cofilin in unstimulated human blood T cells. Inhibition of either MAPK/ERK kinase or PI3K blocks Ras-induced and costimulation-induced cofilin dephosphorylation, whereas transient expression of a dominant negative form of H-Ras inhibits PI3K activation and cofilin dephosphorylation (Wabnitz et al. 2006).

Role of Cofilin in HIV Infection

HIV-1 entry into cells is mediated through binding to CD4 and the chemokine coreceptor, CXCR4 or CCR5. During this entry process, HIV-1 binding to CXCR4 also triggers a transient course of cofilin phosphorylation and dephosphorylation to increase actin dynamics in resting CD4 T cells (Vorster et al. 2011; Yoder et al. 2008). It is suggested that the cortical actin in resting T cells is relatively static in the absence of T-cell activation or chemotactic stimulation. This lack of actin activity limits viral early processes such as entry, DNA synthesis, and nuclear migration (Yoder et al. 2008). Cofilin increases cortical actin dynamics and actin treadmilling, facilitating viral intracellular migration toward the nucleus (Yoder et al. 2008). Cofilin and actin-mediated HIV nuclear localization is suggested to be essential for the establishment of HIV-1 latency in resting CD4 T cells (Cameron et al. 2010; Yoder et al. 2008). Knockout or severe inhibition of cofilin activity in T cells triggers apoptosis, but slight inhibition of cofilin expression through siRNA knockdown increases cortical actin

density, which leads to an increase in HIV DNA synthesis but a decrease in the amounts of HIV-1 nuclear DNA and early transcripts (Yoder et al. 2008). Induction of cofilin activity using a human cofilin-derived peptide (S3), carrying the N-terminal 16 residues, competitively inhibits cofilin phosphorylation through LIMK1, and this induction enhances HIV latent infection of resting CD4 T cells. A pharmacological drug, staurosporine, is also shown to induce gradual cofilin activation that enhances HIV latent infection of resting CD4 T cells following a transient treatment during infection (Yoder et al. 2008). In addition, pretreatment of resting CD4 T cells with chemokines such as CCL19, CXCL9, CXCL10, and CCL20 leads to cofilin activation and changes in actin filaments which greatly promote HIV nuclear migration and DNA integration (Cameron et al. 2010). Interestingly, exposing cells to mechanical shear stress, such as infecting cells under conditions of low-speed spinning or spinoculation, also promotes cofilin activity and actin dynamics, resulting in the upregulation of CXCR4 and a great enhancement of HIV-1 DNA synthesis and nuclear migration (Guo et al. 2011).

Regulation of Cofilin Activity during HIV Infection

HIV-1-mediated cofilin activation in resting CD4 T cells is shown to be through the G α i-dependent signaling from CXCR4; pertussis toxin (PTX), a bacterial toxin inhibiting G-protein-coupled receptors by the ADP-ribosylation of G α i, inhibits cofilin activation and HIV-1 latent infection of resting T cells in the presence or absence of cytokines such as IL-2 and IL-7 (Yoder et al. 2008). Cofilin can be phosphorylated by LIMK, and HIV-1 binding to blood CD4 T cells and macrophages triggers rapid activation of LIMK1, coincident with HIV-mediated early actin polymerization in T cells (Vorster et al. 2011). It is suggested that HIV-1 hijacks LIMK1/cofilin activity to directly regulate actin and CXCR4 dynamics critical for viral entry, postentry DNA synthesis, and nuclear migration. Inhibition of LIMK1 activity through siRNA knockdown

decreases filamentous actin and T-cell chemotaxis toward SDF-1. The decrease in cortical actin density also leads to an increase in CXCR4 internalization and surface recycling. Thus, LIMK-mediated early cortical actin polymerization may result in a temporary block to CXCR4 internalization, facilitating viral fusion and CXCR4 signaling. The LIMK1 knockdown cells also support lower viral entry, DNA synthesis, and nuclear migration. In addition, transient treatment of resting CD4 T cells with a pharmacological agent, okadaic acid, activates LIMK and promotes HIV latent infection of resting CD4 T cells. The signaling pathway that mediates LIMK activation by HIV-1 is identified as the Rac1, PAK1/2, and LIMK pathway in blood resting T cells. The activation is likely triggered by gp120 signaling through both CD4 and CXCR4, as well as from both G α i and G α q (Vorster et al. 2011). HIV gp120-mediated cofilin phosphorylation may also inhibit T-cell chemotaxis toward SDF-1.

In addition to HIV-1 gp120, another HIV pathogenic factor, Nef, is also suggested to regulate cofilin activity (Stolp et al. 2009). Overexpression of Nef in human Jurkat T cells inhibits SDF-1-induced membrane ruffling, actin rearrangement, and cell migration toward CXCL12, CCL3, and CCL19 (Stolp et al. 2009). Overexpression of Nef in hamster CHO cells also inhibits wounding-induced cofilin activation in a cell wound-healing assay. It has been suggested that the Nef-PAK2 complex is involved in the phosphorylation of cofilin, although Nef does not appear to alter the activity of PAK2 in an *in vitro* cofilin phosphorylation assay. Functionally, Nef-mediated cofilin dysregulation may affect migratory behavior of infected T cells (Stolp et al. 2009).

Potential Role of Cofilin in HIV Pathogenesis

A potential pathogenic role of HIV-1-mediated cofilin dysregulation is proposed from studies of cofilin activation in blood resting CD4 T cells treated with HIV or gp120 (Wu et al. 2008). It was shown that HIV-1 or gp120 stimulates cycles of cofilin phosphorylation and dephosphorylation,

suggesting that chronic exposure of CD4 T cells to HIV or gp120 may have a lasting impact on cofilin activity and T-cell functionality. A small-scale clinical study has found that in the peripheral blood of HIV-1-infected patients, levels of active cofilin in their resting CD4 T cells are significantly higher. It is suggested that HIV-1-mediated dysregulation of cofilin may lead to abnormalities in T-cell migration and activation that could contribute to viral pathogenesis (Wu et al. 2008).

Conclusion

Cofilin is a major regulator of actin dynamics in cells. Cofilin activity is normally controlled by extracellular stimuli that transduce signals from surface receptors to regulate cell motility. HIV binding to the chemokine coreceptors also induces a transient cofilin activity that promotes actin dynamics necessary for HIV intracellular trafficking. In addition, other viral proteins such as Nef may modulate cofilin activity to facilitate infection. The interaction between cofilin and components of HIV may lead to dysregulation of normal chemotactic responses in T cells.

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maintained and where pathological consequences, such as inflammation, immune activation, CD4+ T cell loss, collagen deposition, and fibrosis, ensue during HIV infection (Pantaleo et al. 1991; Schacker et al. 2002a). CD4+ T cell count is a hallmark for disease progression in HIV infection and a hallmark for immune reconstitution after initiating combination antiretroviral therapy (cART). In HIV infected individuals who have received cART treatment for many years, CD4+ T cell restoration is heterogeneous (Kelley et al. 2009). Among patients who delayed cART until their CD4 T cell count was less than 200 cells/mm³, a quarter of the population cannot restore their CD4+ T cells to a normal level even after a decade of effective therapy (Julg and Walker 2009; Kelley et al. 2009). This poor CD4 + T cell recovery after long-term cART is related to multiple factors, of which LT collagen deposition and fibrosis is an important one (Julg and Walker 2009; Schacker et al. 2002a, b, 2005; Zeng et al. 2012b). In what follows, the current understanding of LT collage deposition and fibrosis in HIV infection will be reviewed; the underlying mechanism of LT fibrosis and intervention strategies to prevent or to reverse collagen deposition and fibrosis in HIV infection will also be discussed.

Functional Microanatomy of Lymphatic Tissues and Stromal Cells

The secondary lymphatic tissues (LTs), such as lymph node (LN), tonsil, spleen, and Peyer's patches, are strategically located where immune cells encounter cognate antigens to initiate humoral and cellular adaptive immunity. Anatomically and functionally, LTs can be divided into B cell zones (resting primary B cell follicles or activated secondary B cell follicles) and T cell zones. In these zones, stromal cells-fibroblast reticular cells (FRC) in T cell zones and follicular dendritic cells (FDC) in B cell zones-form the three-dimensional network for immune cells to migrate, interact with each other, secrete chemokines to attract and retain immune cells, and produce cytokines to support immune

Collagen Deposition and Fibrosis in the Lymphatic Tissues of HIV-1 Infected Individuals

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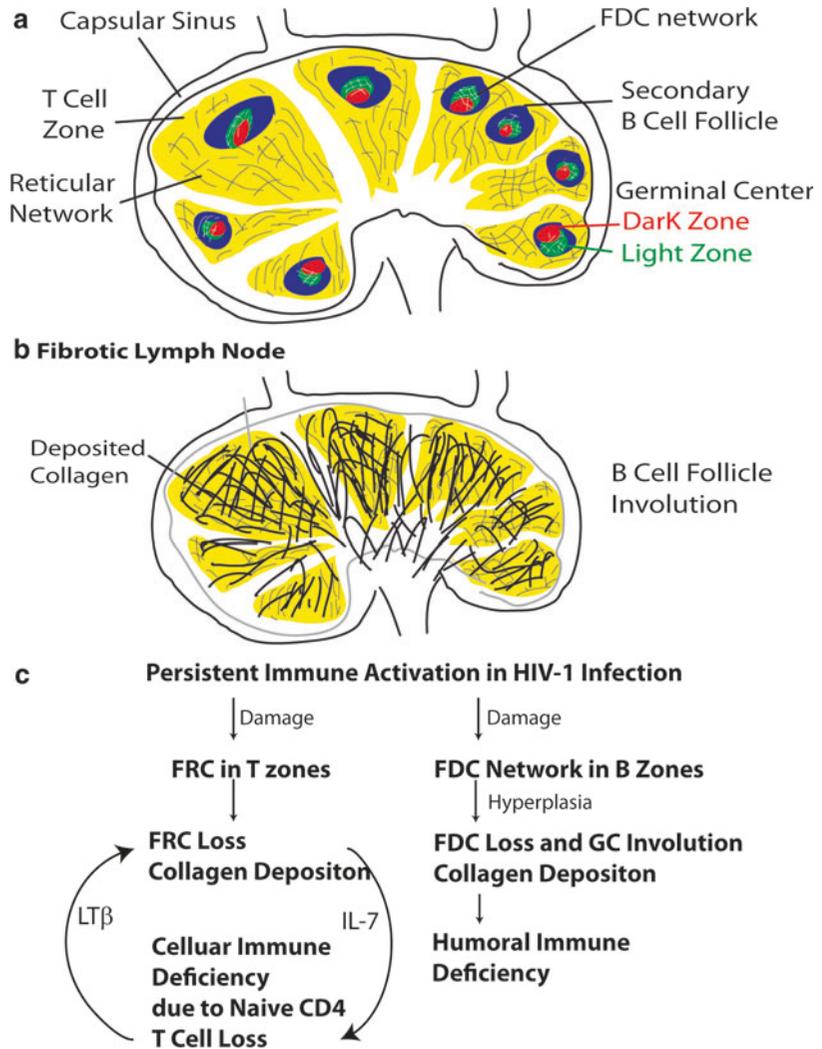
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Definition

HIV/AIDS is primarily “a disease of secondary lymphatic tissues (LTs)” because LTs are the principal sites where HIV is produced, stored, and

Collagen Deposition and Fibrosis in the Lymphatic Tissues of HIV-1 Infected Individuals,

Fig. 1 Collagen deposition and fibrosis in lymphatic tissues during HIV infection. **(a)** The schematic of a normal lymph node. Reticular network in T cell zones and the germinal centers in the secondary B cell follicles are illustrated. **(b)** The schematic of a fibrotic lymph node at the end stage of HIV infection with involuted B cell follicles. **(c)** A simplified model of the collagen deposition and fibrosis in lymphatic tissues driven by persistent immune activation of HIV-1 infection



cell survival (Fig. 1; Victora and Nussenzweig 2012).

During humoral immune response, B cells, follicular helper CD4+ T cells (Tfh), and unprocessed antigens trapped on FDC interact to form germinal centers (GC) of the secondary B cell follicles. Germinal centers can be divided into two morphologically distinct areas: dark zone (DZ) and light zone (LZ) (Fig. 1). B cells undergo clonal expansion and mutate their antibody encoding genes (somatic hypermutation) primarily in DZ, then migrate along FDC networks to the FDC- and Tfh-rich LZ, where B cells actively engage with cognate antigen trapped on FDC, present processed antigen in the form of peptide-MHC II complex to

residential Tfh cells, and are then selected for survival based on the costimulation mainly from Tfh cells (Victora and Nussenzweig 2012). This process is called the germinal center reaction, the products of which are antibody class switching and affinity maturation within B cells. Recent studies using intravital imaging revealed that the germinal center reaction is a highly dynamic and iterative process, and B cells in LT and DZ may be more similar than previously thought (Victora and Nussenzweig 2012). FDC networks exist in both the DZ and LT but are enriched in the LZ. FDC is of importance as an antigen repository to support the GC reaction and also for production of B cell homeostatic cytokines such as BAFF and



IL-7 and secretion of chemokines CXCR4 and CXCL13, which can attract CXCR5⁺ GC B cells and Tfh cells into the germinal centers (Victoria and Nussenzweig 2012).

T cell zones are in the paracortical region between follicles and extend deep into the cortex above the medulla, where the adaptive cellular immune response is initiated by encounters of rare naïve T lymphocytes with cognate antigens on antigen-presenting cells (APC) (Gretz et al. 1996, 1997; Itano and Jenkins 2003). The T cell zones are framed with the infrastructure of a reticular network (RN), a three-dimensional conduit system, of which collagen fibers form the core, which is surrounded by basement membrane extracellular matrix molecules ensheathed by a layer of nonhematopoietic fibroblast reticular cells (FRC) and occasionally dendritic cells (DC) (Gretz et al. 1996, 1997, 2000; Sixt et al. 2005). The RN supports and maintains LN overall structure and provides an organized conduit for naïve T cells and DCs to migrate, interact, and access the paracortex (Bajenoff et al. 2006; Gretz et al. 1996, 1997, 2000) and to transport chemokines and other small molecules (<70 kDa) from the afferent lymph to the T zone and lumen via high endothelial venules (HEV) (Bajenoff et al. 2006; Gretz et al. 2000; Sixt et al. 2005) while excluding high-mass molecules, and particles such as pathogens that would exploit the RT system (Mueller and Germain 2009). Furthermore, The FRC secretes collagens and basement membrane extracellular matrix molecules, and produces naïve T cell and DC chemoattractant CCL19 and CCL21 (Bajenoff et al. 2006; Link et al. 2007; Luther et al. 2000) as well as key naïve T cell survival signal IL-7 (Link et al. 2007; Schluns et al. 2000). This RN microenvironment, providing the central stage for FRC, naïve T cells, and DC trio to interplay, is critical for maintaining T cell homeostasis and in mounting effective cellular immunity.

Damage of the Secondary LTs During HIV-1 Infection

It was well documented in the 1980s that HIV-1 infection can cause histopathological changes in

both B and T cell zones of LTs (Fernandez Richard et al. 1983). There is follicular hyperplasia in acute infection and follicular involution in the end stage of infection and in between during chronic infection. Similarly, the paracortical T zone expands during the acute infection, and the paracortical T cell zone structure depletes throughout the course of prolonged infection. Subsequently, it was observed that FDC is a major reservoir for HIV-1 in LTs and HIV-1 trapped on FDC can infect CD4⁺ T cells (Heath et al. 1995). Although the mechanism of the involution of FDC networks in the later stage of HIV-1 infection remains elusive, the damage to the FDC network contributes to humoral immune dysfunction. Subsequently, it was reported that reticular fibers in LTs of HIV chronically infected individuals became thicker and sometimes collapsed in the paracortical T cell zone (Paiva et al. 1996). However, the linkage between collagen deposition, fibrosis, and CD4⁺ T cell loss in LTs during HIV-1 infection was first demonstrated by Dr. Schacker and his colleague in 2002 (Schacker et al. 2002a). They found that the LT collagen deposition in HIV-1-infected and treatment-naïve individuals were inversely correlated with LT CD4⁺ T cell counts and the extent of LT collagen deposition before initiating cART was also inversely correlated with the changes in the peripheral CD4⁺ T cell counts (Schacker et al. 2002a), indicating that collagen deposition in LTs can perturb the homeostasis of CD4⁺ T cells and impact the extent of CD4⁺ T cell reconstitution after receiving cART (Schacker et al. 2002a). The subsequent study from the same group found that the amount of LT collagen deposition before starting cART can predict the magnitude of recovery of the peripheral CD4⁺ T cells with cART (Schacker et al. 2005); the extent of LT collagen deposition in T zones in HIV-1 chronic infection was inversely correlated with LT- naïve CD4⁺ T-cell population (Schacker et al. 2006), indicating that fibrotic changes in LT have more effect on the homeostasis of naïve CD4⁺ T cells than other CD4⁺ T cell subsets (Schacker et al. 2006). Moreover, it was reported using SIV-rhesus macaque model of HIV infection that at 1 week post infection, type I collagen level was

significantly increased in LTs, which positively correlated with increased LT-immune activation and TGF β 1 expression and inversely correlated with the LT CD4⁺ T cell counts, indicating that LT collagen deposition can occur during acute infection (Estes et al. 2007).

Mechanisms of LT Fibrosis in HIV-1 Infection

Fibrosis is defined by accumulating excessive fibrous extracellular matrix (ECM), such as collagen and fibronectin, and is the final and common pathological consequence of host response to tissue damage during persistent inflammation (Wynn and Ramalingam 2012). Fibrosis can occur in almost every tissue and organ in the body, including liver, lung, kidney, and cardiac and lymph node. Furthermore, FRC damage leading to the disruption of LT architecture has been observed in other viral infections such as LCMV (Mueller et al. 2007).

The fibrotic change in LTs during HIV-1 infection is thought to be one of the consequences of generalized immune activation and inflammation (Estes et al. 2007). Chronic immune activation is a major theme of HIV-1 infection (Grossman et al. 2006) and is manifested as an increase in the turnover of CD4⁺ T cells, CD8⁺ T cells, NK cells, and B cells, increases in production of cytokine and chemokines, and increased frequency of T and B cells expressing immune activation markers, such as CD38, HLA-DR, and Ki67 (Grossman et al. 2006). Furthermore, the extent of generalized immune activation can predict HIV-1 disease progression. The current proposed model of collagen deposition and fibrosis in HIV-1 infection is that persistent immune activation in LTs resulting from HIV-1 infection leads to the activation of T regulatory cells (Treg) to produce TGF β 1, which stimulates collagen production and deposition in the T cell zone. The LT collagen deposition reduces naïve CD4⁺ T cells to gain access to survival cytokine IL-7 secreted by FRC, and the FRC network loss also results in the decrease of IL-7 production, thus resulting in naïve T cell death, which in turn reduces the

secretion of lymphotoxin β , a survival molecule for FRC (Fig. 1; Estes et al. 2007, 2008; Zeng et al. 2011, 2012a). Despite the substantial progress made in this area, more detailed mechanisms remain to be understood since the current model is mainly derived from correlative data.

Conclusion

LTs are the core of the immune system. Non-hematopoietic stromal cells-FDC in B cell zones and FRC in T cell zones-provide the structure of LTs for B cells and T cells to mount adaptive immune responses. HIV-1 infection impacts both types of stromal cells and contributes to immune deficiency. LT collagen deposition as a consequence of persistent immune activation disrupts the architecture of LTs and contributes to the CD4⁺ T cell loss, especially the naïve CD4⁺ T cells. Damaged LTs by collagen deposition also prevent the CD4⁺ T cells from recovery after receiving cART. Further studies delineating the mechanism underlying the stromal cell damage in LTs, especially collagen deposition in the T cell zone and FRC loss, may identify new therapeutic strategies to enable HIV-infected individuals who receive cART to better restore their immune function.

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Combination Approaches to HIV Prevention

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Definition

Combination HIV prevention is defined by UNAIDS (2010) as: “rights-based, evidence-informed and community-owned programs that use a mix of different classes of prevention interventions (biomedical, behavioral, and structural), that operate on the individual, community and societal levels, prioritized to meet the current HIV prevention needs of particular individuals and communities, so as to have the greatest sustained impact on reducing new infections.”

The term *combination prevention* is relatively new but the concept itself is not. Complex packages of prevention interventions have been implemented in different countries and have partially addressed risk factors at multiple levels – individual, dyadic, sexual, or drug use networks and community – within the cultural and epidemiological context (Hankins and deZalduondo 2010). However, well-defined biomedical interventions are often prioritized, overlooking the less definable sociocultural and environmental contexts. Combination HIV prevention strategies are more likely to be effective if they target different points along the pathway to HIV infection, meaning strategies to reduce infectiousness of HIV+ individuals combined with strategies to reduce HIV susceptibility in the HIV- individual. The strategies incorporated in these coordinated prevention packages vary

depending on target population, socio-epidemiological context (Dodd et al. 2010), and levels of evidence for effectiveness.

We review the different components of the combination prevention intervention, discuss the assessment of the combination packages, and address barriers and challenges.

Core Components of Combination HIV Prevention

A combination HIV prevention approach includes different variations and components of three interventions: biomedical, behavioral, and structural. Most of the combination prevention packages need a consistent behavioral component to be effective and are likely to incorporate one or more of the biomedical interventions:

- (a) *HIV Testing*: HIV testing is a critical component of the combination prevention package and remains the gateway to the offering of and linking to appropriate and individualized services for HIV-positive persons. It is well established that most individuals who are aware of their positive status reduce their risky behaviors, thus reducing risk of HIV transmission to others (Marks et al. 2005). However, testing has not resulted in reduced risk behaviors among individuals testing negative. An estimated 21% of people with HIV in the United States are unaware of their status; even fewer know the status of their partners. Furthermore, results of HIV testing conducted as part of the National HIV Behavioral Surveillance System (NHBS) indicated that 19% of men who have sex with men (MSM) who were tested in 2008 were HIV positive; of these, 44% were unaware that they were infected (CDC 2010). Lack of awareness of one’s own status and one’s partner’s status is associated with a 50–66% decrease likelihood of condom use (Celum et al. 2005).

- (b) *Condoms*: It has been well established that the use of condoms reduced HIV transmission in serodiscordant heterosexual couples by 85% (Weller and Davis-Beatty 2002). The correct and consistent use of condoms has been shown to reduce both HIV and STI transmission probabilities. Incorporating motivational and skills components to condom use has been shown to reduce unprotected sex among HIV-positive individuals (Johnson et al. 2006). Condom use remains problematic due to reports of condom slippage and breakage, poor ability to negotiate use with sexual partners, and limited access in developing countries (Rugpao et al. 1997). Additional prevention benefits can be seen when condoms are used with condom-compatible lubricants because they reduce condom breakage and rectal trauma in MSM.
- (c) *Antiretroviral Therapy (ART)*: ART has been shown to markedly reduce HIV infectiousness in HIV-serodiscordant couples (Donnell et al. 2010). Early initiation of ART as compared to delayed therapy led to a reduction of 96% in the number of linked HIV transmissions in serodiscordant couples and a reduction of 89% in the total number of HIV transmissions regardless of viral linkage with the infected partner (Cohen et al. 2011). Reported HIV cases have diminished contiguous with the expansion of ART in British Columbia and San Francisco (Das-Douglas et al. 2010). However, the risk of transmission is not completely eliminated by ART, possibly due to poor ART adherence or persistent replication of the virus in the genital tract despite treatment (Kalichman et al. 2008).
- (d) *Screening, Diagnosis, and Treatment of Sexually Transmitted Infections (STIs)*: It is difficult to demonstrate that syndromic treatment of STIs with antibiotics prevents HIV acquisition. Treatment of STIs to reduce HIV incidence has been evaluated in randomized trials, of which just one showed a substantial decrease in overall HIV incidence due to STI treatment. Bacterial and viral STIs can increase the efficiency of HIV transmission (Chin-Hong et al. 2009). Incident STIs are a clear marker of history of sexual risk and are predictive of future acquisition of HIV infection among MSM (Menza et al. 2009). Therefore, screening, diagnosing, and treating STIs in MSM offer a teachable moment to discuss risky behaviors and risk-reduction strategies. Treatment of STIs is predicted to reduce the infectiousness of HIV+ men.
- (e) *Vertical Transmission Risk Reduction*: There is clear evidence that ART reduces antepartum, intrapartum, and postpartum HIV transmission (Sturt et al. 2010). Alternatives to breastfeeding have decreased postpartum transmission as well. Delivering effective contraception to reduce unwanted pregnancies and coordinating it with HIV testing and ART to pregnant women is particularly challenging in developing countries.
- (f) *Parenteral Transmission Risk Reduction*: Effective interventions to reduce transmission from injection drug use include treatment of the drug use disorder such as the use of opioid substitution therapy for opioid use disorder, needle exchange programs to reduce blood exposure, and behavioral interventions to reduce sexual risk behaviors occurring in the context of substance use (Gowing et al. 2008). Adherence to infectivity-reducing ART is similar among HIV-positive injection drug users and non-injection drug users (Malta et al. 2010). Opioid substitution treatment provided as maintenance therapy is associated with a reduction in the risk of HIV infection among injection drug users (MacArthur et al. 2012).
- (g) *Medical Male Circumcision (MMC)*: MMC is one of the most effective one-time interventions that reduces HIV acquisition risk for men by 50% (Mills et al. 2008). The effects of MMC on HIV risk for women are not well determined. Combining MMC with other strategies, such as HIV testing, condom use, and STI screening and treatment potentially has a highly synergistic effect.

However, their scale-up and implementation are very challenging.

- (h) *Microbicides*: When applied to the vaginal or rectal mucosa, microbicides prevent or significantly reduce the acquisition of HIV or other STIs (McGowan 2010). Vaginal microbicide gel 1% Tenofovir has been shown to reduce HIV acquisition in women by 39% compared with placebo (Abdool-Karim et al. 2010); however there was a trend toward decreasing effectiveness after 18 months possibly due to adherence issues.
- (i) *Other Strategies*: The efficacy of other approaches such as post-exposure treatment with antiretrovirals (PEP) and use of pre-exposure (PrEP) ART for prevention are supported by some evidence (Golub et al. 2010).
- (j) *Structural Approaches*: Social, economic, legal, cultural, political, and environmental factors can significantly affect HIV risk and vulnerability (Gupta et al. 2008). More evidence points to the association between structural factors and HIV risk and vulnerability without a clearly established direct causal effect. Structural factors can act as significant barriers to individual-level HIV prevention, delivery of services, and adoption and implementation of HIV-preventive behaviors. For example, gender-related violence and inequality can affect the ability of some women to negotiate safe sex practices out of fear of physical violence and/or fear of losing financial support (Pronyk et al. 2006). Fear of HIV/AIDS-related stigma and discrimination can deter people from being tested and from disclosing their status to their sexual partner. Structural approaches address these factors – such as gender or income inequality and social marginalization that can be deeply rooted in the societal environment and very challenging to confront (UNAIDS 2010). Structural approaches are viewed as long-term initiatives that should be implemented in a context-specific way. Structural approaches, regardless of whether they are single policies or majorly transformational processes, may address social norms,

regulatory barriers, community engagement, and environmental laws in order to facilitate the development and implementation of HIV prevention packages for vulnerable most-at-risk populations (MARPs) such as women, MSM, injection drug users, or sex workers (Kurth et al. 2011). Structural interventions involving efforts to increase availability of clean needles and syringes by modifying restrictive laws and regulations and outreach to increase pharmacy involvement in syringe sales have been effective in reducing HIV infection in injection drug users (Bertozi et al. 2008). Structural approaches have been minimally incorporated into combination HIV prevention approaches. Formally assessing structural approaches is very challenging due to multiple interacting components and methodological limitations which require more than a simplistic list of individualized approaches, instead an integrated multi-sectoral context-based initiative (Gupta et al. 2008).

Evaluation and Implementation Issues in Combination HIV Prevention

The evaluation of the impact of combination HIV prevention programming has been identified as an urgent global need (Lancet 2010). The *impact* or community-level effectiveness of combination prevention programs is defined as the effect of a program on a population level as measured by changes in incidence, prevalence, mortality, and/or other ultimate outcomes of interests (Lancet 2010). So far, data on impact are limited, and most evaluations assessing the impact of combination programs have failed to implement rigorous cluster randomized designs that have demonstrated any effects on HIV incidence (Padian et al. 2011). Key considerations in evaluating the impact of combination programs include specifically defining the evaluable package and evaluating the entire package or each component of the package. Another key challenge in any impact evaluation is identifying a valid *counterfactual* – the hypothesized scenario of what would have happened if the program had

not been implemented (Padian et al. 2011). Experimental and quasi-experimental methods offer pros and cons in the identification of a counterfactual for a combination prevention program. Furthermore, methodological considerations unique to HIV include the absence of a reliable incidence assay, ethical dilemmas with having a control group, and poor surrogate markers that cannot replace outcomes such as HIV incidence, prevalence, and infections averted, which potentially affect the evaluation process (Padian et al. 2011). The need to integrate monitoring and evaluation strategies into the prevention programs requires collaboration between implementers, evaluators, and policy makers. The fact that effective combination programs cannot be easily randomized or evaluated with the most rigorous methodological designs does not mean that they should not be implemented.

Conclusion

Combination HIV prevention programs have the advantage of delivering biomedical, behavioral, and structural interventions concurrently to maximize synergies among these interventions. If designed and implemented effectively, combination prevention packages can significantly affect the HIV epidemic. However, these types of prevention packages, when implemented at scale, face significant methodological and evaluation challenges, including how best to determine their impact and how and whether to measure the effectiveness of specific component interventions. Continuing to build a better understanding of “what works” in HIV prevention and – particularly in the combination HIV prevention – is necessary to curb the rate of the epidemic (Padian et al. 2011).

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Community Viral Load

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Definition

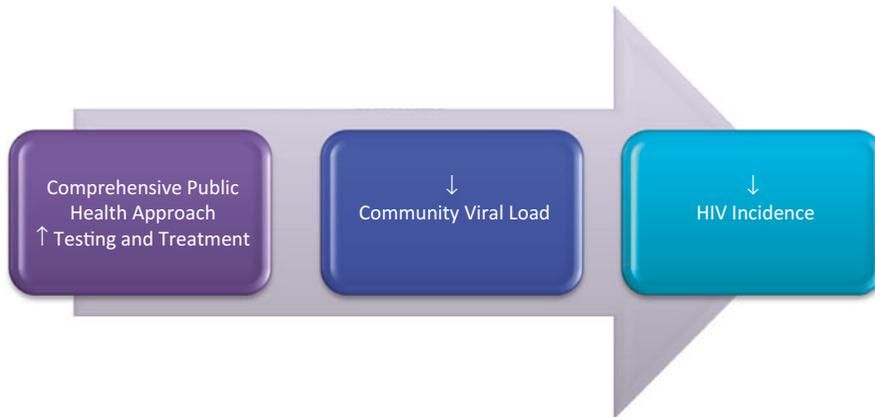
Community viral load (CVL) is defined as an aggregate population-level biomarker of a

community's viral burden over a specific time period and has recently been operationalized as (1) an indicator of a community's level of infectiousness and transmission probability; (2) a measure of the effectiveness of combination of HIV prevention, care, and treatment interventions; and (3) a proximal marker for HIV incidence and potential epidemic propagation.

Background

Accumulating evidence at the individual level has demonstrated both the direct relationship between plasma HIV viremia and HIV transmission and the positive effects of antiretroviral treatment (ART)-mediated virologic suppression in reducing or eliminating perinatal and sexual HIV transmission (Dickover et al. 1996; Garcia et al. 1999; Quinn et al. 2000; Wawer et al. 2005) (Chung et al. 2007; Cohen et al. 2011; Connor et al. 1994; Garcia et al. 1999; Mofenson et al. 1999). It is hypothesized, analogous to the individual case, that the community viral load or CVL is directly related to the magnitude of transmission in the community and that, furthermore, ART-mediated virologic suppression or decreases in CVL at the population level will be associated with reductions in HIV incidence. At the community level, there are currently no definitive randomized controlled trial findings that support this hypothesis (pictured in Fig. 1); however, there are a growing number of prospective observational cohort studies, ecologic analyses, and modeling studies that demonstrate how increasing diagnoses, linkage to care, ART uptake, and engagement in care will ultimately decrease the population-level viral burden, which can be measured by CVL, and therefore reduce HIV incidence (Charlebois et al. 2011; Fang et al. 2004; Granich et al. 2009; Katz et al. 2002; Montaner et al. 2010; Porco et al. 2004; Wood et al. 2009).

In this entry, an overview of the current approaches to measure and apply CVL will be discussed, including an examination of the limitations of the biomarker.



Community Viral Load, Fig. 1 The hypothesis. Increased testing, diagnosis, promoting linkage to care, and therefore increasing treatment will reduce the overall community viral load, and this will ultimately reduce new HIV infections

Definition of “Community”

As noted earlier, CVL is defined as the aggregate biological measure of the viral load (VL) for a population living with HIV. The term “community” refers to either a particular geographic location, such as a city or a particular neighborhood or census tract, or to a particular group of people who share sociodemographic characteristics. Although people who inject drugs (PWID), men who have sex with men (MSM), or particular ethnicities may not necessarily constitute a “community” in the sense of complete social interconnectedness or shared networks, here the term is used broadly to refer to populations defined by demographic, geographic, or behavioral commonality with an elevated probability of connections to other members of the population, including those through needle sharing or sexual partnerships.

Measures

There are several different ways to calculate CVL. Generally, CVL is calculated for a particular time period. The most common reported measure is a measure of the central tendency of the viral load distribution over a particular period of time. For example, the mean annual CVL is the total CVL (sum total of all unique VLs of the population of HIV-positive individuals in a particular jurisdiction during a given year) divided by N , the total

number of HIV-positive individuals. Table 1 below lists the most commonly used CVL measures and their uses, as well as some caveats and limitations:

$$CVL = \left(\frac{\sum_{i=1}^N (VL)}{N} \right)$$

Sources of Data

CVL may be calculated for a clinic population (Geng et al. 2012), for persons participating in cohort studies (Jain et al. 2012a, b; Wood et al. 2009), or from public health surveillance HIV case registries if the VL data are systematically reported and abstracted (Castel et al. 2012; Das et al. 2010; Henard et al. 2012; Montaner et al. 2010; Terzian et al. 2012). A variety of approaches have been used to calculate CVL, often depending on the source of data, number of measures per person, completeness of the data, and distribution of the VLs. For example, Wood et al. demonstrated that the median CVL among a cohort of HIV-positive PWID predicted HIV incidence density among HIV-negative PWID (Wood et al. 2005). As noted in Table 1 above, in samples where over half the viral loads are undetectable, using the mean or log transformed mean CVL for such analyses is more

Community Viral Load, Table 1 CVL measures

CVL measure	Use	Caveats/limitations
Mean (most recent) (Das et al. 2010)	Useful for comparisons between sub-populations (e.g., disparities)	Influenced by outliers
Mean (of the mean) (Das-Douglas et al. 2009b)	Useful for comparisons between sub-populations (e.g., disparities)	Relative contributions of multiple measurements (e.g., those started on ART, trended towards suppression in year)
Median (Wood et al. 2009)	ART uptake and treatment effectiveness	If >50% of VLs are undetectable, then the median is undetectable; limits analyses
Maximum (peak) (Das et al. 2011b; Montaner et al. 2010)	Most conservative estimate of viral burden	Could overestimate true average burden
Minimum (Das et al. 2011b)	Least conservative or most optimistic estimate of viral burden	Could underestimate true average burden
Total (sum of most recent) (Das et al. 2010, 2011b)	The prevalent viremia: takes into account both number of PLWHA and magnitude of the most recent VL	The proportion missing VLs can have greater influence
Time-weighted average (Das et al. 2011c)	Alternate approach to handling multiple measures in year	
Population virologic suppression (Das-Douglas et al. 2009a, c)	Evaluate universal treatment policies at the population level	Includes all comers, including those not on treatment, more helpful for estimate of transmission probability
Maximal virologic control (Horberg et al. 2010)	Evaluate HIV quality of care among those on ART for a given time	Includes only those on treatment for particular time period, more helpful for evaluating quality of care
Log transformation of any of the above measures (Das et al. 2010)	Reduces the influence of outliers	

useful than examining the median (which would be undetectable). In samples where individuals have repeated measures during a calendar year or particular time period, investigators have either used the most recent VL value or have employed traditional statistical approaches for handling repeated measures.

Applications

CVL has been used as (1) an indicator of a community's level of infectiousness and transmission probability, (2) a measure of the effectiveness of combination HIV prevention care and treatment interventions, and (3) a proximal marker for HIV incidence and potential epidemic propagation.

Comparing the mean CVLs of different sub-populations and communities can be useful to

evaluate disparities by specific demographic characteristics, such as race/ethnicity, transmission risk, or other clinical attributes. Mean CVL can be mapped using geographic information systems (GIS) software by neighborhood or census tract to demonstrate geographic differences within a jurisdiction. Neighborhoods with higher mean CVLs also tend to have higher rates of both AIDS mortality and poverty (Das et al. 2011a).

Establishing overall and subpopulation or neighborhood CVL baselines and following trends over time can be helpful for municipal health departments making decisions regarding the effectiveness of current HIV/AIDS strategies and/or the reallocation of resources for HIV prevention and treatment.

Disparities in mean CVL among different sub-populations may help to explain disparities in access to and engagement in care among these different groups and may be a possible

explanation for disparities in HIV incidence. For example, numerous studies have demonstrated that African-American MSM do not have higher levels of sexual risk and drug-using behavior than white MSM; however, HIV incidence is disproportionately higher among African-American MSM (Millett et al. 2007). Higher CVL among African-American MSM may be a potential explanation (Christopoulos et al. 2011). Jurisdictions can examine the relationship between temporal trends in CVL and estimated HIV incidence using public health surveillance data. Planned cluster randomized controlled trials evaluating “treatment as prevention” or “test and treat” can examine CVL as a marker of transmission probability, to evaluate the effectiveness of the strategy, as well as a proximal marker for HIV incidence (Granich et al. 2011). As clinics or health systems increase efforts to increase the prompt initiation of ART to achieve durable virologic suppression, CVL measures for a provider panel, different clinics, or parts of health systems can be used to monitor quality of HIV care.

Critiques and Limitations

There are several potential limitations about the estimation of CVL using different data sources as well as critiques regarding the interpretation of the applications of CVL.

Case Registry and Surveillance Data

When CVL is calculated using an HIV/AIDS case registry from public health surveillance data, the accuracy of the CVL estimate is affected by the testing efforts and the timeliness and completeness of HIV case reporting in that jurisdiction. The CVL estimate is further limited by the fact that the HIV case registry contains only the information from those individuals who have been diagnosed and reported in a jurisdiction, presenting two significant issues. First, a public health case registry does not include persons who are unaware of their HIV status, which includes any persons in the acute phase of infection who may have a negative

HIV antibody and high VLs – a population that may account for a disproportionate number of onward infections (Cohen et al. 2012; Das et al. 2010; Smith et al. 2012; Wilson 2012). These gaps in the calculation of CVL cannot be addressed using current standards of public health surveillance but may change in the future as HIV testing technology advances and policies are updated. However, it will continue to be very difficult to include estimates of VL from acutely infected persons who will rarely be detected in population-based surveys or public health surveillance systems. For those individuals who are chronically infected but as of yet undiagnosed, population-based surveys – such as National HIV Behavioral Surveillance, a CDC-coordinated series of population-based, and cross-sectional surveys among populations at risk for HIV in 21 US cities (Gallagher et al. 2007) – can provide an estimation of the VL distribution of those chronically infected but unaware of their HIV-positive status. Second, there may be individuals who are in the case registry but who may be missing VL data. This may be because the individual is in care but not having VL testing for a variety of reasons, e.g., difficult venipuncture, not on ART, or because the individual is out of care and not being monitored. Individuals in a case registry may also be missing VL data because surveillance departments have historically defined HIV/AIDS case ownership for a particular jurisdiction as those diagnosed within that jurisdiction. This essentially means that an individual diagnosed and reported in a particular jurisdiction remains on the case registry for that jurisdiction, even if they move to another jurisdiction. Earlier in the epidemic, especially when many jurisdictions primarily had AIDS-only case reporting, this attribution of cases was reasonable. In the current era, many people diagnosed with HIV have much longer life expectancies and can feasibly be expected to move out of the jurisdiction in which they were diagnosed. Current laws and policies around exchanging information regarding HIV cases between jurisdictions, especially between states, hamper the ability of surveillance registries to confirm whether a person has moved out of the

jurisdiction. As a result, CVL estimates derived from surveillance case registries may (1) exclude those who live and are receiving care in the jurisdiction but were diagnosed elsewhere and therefore will remain a case within their jurisdiction of diagnosis and (2) be hampered by jurisdictional HIV cases which are missing data because the individual has moved out of the jurisdiction and no longer are having clinical data such as VLs reported to the public health department. The former limitation can be addressed by simply including VL data from those living in the jurisdiction but diagnosed elsewhere. The latter limitation can potentially be addressed with statistical techniques such as multiple imputation to predict VL values for those missing, assuming that individuals who are missing data are missing VL data at random. The assumption that the data are missing at random holds for the majority of cases if most VLs are missing because individuals moved. However, if most individuals are missing VLs because they are out of care, there is a systematic bias and multiple imputation should not be used. Lastly, there will be individuals who were diagnosed elsewhere who have moved into a particular jurisdiction but are not in care. There is not a way to quantify those individuals, who most likely have detectable VLs and are in need of care, unless surveillance registries are allowed to share information across jurisdictions, including across state and other territorial lines. In calculating and interpreting CVL estimates from surveillance case registry data, it is vitally important to have estimates of completeness and timeliness of case reporting, as well as completeness of current address data, and status of efforts to de-duplicate cases across jurisdictions.

Clinical or Cohort Data

Many of the concerns raised above could potentially be addressed by calculating CVL estimates from clinical databases or cohort studies of HIV-positive individuals. These estimates are likely to be more complete than estimates from the surveillance registry. However, these estimates are less generalizable beyond the clinical system or

cohort study and may reflect more the quality of care in a particular clinic system than the effectiveness of combined HIV prevention, treatment, and care interventions in an entire jurisdiction. Exceptions to this concern would include a cohort which purportedly contains all HIV-positive individuals in a geographic area, for example, all PWID in Vancouver (Wood et al. 2009), which demonstrated that the cohort VL predicted HIV incidence density 6 months later; however, there have been critiques challenging whether CVL can be used as a proximal marker for HIV incidence and potential epidemic propagation.

Alternative Explanations

The aforementioned hypothesis that increases in testing and treatment uptake will reduce population-level viremic burden, as measured by CVL, which will reduce HIV transmission, has been challenged (Cohen et al. 2012; Smith et al. 2012; Wilson 2012). Alternative explanations to account for reductions in HIV incidence have been posited, such as ecologic fallacy or reductions in risk behavior including serosorting or decreased syringe sharing. Cohort studies are able to adjust for individual-level risk behavior and address these critiques. In analyses conducted with HIV case registry data, triangulating data from other public health surveillance data – such as trends in rectal gonorrhea cases which reflect rates of unprotected anal intercourse among MSM – can be examined to evaluate the role of changing sexual risk behavior. Finally, while neither the biologic plausibility of the relationship between HIV viremia and HIV transmission nor the role of ART-mediated virologic suppression in reducing HIV transmission has been challenged, questions have been raised as to whether, at the population level, it is prevalent viremia rather than HIV prevalence driving HIV infection.

Conclusion

It has long been recognized that examining trends in newly diagnosed HIV cases and HIV

prevalence is no longer sufficient to track the full burden of HIV disease or the potential for further transmission; this point is all the more true in the current era of wider use of ART and reduction in transmission due to ART-mediated virologic suppression (Cohen et al. 2011). Moreover, direct measures of HIV incidence and acute infection face logistical and theoretical challenges and are consequently rare on a population level. In the context of expanded efforts to promote earlier and durable viral suppression, the measure of CVL provides additional insight to mitigate the effects of HIV and thereby reduce transmission. In the United States with a well-characterized epidemic and mature surveillance systems, establishing a baseline for CVL and population-level virologic suppression rates and examining temporal trends in these measures can help evaluate the impact of the National HIV/AIDS Strategy and the Affordable Care Act on the health outcomes of people living with HIV. Internationally, mapping CVL trends may contribute to understanding the stage, progression, and spread of HIV as well as the effectiveness of ART scale-up. Additionally, mapping the spatial distribution of CVL may delineate new “hotspots” or areas that have high HIV incidence, allowing a rapid response to target resources and interventions to populations at greatest risk. In summary, monitoring trends in CVL can help to characterize the burden of disease within a community, efficiently target resources, identify disparities, and help set goals for maximizing outcomes along the implementation cascade and ultimately achieving health equity. Examining CVL trends in upcoming international individual and community cluster randomized trials of ART as prevention (Granich et al. 2011) will further refine the current understanding of the relationship between population-level viremic burden and HIV transmission.

Cross-References

- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Behavioral Aspects of HIV Treatment as Prevention](#)

- ▶ [Clinical Ethics in HIV/AIDS Prevention, Care, and Research](#)
- ▶ [Combination Approaches to HIV Prevention](#)
- ▶ [Healthcare Workers, Shortage and Task Shifting of](#)
- ▶ [HIV Counseling and Testing, Prevention of HIV](#)
- ▶ [HIV Prevention and African Americans](#)
- ▶ [HIV Prevention and Hispanics](#)
- ▶ [HIV Prevention and Women](#)
- ▶ [HIV Prevention Efforts Within Substance Use Disorder Treatment Settings](#)
- ▶ [HIV Prevention for MSM](#)
- ▶ [HIV Prevention in Transgender Persons](#)
- ▶ [Housing as HIV Prevention](#)
- ▶ [Multilevel Interventions/Structural Approaches to HIV Prevention](#)
- ▶ [Positive Health, Dignity, and Prevention \(PHDP\)](#)
- ▶ [Prevention for People Living with HIV](#)
- ▶ [Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#)
- ▶ [Surveillance Case Definition](#)

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Comorbidity: Opioids

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Definition

Opiate abuse is a major global health concern, due to the fact that the HIV infection frequently occurs in individuals who abuse opiates. Both clinical and preclinical evidences show that opiate abuse impairs resistance to opportunistic infections and alters both innate and adaptive immunity. Moreover, opiate use results in greater susceptibility to the development of neurodegeneration that can occur in association with HIV infection. Opioid drugs, most notably morphine and heroin, exert both pro- and antiinflammatory activities for the immune system and, following HIV infection, can exert direct neurotoxic effects on neuronal cells. In addition, opiate administration exacerbates the neurotoxic properties of some of the HIV products, and these drugs can alter blood-brain barrier integrity. Finally, the activation of opioid receptors by morphine or heroin can alter both the expression and function of the major HIV coreceptors CCR5 and CXCR4. Taken together, opiate abuse has the potential to contribute substantially to the pathology associated with this infection.

Introduction

Opiate drug abuse is a major contributor to the global AIDS epidemic, and approximately 35% of HIV infections in the USA are linked to intravenous drug abuse. Moreover, global estimates show that 20% of intravenous drug abusers are infected with HIV (Mathers et al. 2010). Due to the increase in the misuse of prescription opioid drugs in the USA, chronic opioid abuse is a growing problem. Both clinical and basic research

shows that heroin or morphine inhibits elements of both adaptive and innate immune responses, and this leads to a decline in resistance to a number of opportunistic infections (Dutta and Roy 2012). For patients who become infected with HIV, the use of shared needles offers a portal of entry for the virus. It is not clear that the opiate use necessarily alters susceptibility to HIV infection by this route. However, clinical evidence indicates that opiate abuse leads to higher rates of encephalopathy following HIV infection when compared to infected nondrug abusers (Bell et al. 1996).

Opiates and Opioid Receptors

The use of alkaloids derived from the seedpods of the opium poppy *Papaver somniferum* has been common for centuries. Heroin, or diacetylmorphine, is chemically synthesized from opium, and morphine is the major bioactive metabolite of heroin. Three opioid receptors have been identified (designated μ -, κ -, and δ -opioid receptors), and while morphine is primarily an agonist for the mu-opioid receptor (MOR), this drug can also activate the δ - and κ -opioid receptors (DOR and KOR). This is an important issue since MOR and KOR can mediate opposing activities in cells of the immune system. The opioid receptors are expressed in both the central and peripheral nervous system by most of the neuronal cell types, and it is now clear that these receptors are also expressed by hematopoietic cell populations. It is important to appreciate that levels of expression vary depending on the tissue location and leukocyte subtype.

Opioid-Mediated Neurotoxicity

Opiates exert both direct and indirect toxic effects on neuronal cells, and this is important given the higher rates of encephalitis in opiate abusers who are infected with HIV. For example, morphine exerts a direct toxic effect on Purkinje cells in vitro, and the MOR agonist fentanyl induces damage to the limbic system when administered to rats in vivo. MOR-selective opiates also

exacerbate cerebral ischemia in the forebrain, and this may be due, at least in part, to the capacity of these drugs to exert a proapoptotic effect when combined with other apoptotic agents. This is consistent with findings which show evidence of astrogliosis and inhibition of dopaminergic function of tyrosine hydroxylase terminals in the nucleus accumbens in heroin abusers. Moreover, chronic administration of either morphine or heroin in rodents results in reduced striatal levels of synaptic dopamine and dopamine transporter. These findings suggest that opiate administration, particularly when given chronically, exerts a neurotoxic effect, and these effects have been associated with the accumulation of perivascular macrophages and lymphocytes (Hauser et al. 2012).

This suggests that the opiate-mediated toxicity is the result of an inflammatory response, and this is somewhat surprising given the well-documented capacity of opiates to exert immunosuppressive activity (Dutta and Roy 2012). It is now apparent that MOR activation can have pleomorphic effects on the immune response, and this is particularly apparent with respect to the inflammatory response. Morphine administration inhibits the expression of several proinflammatory cytokines, including interferon- γ (IFN γ), interleukin-2 (IL-2), tumor necrosis factor- α (TNF α), IL-1, and IL-12 (Roy et al. 2006). Morphine also upregulates the expression of the immunosuppressive cytokine transforming growth factor- β (TGF β). In contrast, administration of morphine or other MOR agonists has been shown to induce increased expression of the proinflammatory chemokines CCL2, CCL5, and CXCL10 (Wetzel et al. 2000) and the proinflammatory chemokine receptors CCR5 and CXCR4 (Steele et al. 2003). These apparently opposing effects of opiates on the inflammatory response suggest that opiates exhibit a complex immunomodulatory effect on the immune response.

Opiate administration may also promote neurodegeneration by degrading the integrity of the blood-brain barrier (BBB). Morphine has been shown to inhibit the expression of the tight-junction zona-occludin proteins and increase the

production of the junctional adhesion molecule-1, resulting in increased BBB permeability. Morphine also upregulates the production of platelet-derived growth factor (PDGF) by brain microvascular endothelial cells, a mediator which can contribute to ischemic stroke, and this cytokine weakens BBB stability. This cytokine is also produced by a subset of macrophages which are referred to as “alternatively activated” or “M2” cells, and in general these cells exert a pro-fibrotic effect in inflamed tissues. The accumulation of macrophages in the perivascular space in the brain in HIV-infected patients may provide a source of PDGF which can act locally to promote additional recruitment of inflammatory cells.

Opioid-HIV Interactions Associated with Neurodegeneration

Recent evidence shows that the combination of opioid abuse with HIV infection creates a greater level of neurodegeneration than observed with virus infection or opiate use alone. It is well established that MOR opiates directly induce functional activation of neuronal cells including neurons, microglia, astrocytes, neuronal precursors, and oligodendrocytes, as one would expect since each of these cell populations express MOR. However, when HIV infection is combined with mu opiates, a greater degree of toxicity is induced, and astrocytes and microglia are the primary targets (reviewed in (Hauser et al. 2012)). HIV products, including gp120 and tat, which are released from infected cells into the surrounding brain tissue, exert potent proinflammatory direct effects on microglia and astrocytes. It is clear that other neuronal cells, including neurons, are susceptible to the toxic effects of these inflammatory mediators, and altered neuronal function is the end result of the combination of these direct and indirect effects of opiates and HIV products.

The activation of both microglia and infiltrating perivascular macrophages is a hallmark of the histopathology that is observed with HIV infection (Hauser et al. 2012), and the degree of activated microglial/macrophage infiltration correlates with the severity of HIV-associated

neurocognitive disease (HAND). Research carried out with experimental animals shows that systemic administration of morphine induces an increase in the infiltration of inflammatory cells at sites of HIV Tat injection. This is consistent with the observation that the abundance of both macrophages and activated microglia in the gray matter of the thalamus and hippocampus is greater in opiate abusers.

Astrocytes are not targets for productive infection with HIV, but these cells are susceptible to the potentially toxic products of HIV that are released from infected microglia and macrophages. Astrocytes perform a number of critical functions in the maintenance of neuronal cells and are essential for the integrity of the BBB. Toxic products elicited from HIV-infected cells lead to astrocyte damage, and this can exacerbate the neurodegenerative effects of HIV infection through the release by astrocytes of potentially toxic mediators such as nitric oxide and proinflammatory cytokines. This mix of HIV products and astrocyte mediators collectively serves to promote HIV-mediated neuropathology. Moreover, the combination of morphine and HIV Tat can augment cytokine and chemokine expression and astrocyte death (Hauser et al. 2012). Both HIV Tat and gp120 induce astrocyte activation, and this results in the production of elevated inflammatory cytokines and chemokines. The combination of these HIV products with elevated MOR opiates boosts astrocyte activation, leading to greater glutamate release and a drop in the glutamate excitation threshold.

Neurons are also directly susceptible to the toxic effects of HIV products including Tat, gp120, and Vpr, and their release into the brain interstitium results in neuronal damage. As these HIV proteins are produced by macrophages and microglia, the expression of opioid receptors by these cells drives a response to endogenous and exogenous opioids which promotes HIV-related toxicity. Current evidence suggests that opiate exposure reduces the threshold required for HIV product-mediated neuropathology. There is growing evidence that opiate abuse leads to an exacerbation of HIV-induced synaptodendritic damage, and this damage directly contributes to the

development of neurobehavioral abnormalities. The administration of MOR agonists in the presence of products of HIV infection leads to a reduction in the complexity of dendrites and lessens the density of dendritic spines.

Immune Activation

It is clear that the opiate contribution to neurodegeneration is due in part to the MOR-dependent induction of a proinflammatory immune response. This may initially seem surprising since MOR agonists are capable of exerting antiinflammatory activity in many circumstances. It is well established that MOR agonists, including heroin, morphine, and endogenous opioid neuropeptides (including the MOR-selective agonists endomorphin-1 and endomorphin-2), are all capable of modulating proinflammatory cytokine expression. The degree of immunomodulation can be substantial, and this has led some to speculate that a major mechanism for the immunomodulatory activity of opioids is mediated through altered cytokine expression (Dutta and Roy 2012). Altered cytokine expression could provide a common basis for the well-documented opioid-mediated modulation of innate immunity and both cell-mediated and antibody-mediated adaptive immune responses. Based on both *in vivo* and *in vitro* studies conducted using both human and experimental animal systems, it is clear that MOR opiate administration alters both pro- and antiinflammatory cytokine expression (Rogers and Peterson 2003). For example, morphine induces a significant inhibition of the human peripheral blood leukocyte expression of the very proinflammatory cytokines IFN γ and IL-2. Morphine has also been reported to inhibit the human and murine leukocyte production of the highly proinflammatory cytokines IL-1 β , TNF α , and IL-12, although under certain conditions the effect of morphine treatment on the expression of these cytokines may be the opposite. Overall the activation of MOR leads to alterations of both pro- and antiinflammatory cytokine expression, and the selectivity of these immunomodulatory activities is not fully understood.

Balance in the immune response, and control of inflammatory responses, is maintained in part through the production of antiinflammatory cytokines which include both IL-10 and TGF β . These cytokines are typically produced during an inflammatory response, and while they may not be sufficient to terminate an inflammatory response, they often reduce the intensity of the inflammation and attenuate the expression of some of the proinflammatory cytokines. The levels of TGF β in the CNS are elevated in AIDS patients, as well as several other neurodegenerative disease states, including multiple sclerosis and Alzheimer's disease. The astrocytosis which is frequently observed in the brain tissue of HIV-related dementia patients is attenuated by TGF β , and this is likely due to the ability of this cytokine to inhibit the response of astrocytes to growth signals. In addition, TGF β reduces the expression of several proinflammatory cytokines in the CNS, including IL-1 and TNF α , and downregulates the production of potentially toxic glutamate and nitrogen oxide. These anti-inflammatory activities are beneficial for the brain tissue in the HIV-infected patient and serve to limit the toxicity of proinflammatory cytokines and HIV products.

The administration of MOR opiates upregulates TGF β expression, and the production of this cytokine has been shown to be responsible for the downregulation of TNF α under conditions of immune activation. However, TGF β is also a cytokine that exhibits proinflammatory activity under certain circumstances. For example, MOR opiates induce the expression of the proinflammatory chemokine CCL5, and the opioid-induced upregulation of this chemotactic cytokine is dependent on the initial induction of TGF β expression (Happel et al. 2008). Moreover, TGF β has been shown to enhance the adhesive properties of monocytes by inducing the expression of both lymphocyte function-associated antigen-1 (LFA-1) and the fibronectin receptor. These effects are likely to promote the adherence of monocytes to vascular endothelial cells and enhance the traffic of these cells across the blood-brain barrier.

Studies conducted on the expression of the chemokines have, for the most part, shown that

treatment with MOR agonists increases expression. The consequences of the influence of opiate administration are particularly significant, given the critical role of chemokines in the neurodegeneration associated with HIV infection. These cytokines are essential for the migration of infected and noninfected but activated monocytes into brain tissue where these cells reside in the perivascular space. The most important of the chemokines for the traffic of infected monocytes across the BBB is CCL2, but other chemokines including CCL3, CCL4, CCL5, and CX3CL1 are also involved. Recent evidence suggests that a relatively minor subpopulation of monocytes which express both CD14 and CD16 (designated “nonclassical monocytes”) are preferentially susceptible to infection by HIV, and the migration of these cells across the BBB appears to be driven in part by CX3CL1. The receptor for CX3CL1, CX3CR1, is strongly expressed by CD14+16+ monocytes, and this population expresses viral pattern recognition receptors. By virtue of the “viral-response” nature of their receptors, this monocyte subpopulation performs “patrolling” functional activity as a part of antiviral immunity (Geissmann et al. 2010). Nevertheless, the expression of these chemokines, particularly CCL2, increases in the brain as the infected monocytes differentiate into macrophages and the levels of CCL2 increase.

The expression of the critical chemokine CCL2 increases with exposure to elevated levels of MOR agonists, including morphine (Wetzel et al. 2000). Opiate administration also induces significant increases in the expression of chemokines CCL5 and CXCL10 from both HIV-infected and noninfected monocytes and macrophages. The production of both of these chemokines, particularly in the presence of elevated levels of CCL2, would be expected to promote the attraction of additional monocytes into the infected tissue. In addition, the combination of morphine and HIV Tat administration has been shown to upregulate the production of CCL2, CCL3, and CCL5 from astrocytes (El-Hage et al. 2005). As the HIV infection proceeds in the brain, the accumulation of HIV products such as Tat and gp120 promotes a

proinflammatory cytokine response, and much of this response is augmented in the presence of elevated levels of MOR opiates.

The localized inflammation in the brain tissue of HIV-infected patients is an important contributor to the progression of disease. It is also important to consider that systemic inflammation is also an important aspect of HIV pathogenesis, both for the neurodegenerative disease and for peripheral manifestations of the disease. There is good evidence that systemic inflammation contributes to the development and severity of HAND, and biomarkers of systemic inflammation can correlate well with symptoms of neurodegeneration. Of course, systemic inflammatory processes are well established to contribute to HIV replication both in the brain and in the periphery, but systemic inflammation promotes neurodegeneration independent of the replication of the virus. For example, recent clinical studies show that circulating levels of the proinflammatory biomarkers C-reactive protein (CRP), D-dimer, IL-6, and fibrinogen are significant predictors of mortality in HIV-infected patients. Moreover, systemic proinflammatory biomarker levels exhibit predictive significance independent of circulating viral titers or CD4 T cell counts.

Most HIV-infected patients exhibit detectable evidence of systemic “immune activation,” and this is consistent with the systemic biomarker evidence described above. Immune activation in these patients is, in large part, the result of microbial translocation in the gut, and this is likely to be a common element of the pathogenesis of this infection. Results from a number of studies show that deterioration of the gut wall occurs following infection, which compromises the integrity of the gut wall epithelium, leading to translocation of gut flora through the wall and the appearance of microbial products in the blood. This process leads to elevated levels of gram-negative bacterial endotoxin in the bloodstream and the activation of leukocytes through endotoxin-responsive toll-like receptors. The levels of endotoxin in the plasma obtained from these patients significantly correlate with the progression of the disease, and circulating monocytes can exhibit impaired (or refractory)

responses *in vitro* to endotoxin suggesting that these cells were pre-stimulated with endotoxin in the bloodstream. There is also a loss of lymphocytes from the gut-associated lymphoid tissue as a part of this process, and the reduction in the levels of these cells contributes substantially to the drop in circulating lymphocytes in a portion of these patients.

The contribution of opiates, particularly the chronic use of MOR agonists such as heroin or morphine, on the bacterial translocation process in HIV-infected patients is largely unknown. However, studies carried out with slow-release pellet delivery of morphine in mice show that a high percentage of these animals develop bacterial sepsis as a result of bacterial translocation (Hilburger et al. 1997). This result is very significant given the critical role of immune activation in the progression of HIV-related disease. Additional work will be necessary to evaluate the effect of the combination of both HIV infection and chronic opiate administration on bacterial translocation and the resulting immune activation.

Interactions Between Opioid and Chemokine Receptors

The major coreceptors for HIV-1 are CCR5 and CXCR4, two chemokine receptors which are also important for inflammatory responses. The HIV-1 envelope protein, gp120, binds to either CCR5 or CXCR4, or both, and this is required for attachment and subsequent internalization of the virus. HIV gp120 is a highly polymorphic protein, and those strains of HIV-1 which express a gp120 sequence which binds predominantly to CCR5 (rather than CXCR4) are monocyte/macrophage tropic (R5 strains). Virtually all HIV-1 strains isolated from the brain are R5 tropic, and this is somewhat surprising since the expression of CXCR4 in the brain is upregulated in patients with HAND, while the expression of CCR5 in these patients is reduced. This is in contrast to isolates obtained from the blood during the later stages of AIDS, which are frequently either CXCR4-dependent T cell tropic (X4 tropic) or dual R5/X4 tropic.

The acute administration of MOR opiates induces the leukocyte expression of both CCR5 and CXCR4, and this effect appears to be most prominent for monocytes and T cell lymphoblasts. As expected, the increase in expression of CCR5 and CXCR4 is closely associated with an increase in susceptibility to infection by R5 and X4 strains of HIV-1, respectively (Steele et al. 2003). Moreover, the opioid-induced increase in expression of CCR5 is likely to promote the capacity of monocytes and T cells to migrate within and between tissues and would increase the distribution of infected cells in the body.

The activation of MOR by opiate administration also leads to the regulation of chemokine receptor functional activity through a process of cross-desensitization (or “heterologous desensitization”). Cross-desensitization is believed to be a common element of the regulation of G protein-coupled receptor (GPCR) functional activity. This process takes place when a GPCR is activated and initiates a signaling pathway which leads to the inactivation (or desensitization) of an unrelated GPCR. There is a hierarchy of GPCRs which has been documented, so that there is a group of GPCRs that are strong inducers of cross-desensitization, while other GPCRs are moderate or weak inducers. Similarly, the strongest inducers are typically the least susceptible to the process of cross-desensitization (Steele et al. 2002). It is apparent that the activation of MOR leads to the cross-desensitization of susceptible GPCRs, including CCR5, CCR1, CCR2, CXCR1, and CXCR2, but not the formyl peptide receptor or CXCR4 (Szabo et al. 2003). Additional works show that the MOR-induced desensitization of CCR5 also results in a loss of the ability of CCR5 to function as an HIV coreceptor. The signaling pathway responsible for the cross-desensitization of CCR5 is dependent on the activation of PKC ζ , and this signaling pathway is induced within the first 5–10 mins following activation MOR. Recent results show that an unrelated signaling pathway is also induced by MOR in the CNS which results in the inactivation of neuronal CXCR4 functional activity, and while this pathway requires a longer induction period, the desensitization of CXCR4 leads to a loss of the

neuroprotective activity of CXCR4 (Patel et al. 2006).

Taken together, the interaction of MOR with CCR5 is particularly significant given the impact on susceptibility to HIV infection. It appears the administration of MOR opiates induces a change in CCR5 functional activity that is composed of two phases. In the first or early phase, MOR activation induces a rapid desensitization of CCR5 functional activity. This occurs without loss of CCR5 from the outer membrane and persists for several hours. This period can be sustained for longer periods of time with prolonged exposure to the MOR opiates. This first phase is followed by a delayed phase, which begins at about 24–48 h, and is composed of elevated CCR5 and CXCR4 expression. The increase in the expression of CCR5 in the second phase is associated with elevated susceptibility to infection with R5 HIV, while the decrease in CCR5 functional activity in the first phase is associated with decreased susceptibility. More work is necessary to evaluate the impact of long-term or chronic exposure to MOR opiates on the HIV coreceptor activity of CCR5.

Conclusion

Recent advances have improved our understanding of the impact of opiate administration on the neurodegeneration associated with HIV infection. It is now clear that opiates participate in this process at several levels. First, the exposure to MOR opiates alters the expression and functional activity of the HIV coreceptors, and this is likely to alter the progression of the infection both in the periphery and in the CNS. Second, MOR opiates alter the transit of infected leukocytes, and activated monocytes, across the blood-brain barrier. Third, opiate exposure increases the degree of immune activation as a result of the deterioration of the gut mucosa (see chapter on ► [Chronic Immune Activation in HIV](#)). And fourth, MOR opiates contribute to the pathology in the CNS that is promoted by products of HIV infection. The combination of these effects on the progression of neurodegeneration is clearly very

complex, and the variability that occurs between individuals is likely to be due to individual variances in the degree or intensity of one or more of these individual opiate effects. Finally, there is still a great deal that we need to learn in order to have a complete understanding of the comprehensive effects of chronic opiate abuse. For example, research at this point has placed emphasis on our understanding of the effects of opiates alone on the pathology associated with HIV infection. However, the actual clinical situation is much more complex, and we need to evaluate the impact of chronic opiate exposure in the context of the use of other drugs, including both long-term tobacco use and alcohol abuse. Of course, these additional considerations create a much more challenging research area, but in the end should yield information which is more clinically valid.

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Comorbidity: Progressive Multifocal Leukoencephalopathy

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Introduction

Progressive multifocal leukoencephalopathy (PML) is a progressive infection of the brain by reactivation of the JC virus that manifests

clinically as a subacute decline in neurologic function in the setting of significant immune suppression. It was first named in 1958 after histological definition of autopsy cases. All patients suffered underlying lymphoproliferative disorders (Astrom et al. 1958). PML has since continued to be a rarely occurring complication of these diseases, such as myelodysplastic syndrome, chronic lymphocytic leukemia, and Hodgkin disease. Reviewing the world's publications through 1984 and adding cases from their own extensive experience, Brooks and Walker were only able to identify 230 cases (Brooks and Walker 1984). With the emergence of the HIV pandemic, PML became exponentially more common, typically emerging in the HIV-infected when CD4 counts waned below 200 cells/ μ L. In fact, it was diagnosed in 4% of the HIV-infected population (Berger et al. 1998), and mortality was high, with only 10% survival at 1 year. With the advent of combination antiretroviral therapy (cART), JC virus (JCV) was recognized to be a target for the reconstituting immune system, provoking the immune reconstitution inflammatory syndrome (IRIS) in a subset of those recently started on treatment. This created a complicated conundrum, as the only unequivocally effective treatment for PML is restoration of immune function.

PML reached its next stage of recognition in 2005 when three cases were discovered complicating the use of natalizumab, an α 4 integrin inhibitor used in the treatment of multiple sclerosis and Crohn's disease. This precipitated the drug's temporary removal from the market, but in 2006, it was reintroduced with intensive ongoing scrutiny. Three factors have been identified as increasing the risk of natalizumab-induced PML, namely, JCV serostatus, duration of natalizumab treatment, and prior immunosuppressive therapy. Those without JCV antibodies had a risk of <0.09/1,000 patient years, but those with all three risk factors had a risk of 11.1/1,000 patient years (Bloomgren et al. 2012). Once patients developed PML associated with its use, the standard protocol is immediate discontinuation of the drug. Plasma exchange has been used frequently in order to remove drug expeditiously from the patient's circulation, but this may precipitate an

IRIS phenomenon, which in some cases can be fatal.

Concomitantly with the introduction of natalizumab, the use of other biologic immunosuppressive therapies for the treatment of a variety of autoimmune diseases and malignancy was recognized to potentially increase the risk of PML. These medications include rituximab, efalizumab, adalimumab, infliximab, etanercept, brentuximab vedotin, dimethyl fumarate, and ruxolitinib among others. Mycophenolate mofetil has also been implicated, and it is likely that careful study will identify a risk, albeit small, for widely used immunosuppressive agents. Notably, the association of rituximab and PML actually predated that of natalizumab (Goldberg et al. 2002), but until the latter entered the limelight, little attention was paid to the former. Although currently still dwarfed by the number of AIDS-related cases, in developed countries where HIV control has substantially improved from early in the disease and autoimmune disease is common, the growth in number of iatrogenic PML cases has spawned new fear of this disease (Fig. 1).

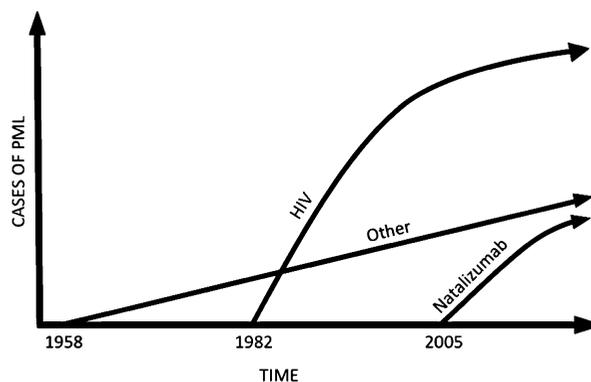
Epidemiology and Evolution of JC Virus

The JC virus is ubiquitous throughout the world. It has been divided into seven types by sequencing

complete genomes and determining coding region polymorphisms. The types are labeled 1–8, with several subtypes (indeed, type 5 is actually a subtype of 3) (Ferenczy et al. 2012). Virus types have been used to track human migration patterns, and all seem to have originated in sub-Saharan Africa.

Type 6 is thought to be the original virus from which other types evolved (Hirsch et al. 2013). The major types in North America are 2A, coming from Asia via the Northwest, and 4, which came trans-Atlantically from Europe. The different types appear to impact clinical disease expression. Differences in VP1 between type 4 and type 2B, the latter of which is common in Eurasia, are associated with lower and higher PML risk, respectively (Ferenczy et al. 2012; Hirsch et al. 2013).

Around 70–80% of adults are found to be seropositive for JCV antibodies (Berger 2010), and most initial infections are thought to occur during childhood. Since seroprevalence increases with advancing age, it follows that those not infected as children have a high risk of asymptomatic infection at some point. A single negative titer in an adult may need to be repeated in the future if there is new concern for risk, as there remains both a risk of JCV infection into adulthood and currently employed serological tests have substantial false-negative rates (Berger et al. 2013). Since the majority of all populations regardless of demographics are exposed to JCV, it



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 1 Graphic representation of cumulative PML cases (X axis) over time by associated condition. PML was first described in 1958 as a complication of hematologic malignancy and has been steadily associated with this and several autoimmune conditions at a low rate

since. In 1982, HIV heralded an exponential increase in the diagnoses of PML due to the AIDS pandemic. With the use of biologics, most notably natalizumab for relapsing-remitting multiple sclerosis, came the inception of a third wave of PML diagnoses

logically follows that the epidemiology of those who develop PML reflects that of the underlying predisposing condition. For instance, in the 1980s and 1990s, mostly men aged 20–50 were affected (Berger et al. 1998), pursuant to the epidemiology of HIV. Since 2005 when natalizumab became a dominant risk factor, the epidemiology in that population follows that for MS in general, being mostly young adult females (Bloomgren et al. 2012).

JCV antibody positivity is measured by a variety of mechanisms all targeted at the main capsid protein, VP1. Seropositivity is higher in those above the age of 60 and in men. Reported false-negative rates vary from 2.5% to 2.7% (Hirsch et al. 2013) to more than 30% (Berger et al. 2013). This latter observation is important in iatrogenically immune-suppressed populations at risk for PML such as those with MS receiving natalizumab, as a negative JCV antibody could be misconstrued as conferring lower risk of disease (Major et al. 2013). However, the JCV antibody is not protective, and the fact that deficiencies in cell-mediated immunity predispose to PML demonstrates that humoral immunity has no or little role in clearing reactivated virus.

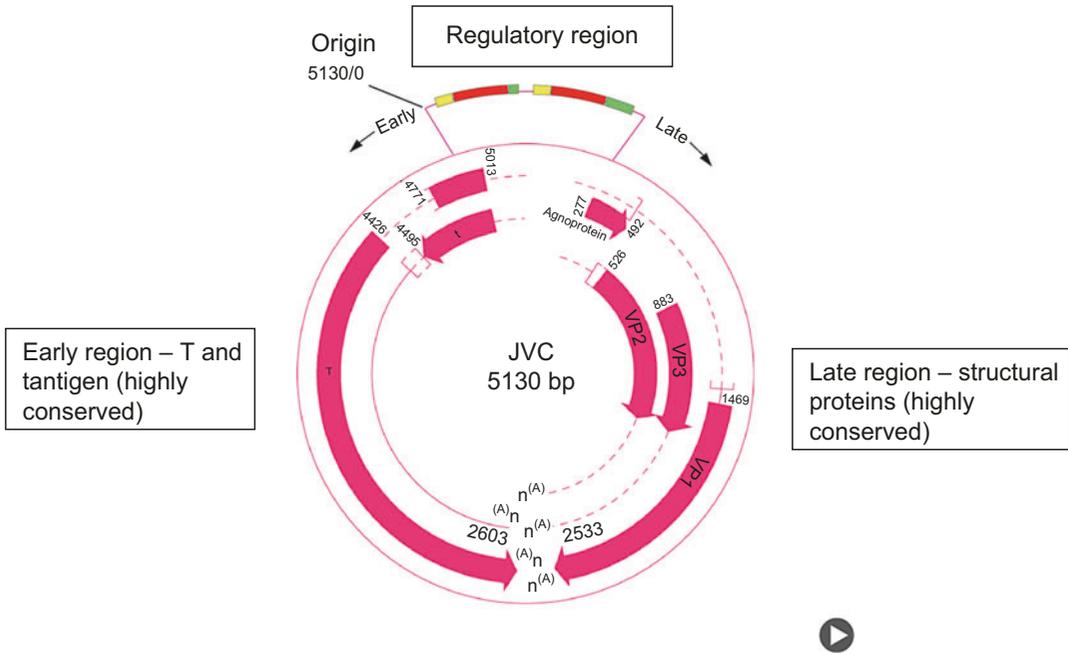
Molecular Biology of JC Virus

PML was named in 1958 when Aström, Mancall, and Richardson described it pathologically; they are credited for initial pathological descriptions, although in their review in the literature, they raise the possibility that earlier investigators may have reported the same disease (Astrom et al. 1958). The initial descriptions were all entirely pathologic. They detailed large plaques of myelin breakdown in the subcortical white matter involving U fibers and even the deepest layers of cortex, but largely there was axonal sparing. These plaques started small and often posteriorly, but became progressively larger to the point of confluence. The investigators also remarked about reactive astrocytes of bizarre shapes and sizes as a commonality among cases. Macroscopic exam did not show lesions in the brainstem or cerebellum. The precise etiology of the lesions was

unknown. In 1959, Cavanagh and Greenbaum proposed a viral etiology given the electron microscopic appearance of nuclear inclusion bodies in the enlarged oligodendrocytes (Cavanagh et al. 1959) that appeared to be what is seen in papovavirus infection. This virus was not previously known to result in central nervous system disorders. Subsequently, Padgett described confirmation of papovavirus as the culprit after viral isolation from glial cell cultures of a patient with PML named John Cunningham (Padgett et al. 1971), whose initials became attached to this new polyomavirus, formerly a genus of the *Papovaviridae* family. Other polyomaviruses include Merkel cell, BK, and SV40. Although there previously have been descriptions of PML associated with these latter two viruses, those reports remain questionable (Stoner and Ryschkeiwitsch 1995).

JCV replication is only possible in human cells, and therefore, there are no animal models for PML. Among the attempts to understand the biology of JCV in an animal model is the development of a humanized mouse model of the virus (Tan et al. 2013). Immune-deficient, irradiated mice transplanted with human fetal bone marrow, thymus, and liver produce a replica of the human immune system, after which they are inoculated with either archetype virus (whose noncoding region matches that from JCV isolated from urine of asymptomatic individuals) or prototype JCV (whose noncoding region matches that from subjects with PML), also known as Mad-4. Inoculated animals remained asymptomatic, but JCV was detected in urine and/or blood periodically from several of the mice. This provides a basic framework for animal study of JCV and promises future advances in the study of PML specifically. Additionally, it may provide a platform in which to study therapeutics given that currently there are no specific effective medications to combat PML. Another animal model that has been proposed for the study of CNS infection with polyomavirus is the SHIV-infected rhesus monkey (Axthelm et al. 2004). Despite these efforts, there remains no animal model of PML.

JCV is a non-enveloped, double-stranded, circular DNA virus within an icosahedral capsid



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 2 The JC virus genome

(Fig. 2). The genome encodes early and late transcripts by counterclockwise and clockwise transcription, respectively. A total of six proteins are encoded, and additionally there is a 200-base pair noncoding region that helps orchestrate tropism to the nervous system and neurovirulence. Notably, differences in the noncoding region are found in the isolates of JCV from urine of asymptomatic individuals and those from the brains of patients with PML. Although it is unknown which type causes primary infection, the ubiquity of the archetype JCV in urine strongly implies that most, if not all, persons are initially infected with that form of the virus and in those individuals developing PML, alterations in the genomic sequence in the noncoding control region (NCCR) convert the virus to a neuropathogenic strain (Berger and Khalili 2011). T antigen, an early, nonstructural protein, directs expression of the alternatively spliced transcript, which encodes three structural proteins and agnoprotein. T antigen also hijacks cell cycle machinery for replication, arresting the cell in S phase. This has potential for oncogenesis, but the role of JCV in promotion of tumors in humans remains

controversial (Beltrami and Gordon 2013) despite its association in vitro (Walker et al. 1973). The three structural proteins, VP1, VP2, and VP3, comprise the capsid. VP1 is the principal component of the virus's structure, making pentamers that assemble into an icosahedral capsid. It is responsible as such for viral binding to host cells; notably, a mutation in VP1 appears to be responsible for the ability of JCV to infect cerebellar granule cells, producing a clinically distinct clinical syndrome from PML referred to as JC virus granule cell neuronopathy (Dang and Koralknik 2006). VP2 and VP3 are present at a fraction of the amount of VP1 and are integral in viral assembly and subsequent uncoating and delivery. Agnoprotein assists in cell cycle dysregulation and interferes with DNA repair in order to promote viral replication; mutant forms of this protein enable JCV to productively infect cortical pyramidal neurons, producing JCV encephalopathy, another clinically distinct entity from PML (Dang et al. 2012). A small T-antigen protein is an early protein associated with JCV pathogenicity (Beltrami and Gordon 2013).

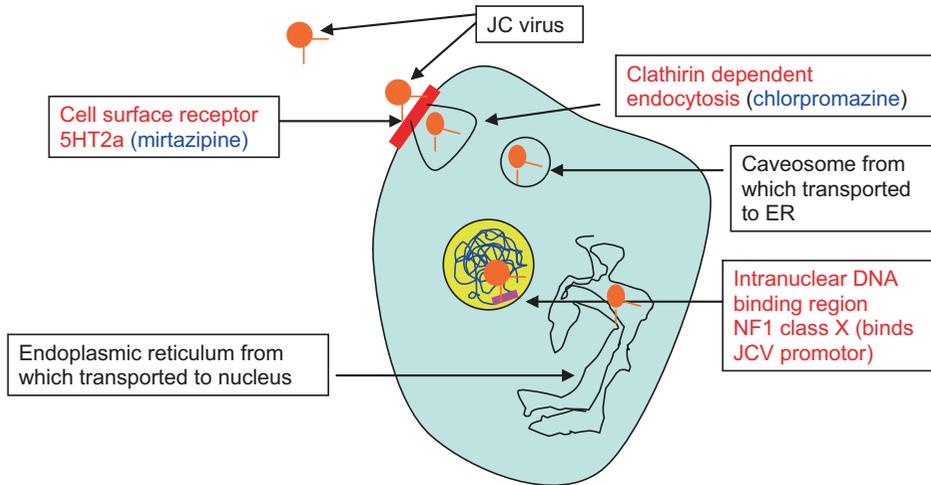
Pathogenesis of PML

Initial infection by JCV generally occurs within the first two decades of life, based on antibody seropositivity of up to 70–80% in adults (Berger 2010), and is asymptomatic. The inoculation site is thought to be along the oral or respiratory routes; JCV DNA has been detected in tonsils (Monaco et al. 1998; Kato et al. 2004) and gastrointestinal tissues (Laghi et al. 1999; Ricciardiello et al. 2000) of infected individuals. As mentioned above, it is presumed that archetype is the principal infecting agent, but conceivably prototype may initiate infection in some individuals. The mechanism by which people are infected and whether repeated infection can occur remains uncertain. The virus is asymptotically detected in blood and urine periodically regardless of HIV status; its reservoir is debated but thought to be principally the renal system or peripheral blood mononuclear cells (Berger 2010). However, JCV has been detected in the brain of patients without PML, and so clinically silent brain infection may be possible. Notably, despite the predominance of population-wide infection with JCV, only a vanishingly small fraction develops PML, indicating that establishing symptomatic brain infection is a formidable task for the virus. That is to say, in order for an individual to develop PML, he or she must have had primary infection by the virus followed at some point (and not necessarily in this order) by loss of immune capability of suppressing viral spread, noncoding viral genome alteration to the neuroinvasive form, dissemination of virus to the brain, establishment of productive infection in susceptible cells, and lesion expansion to the point of symptomatic presentation.

Where latent virus is cached and the events leading up to clinically overt brain infection and PML are incompletely understood. Primary infection gives rise to viremia, which could allow for virus to be deposited at multiple sites throughout the body. Hence, JCV DNA can be occasionally detected in the tonsil, kidney, white blood cells, and brain without detection of proteins, indicating a dormant infectious state, unless the virus has attached to or been phagocytosed by the cell, not

truly infecting it (White and Khalili 2011). While this may argue against B lymphocytes as a reservoir for subsequent JCV infection of the brain, there is compelling evidence for the role of the B lymphocyte in the pathogenesis of PML. For instance, intact JCV DNA without viral progeny has been found in CD34+ hematopoietic stem cells in the bone marrow, and experimentally these cells have correspondingly expressed a nuclear factor required for JCV replication in the brain (Monaco et al. 2001). Additionally, intact JCV virions can be detected inside B cells and have been demonstrated to be able to infect naïve glial cells in vitro (Chapagain and Nerurkar 2010). Furthermore, the B cell is the one somatic cell that has the genetic machinery necessary to rearrange the viral genome. A leading hypothesis for the pathogenesis of PML is that latent archetype JCV in the kidney and renal pelvis seeds B lymphocytes where the virus is transformed to a prototype virus by genomic rearrangement of the NCCR and is rendered capable of replicating effectively in glial cells. At the time of immune suppression, the virus enters the brain establishing active infection. An alternative explanation is that the latency lies actually in the brain itself, and periodic replication, perhaps influenced by Tat, could result in productive demyelinating infection. However, there has been evidence of JCV DNA peripherally circulating prior to the onset of PML that was genetically identical to that isolated from the brain after the emergence of clinically manifest disease, which argues against this postulate (White and Khalili 2011).

How JCV enters the brain is another topic of debate. Several plausible mechanisms have been proposed. First, as infected B lymphocytes can be found in the vicinity of PML plaques, there is potential for a “Trojan horse” mechanism whereby these infected cells are chaperoned across the unsuspecting blood-brain barrier (BBB) by cytokines and chemokines. This is a scenario whereby the most parsimonious explanation for latency is indeed in B cells. Additionally, however, there is evidence that brain microvascular endothelial cells can harbor virus, which may allow for cell-free virus to cross the BBB.



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 3 Steps to viral entry and transfer to the nucleus

In order to gain entry into cells for initial infection and spread, JCV binds sialic acid moieties on cell surface glycoproteins and glycolipids (Fig. 3). It also secondarily uses the 5HT_{2A} receptor. This leads to clathrin-mediated cell entry, delivery into the endoplasmic reticulum, and retrograde transport to the cell nucleus. The virus subsequently replicates and packages progeny virions, ultimately lysing the cell and propagating infection. In the brain, oligodendrocytes are usually the primary targets of productive infection. Astrocytes and even cortical neurons can have viral proteins identified within them, suggesting potential for infection. Additionally, productive infection of cerebellar granule cells (Dang and Koralknik 2006) and even cortical pyramidal neurons (Dang et al. 2012) require VP1 and agnoprotein mutations, respectively, and produce distinct clinical entities. The time to development of PML from infection of brain cells is unknown given recent work unmasking asymptomatic infection, but regardless, the disease is strongly associated with immune compromise-mediated reactivation of latent virus. As cytotoxic CD8 T cells are responsible for clearance of infection and CD4 T-cell trafficking and signaling to these cells is important in keeping infection at bay, a backdrop of low CD4 counts in the HIV-infected population predominantly precedes the development PML. The obverse is that in IRIS, once CD4 and CD8

cells are disproportionately rebounding, antigen detection can precipitate an exaggerated cytotoxic response, which in turn mediates potentially severe bystander damage.

Outside of the context of IRIS, however, PML is actually characterized by a lack of inflammation. In large measure, this is due to the nature of the disorder, but the inability to mount an effective immune response in affected individuals is likely contributory. There are several points along the pathway of JCV clearance that are susceptible to failure. CD4 T cells are charged with antigen recognition of JCV, mostly of the VP1 protein; any physical or functional infirmity of these cells will cause a decline in their surveillance ability. In HIV, infection of CD4 cells results in massive loss. Next, CD4 cells present recognized antigen to cytotoxic CD8 cells, which target and destroy infected cells to prevent viral propagation. In monoclonal antibody treatment, one theory is that these cells are either sequestered in the periphery and therefore are unable to cross the blood-brain barrier to reach infected cells (Diotti et al. 2013). As such, JCV infection itself leads to necrosis of the oligodendrocyte, releasing virions to neighboring cells and causing death to the myelin sheath of neurons, thereby exposing axons and impairing conduction. The result is clinical symptomatology depending on site and extent of demyelination. Histologically, infected

oligodendrocytes display viral inclusions and nuclear enlargement prior to death. Viral particles are spread to adjacent cells, and infection spreads progressively. Astrocytes, also infected by JCV but incapable of supporting complete viral replication, develop bizarre cellular forms with mitotic activity. Histiocytes and macrophages enter the scene to phagocytose the myelin debris.

Host Factors and Underlying Diseases

As a general rule, PML does not occur in young, otherwise healthy individuals. However, profound immune suppression due to HIV or iatrogenic immune modulation is not always required for its emergence, although these are the most common scenarios. Indeed, the first cases were reported in the setting of non-Hodgkin lymphoma and chronic lymphocytic leukemia. While any hematologic malignancy may predispose to PML, B-cell malignancies appear to carry the greatest risk. Additionally, there have been reports of PML in patients with otherwise subtle forms of immune suppression not typically thought to be overwhelming enough to place one at risk for PML, such as idiopathic CD4 cell lymphocytopenia, pregnancy, dermatomyositis, alcoholic liver cirrhosis, dementia, renal failure, or advanced age (Gheuens et al. 2010; Christakis et al. 2013). That said, a PML diagnosis in a patient without HIV or predisposing treatment with immunosuppressive or biologic agents for autoimmune disease, malignancy, or organ transplantation is extremely rare and noteworthy.

Far and away, the most common scenarios for PML to manifest are that of advanced HIV followed by autoimmune disease treated with biologic agents, in particular, multiple sclerosis treated with natalizumab. The contribution of the underlying autoimmune disease to the development of PML is often difficult to separate from that of the immunosuppressive treatment used for the condition, but it clearly appears that some disorders predispose to PML, including rheumatoid arthritis, sarcoidosis, and systemic lupus erythematosus (Brooks and Walker 1984; Amend et al. 2010). Solid tumor malignancies

treated with chemotherapeutic agents such as cyclophosphamide have also been a backdrop for the development of PML (Marshall and Major 2010; Pugnet et al. 2013), and PML is seen as well in the setting of hematological and solid organ transplantation (Mateen et al. 2011; Kaufman et al. 2014).

PML in the Era of AIDS

The HIV pandemic greatly increased awareness of PML. Prior to, PML was extremely rare, but it became much more common after the appearance of HIV. Before the advent of cART, studies showed that 4% of patients with AIDS had PML (Berger et al. 1987, 1998); some studies showed even higher numbers of 7% (Lang et al. 1989) and 9.8% (Kuchelmeister et al. 1993), although referral bias may have been at play in the higher numbers. It is an “AIDS-defining illness,” hallmarking advanced infection. Although PML epidemiology is evolving, given the sheer numbers of people with HIV worldwide, PML remains most commonly observed in this population.

There are several potential reasons why PML is disproportionately seen in HIV+ individuals due to many aspects of the nature of HIV infection. First are the specifics of immune suppression elicited by HIV. CD4 cells, infected and destroyed by HIV, are important in prevention of PML, as although it is the cytotoxic CD8+ cell that clears the virus, HIV-mediated loss of CD4+ surveillance, and subsequent activation of CD8+ cells likely heralds PML. Next is the long duration of HIV infection prior to overt evidence of profound CD4 loss that seems to be necessary to develop PML. This is corroborated by the data on PML in MS patients treated with natalizumab, as the risk increases with duration of treatment (Bloomgren et al. 2012), suggesting that prolongation of immune suppression increases the likelihood of PML. Next, HIV enters the brain early in the course of infection and from there establishes a state of chronic infection, immune dysregulation, and/or BBB disruption, which may facilitate JCV entry into the brain and/or promote viral replication. Additionally, the HIV

protein Tat, which is constitutively active in HIV infection and can be released extracellularly, has been shown to increase JCV replication and transcription (White and Khalili 2011). Finally, HIV infection activates B cells, which have been proposed to serve as a reservoir of JCV as well as a potential site for the evolution of the neurotropic form of the virus from the archetype.

With the advent of cART, not only has survival from PML improved, but also the incidence overall has dropped. Although the numbers of those with HIV continue to swell, those with AIDS-defining illnesses have substantially diminished (Deeks et al. 2013); this is indicative of better control of HIV overall and less profound immune suppression, thereby greatly reducing the risk of PML. Additionally, with improved diagnostics and understanding of PML and HIV, prompt initiation and efficacy of antiretroviral therapy have also played a role. In a study of AIDS patients diagnosed with PML divided into groups based on cART era (1996–2000, 2001–2006, and 2007–2011), the incidence of PML diminished, and the survival increased in the later cART groups (Casado et al. 2013). Incidence dropped from 14.8 cases/1,000 persons/year to 2.6 and 0.8, respectively, for each of the groups. Additionally, the 2007–2011 group had higher CD4 counts at the time of diagnosis and a more inflammatory CSF profile, which were also linked to prolonged survival. Other studies have borne out that higher CD4 counts at the time of PML diagnosis in AIDS, along with lack of prior AIDS-defining illness, portend prolonged survival (Berger et al. 1998; Antinori et al. 2003a). With this as an example, as we continue to improve on initiation of antiretrovirals earlier in HIV infection, the statistics for AIDS-associated PML may continue to improve.

PML in Other Populations

After the shift in PML epidemiology from being rare and associated with hematologic malignancy to being relatively common in association with advance HIV in the 1980s, the face of PML

again changed in 2005 after three cases were reported as complications of the monoclonal antibody to $\alpha 4$ integrin used in the treatment of multiple sclerosis and Crohn's disease (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005; Van Assche et al. 2005). This opened a Pandora's box of PML cases in association with various scenarios of immune suppression. Biologic agents targeted at specific points of the immune system seem to carry the highest risk (Berger 2010), particularly, the $\alpha 4\beta 1$ integrin inhibitor, natalizumab, and the antilymphocyte function-associated antigen antibody, efalizumab (Zaheer and Berger 2012). Less specific immunosuppressive medications, such as chemotherapeutic agents, mycophenolate mofetil, and even dysregulated immune systems due to underlying autoimmune diseases have been implicated. As the immune system in these individuals is not irreversibly compromised as in HIV, survival rates have been better than with AIDS-associated PML provided the offending agent can be removed and its effects reversed.

Natalizumab is the most efficacious medication for preventing MS relapses, and it is thought to do so by preventing immune cell diapedesis across the blood-brain barrier. This may also be the predisposing factor for development of PML, as periodic immune surveillance in the CNS is critical to prevent infection. Once there were cases of PML linked to natalizumab, it was removed from the market but subsequently reinstated with a strict surveillance program, and cases of PML were scrutinized for risk factors and outcomes. The major risk factors for developing PML in natalizumab treatment were three: evidence of prior JCV exposure as measured by JCV antibody positivity, prior use of immunosuppressive agents, and duration of natalizumab therapy longer than 24 months. Five categories of risk were then created. The first was those who did not have JCV antibody positivity. Their risk was 0.09 or less/1,000 patient years. Those with JCV antibodies but who did not have prior immunosuppressant use and had less than 24 months of natalizumab treatment had a risk of 0.56/1,000 patient years. For those who had prior immunosuppressant use in addition to JCV antibodies but

who had less than 25 months of natalizumab, the risk was 1.6/1,000 patient years, regardless of type or duration of prior immunosuppressant. Next, those with JCV antibodies, no prior immunosuppressant use, and 25 or greater months of natalizumab therapy had a risk of 4.6/1,000 patient years. Finally, those with all risk factors, that is to say JCV antibody positivity, prior immunosuppressant use of any sort, and 25 or greater months of natalizumab therapy, had the highest risk of PML at 11.1/1,000 (Bloomgren et al. 2012). This information has helped select the patient appropriate for natalizumab therapy.

Other biologic agents and immunosuppressive agents have been associated with PML, but not nearly to the degree of natalizumab, as mentioned above. Dimethyl fumarate, approved in the United States in 2013 for the treatment of multiple sclerosis, is attractive because of its oral route of administration and superior efficacy and adverse effect risk profile over the other oral agents. As such, it promises widespread use for MS treatment, but soon after its release, it had already been linked to PML (Ermis et al. 2013).

The majority of PML cases occur in the setting of HIV and natalizumab treatment. A study that predated the natalizumab era of PML found that between 1998 and 2005, 80% of the nearly 10,000 cases identified were in the setting of HIV. Less than 10% were due to hematologic malignancies, and diagnoses of autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, accounted for less than 1% each (Molloy and Calabrese 2009). From a database of nearly 175,000 patients with autoimmune disease, non-Hodgkin lymphoma, chronic lymphocytic leukemia, and transplants, frequencies per 100,000 cases of 2.4 for SLE, 10.8 for autoimmune vasculitis, 8.3 for NHL, and 11.1 for BMT were reported (Amend et al. 2010).

Clinical Disease

The clinical manifestations of PML are protean, but the most common threads in patient

presentations are cortical or cerebellar dysfunction. Hemiparesis, sensory ataxia, limb apraxia, gait disturbance, hemianopia, and cognitive decline are typical and owe to the large areas of subcortical white matter involvement in corresponding areas, such as frontal lobes for motor weakness, occipital lobes for visual field deficits, and cerebellar inflow tracts for gait ataxia. Cortical weakness occurs in about 60% of PML presentations, gait disturbance in 65%, and visual field cut in 20% (Aksamit 2012). The most common cognitive complaints are memory loss and behavior disturbances and are the presenting symptoms about 30% of the time. Headache often accompanies. Focal seizures that can secondarily generalize occur in as many as 10% of patients. Although white matter in the basal ganglia and brainstem can be involved, referable symptomatology is relatively uncommon, especially in PML that is not associated with HIV. Additionally, as the spinal cord is spared, presentations indicative of transverse myelitis are essentially never seen.

Historically, PML has been the AIDS-defining illness about 1% of the time (Berger et al. 1998). The overall prevalence is estimated at up to 4–5% in presumed clade B cohorts (Berger et al. 1987). As most research is with HIV-1 clade B, statistics for HIV-2 and other HIV-1 clades are largely unknown, but a study of a clade C cohort found a similar prevalence of PML at 2.8% (Netravathi et al. 2013). In a series of HIV-associated PML patients, PML was the AIDS-defining illness for 27% (Berger et al. 1998), indicating that the disorder may herald the diagnosis of HIV.

As mentioned above, JC virus genetic variants give rise to disparate cellular tropism and, as such, produce distinct clinical presentations. JCV encephalitis, produced by JCV infection of cortical pyramidal neurons, to date has only been described in a single HIV-negative lung cancer patient who presented with aphasia and cognitive decline (Wuthrich et al. 2009). JCV cerebellar granule cell neuronopathy spares supratentorial brain to preferentially infect cerebellar granule cells, producing cerebellar atrophy that manifests as ataxia, speech disturbance, and gait abnormalities (Koralnik et al. 2005).

Neuroimaging

In the appropriate clinical context, characteristic neuroimaging can be used with CSF studies to diagnose PML (Berger et al. 2013). Computed tomography (CT) of the head is not as sensitive as magnetic resonance imaging of the brain but can reveal patchy hypodensities in the affected subcortical white matter (Fig. 4). There may be sub-gyral scalloping where there is involvement of U fibers.

MRI is extremely sensitive for demonstrating JCV-mediated abnormalities in symptomatic patients and is the imaging modality of choice for PML. A normal MRI in fact argues strongly against the diagnosis. Characteristic MRI findings are of bilateral, asymmetric, multifocal white matter plaque-like lesions that are T1 hypointense and T2/FLAIR hyperintense. Unifocal lesions affecting but one hemisphere are observed and may be more common with natalizumab-associated PML than with HIV. Notably, in the absence of PML-IRIS, there is no edema or mass effect (Fig. 5). Diffusion

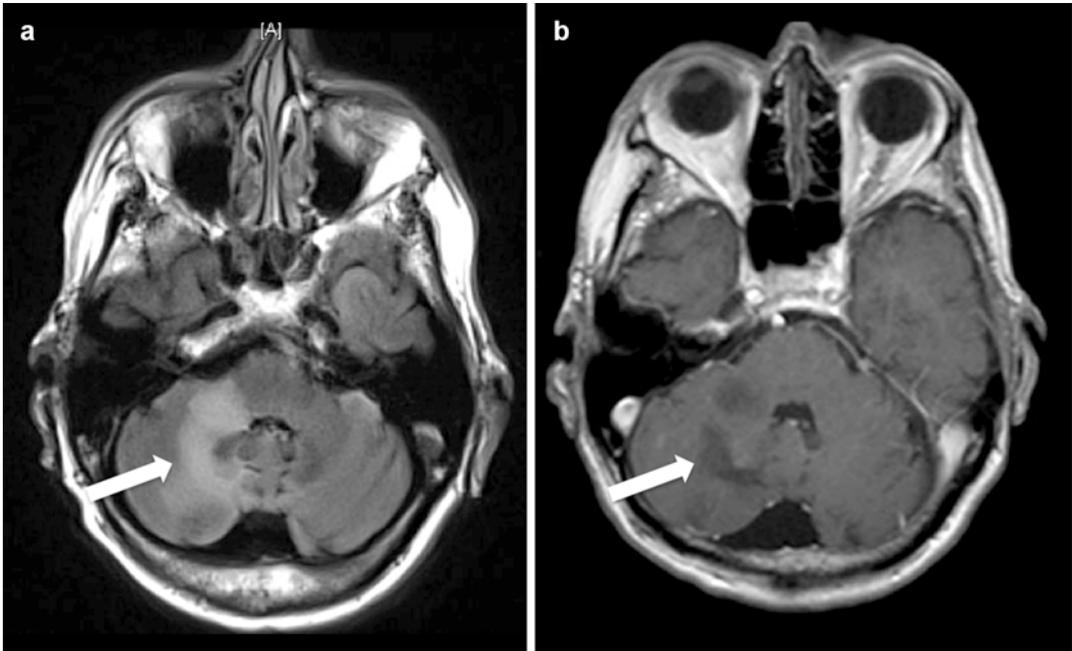
restriction is common at the advancing edge of the lesions, but this finding wanes with time (Sahraian et al. 2012). Additionally HIV-associated PML demonstrates enhancement in only about 15% of cases (Berger et al. 1998). Enhancement is more commonly seen in natalizumab-associated PML or PML-IRIS, although even in this scenario, enhancement is only seen about 40% (Yousry et al. 2012) and 50% (Tan et al. 2011) of cases, respectively.

MR spectroscopy, a modality that evaluates parenchymal health by measuring metabolites and often comparing to creatine as an internal control, demonstrates low ratios of N-acetylaspartate to creatine and high ratios of choline to creatine and lactate to creatine (Sahraian 2012). These findings reflect increased cell death and membrane turnover. A longitudinal MRS study showed less neuronal loss, but higher membrane turnover at the periphery of a PML plaque in a single patient, suggesting increased activity of the virus at the leading edges of the lesion (Yoon et al. 2007); this finding logically follows the pattern of pathologic advancement of PML lesions.

Magnetic resonance neuroimaging in PML is usually quite distinct from other intracranial lesions that are seen in HIV when the CD4 count is low (Fig. 6). Toxoplasmosis and primary CNS lymphoma (PCNSL) are typically associated with surrounding edema and mass effect, and enhancement is not uncommon. Deep gray structures are commonly affected. Toxoplasmosis lesions frequently have blood products evident on gradient echo imaging, and rim enhancement is common. PCNSL can have variable enhancement patterns, and because the middle cerebellar peduncle (MCP) is a common site for lymphoma, a well-placed solitary PML lesion in the right MCP has been reported as an imaging mimic of PCNSL (Westwood et al. 2013). Infarcts obey vascular boundaries and also tend to involve the cortical ribbon, both in contradistinction to PML. Also unlike PML, meningitides commonly seen in immune suppression, such as tuberculosis and *Cryptococcus*, can show sulcal and basilar meningeal abnormalities on T2/FLAIR or after the administration of gadolinium, but the imaging in these maladies can also be normal. Other



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 4 Axial computed tomography imaging of the head in PML demonstrating diffuse, confluent subcortical white matter hypodensities bilaterally (arrows)



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 5 Magnetic resonance brain imaging in PML. (a) Axial T2-weighted fluid-attenuated inversion recovery (FLAIR) sequencing at the level of the cerebellopontine angle in a 63-year-old with untreated HIV and no prior AIDS-defining illness demonstrates

hyperintensity without mass effect in the *white matter* tracts of the middle cerebellar peduncle on the right. (b) Axial T1-weighted imaging after the administration of gadolinium at the same level demonstrates characteristic hypointense signal without enhancement

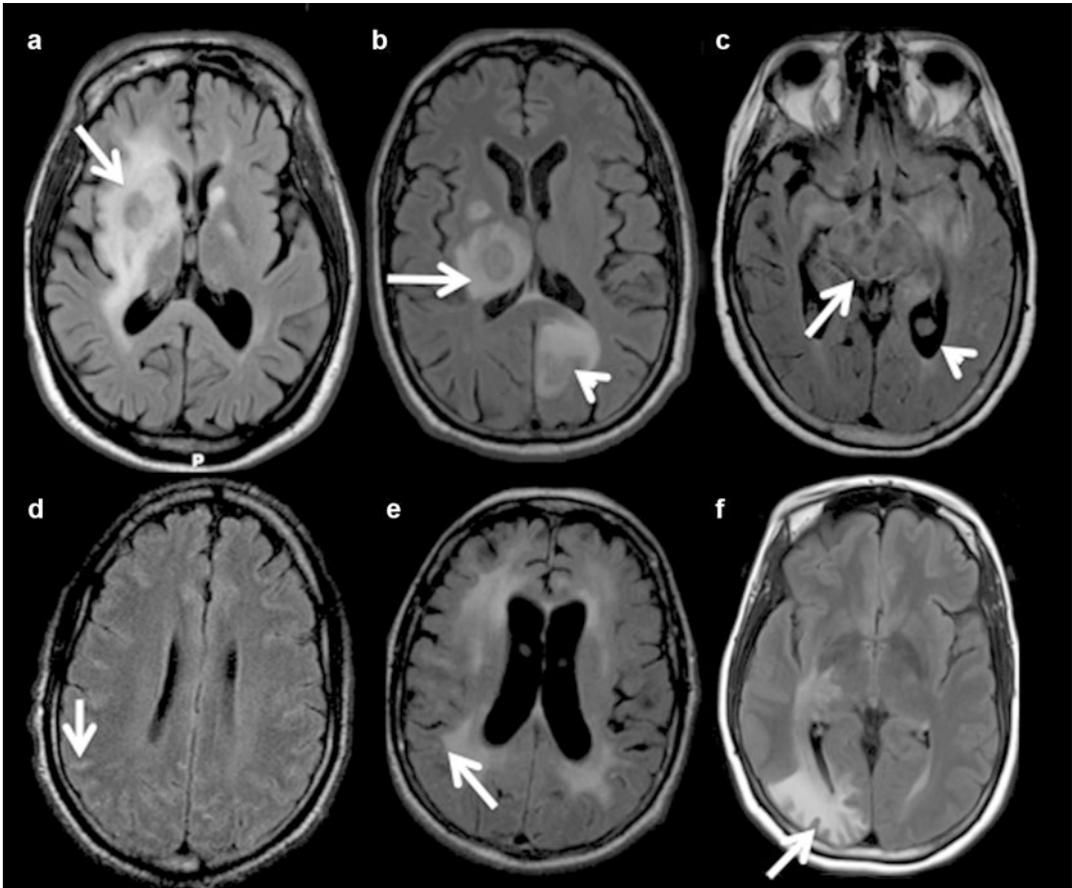
infections, such as bacterial brain abscesses, tuberculomas, or cryptococcomas, also differ from PML in both the presence of one or more mass lesions, the presence of surrounding edema, and the presence and characteristics of contrast enhancement. In differentiating from other demyelinating diseases, such as multiple sclerosis (MS) or acute disseminated encephalomyelitis (ADEM), the large size and confluence of plaques, predominantly subcortical location with involvement of subcortical U fibers, and sparing of spinal cord coupled with the appropriate clinical presentation argue in favor of PML. Finally, HIV infection alone frequently causes white matter hyperintensities on T2/FLAIR imaging, but these are typically diffuse, are T1 isointense, and heavily involve basal ganglia and periventricular white matter, sparing the U fibers. There is also a significant amount of global atrophy seen secondary to HIV infection although this can be seen in PML owing to the fact that there is

underlying HIV that likely also is affecting the brain.

Laboratory Studies

In HIV-associated PML, advanced disease causing immune suppression to a CD4 count below 200 cells/mL or 14% in blood is the general tenet. Although in some series, the mean has been around 90 cells/mL (Berger et al. 1998), numbers can be higher. According to one PML series, about 10% had CD4 counts greater than 200 cells/mL (Berger et al. 1998). In HIV, there have been no studies on JCV antibody positivity and risk, but in natalizumab-associated PML, JCV antibody positivity was found in nearly all patients with PML (Bloomgren et al. 2012; Major et al. 2013).

CSF studies are helpful both in diagnosis of PML and in evaluation of other diseases. The



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 6 Characteristic magnetic resonance fluid-attenuated inversion recovery (MR FLAIR) findings in the brain for various infections complicating advanced HIV. (a) EBV-associated primary CNS lymphoma typically demonstrates solitary deep lesions, here seen in the right basal ganglia (*arrow*), with significant surrounding mass effect and rim enhancement on T1 post-gadolinium imaging (*not shown*). (b) Toxoplasmosis typically demonstrates multifocal deep lesions, here seen in the right thalamus (*arrow*) and left temporal/parietal subcortical gray matter (*arrow head*), which rim-enhance on T1 post-gadolinium imaging (*not shown*) and may have elements of microhemorrhage on susceptibility-weighted imaging (*not shown*). (c) Tuberculous meningitis typically shows

brainstem leptomeningeal hyperintensity (*arrow*), better visualized on T1 post-gadolinium imaging, with ventricular enlargement (*arrow head*), indicating obstructive hydrocephalus. (d) Cryptococcal meningitis may show sulcal hyperintensity (*arrow*) and cortical leptomeningeal enhancement on T1 post-gadolinium imaging (*not shown*), with ventricular enlargement (*not shown*), but imaging may also be entirely normal. (e) HIV leukoencephalopathy demonstrates diffuse atrophy and periventricular *white matter* hyperintensity without mass effect that spares the subcortical U fibers (*arrow*). (f) PML demonstrates confluent subcortical *white matter* hyperintensity without mass effect, which involves the subcortical U fibers (*arrow*) and may not demonstrate concomitant ventricular enlargement, as shown here

typical profile is an acellular or perhaps lymphocytic-predominant paucicellular (up to 20 cells/mL) CSF with normal to moderately elevated protein and normal glucose (Berger et al. 1998). Oligoclonal bands can also be detected but are usually best ascribed to concomitant HIV infection. Other viral etiologies or

syphilis, especially in advanced immune suppression, may have a similar profile. Notably, HIV by itself can give this CSF profile. Marked pleocytosis, especially if polymorphonuclear cell predominance and/or with hypoglycorrhachia, should raise suspicion of other diagnoses. Bacterial meningitis, for example, tends to give a very

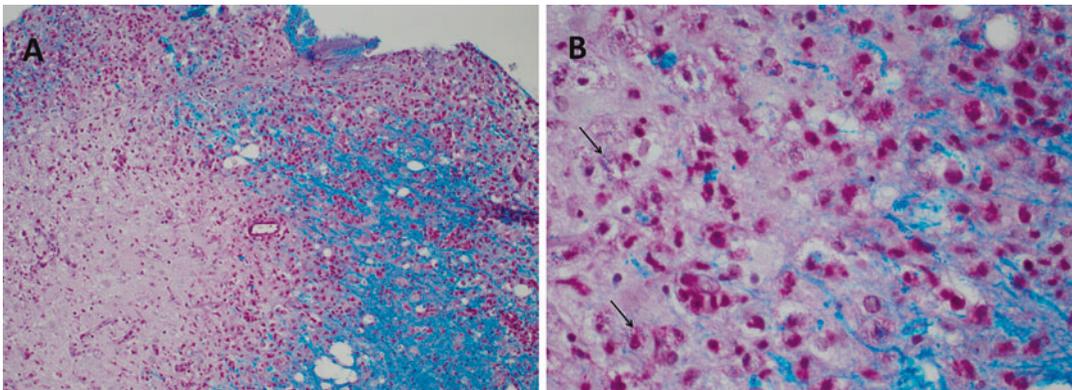
high, neutrophil-predominant CSF pleocytosis with low to undetectable glucose and extremely high protein. Tuberculosis and fungal infections also typically show a moderate to high pleocytosis and low glucose. As such, evaluation for HIV RNA, cryptococcal antigen, acid fast bacillus PCR, Venereal Disease Research Lab (VDRL) testing for syphilis, varicella zoster DNA, cytomegalovirus DNA, herpes simplex 1 and 2 DNA, and/or Epstein-Barr virus DNA may be included in the CSF studies where clinically appropriate. In 1992, polymerase chain reaction (PCR) detection of JCV DNA from CSF is available (Telenti et al. 1992), and this has proved to be extremely useful. In fact, detection of JCV DNA in CSF coupled with characteristic imaging in the correct clinical setting obviates the need for biopsy to diagnose PML. The sensitivity is only about 75–90%, owing to one or more of low copy number, specimen handling, or specimen quantity. Specificity is upward of 100% (Koralnik et al. 1999). Recent quantification assays boast ability to detect JCV in CSF at ten copies/mL (Ryschewitsch et al. 2013).

Pathology

Historically, PML has been a pathological diagnosis. The initial descriptions depict widespread multifocal demyelination (Fig. 7), bizarre

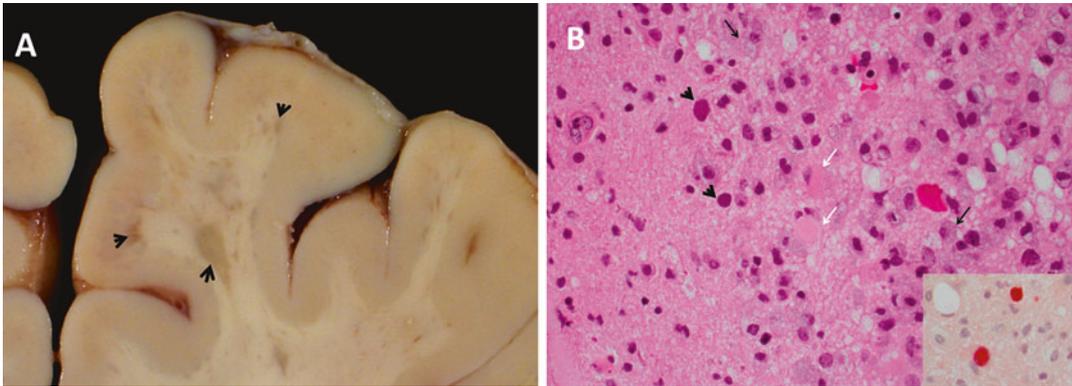
astrocytes with lobulated nuclei, and enlarged nuclei in oligodendroglial cells by immunohistochemistry stain for JCV (Fig. 8). There is also characteristically a lack of inflammatory cells in the lesions, and neurons are spared of infection. Astrocytes may harbor infection but largely are thought to be reactive (Astrom et al. 1958). As noted elsewhere, the combination of neuroimaging, CSF studies, and clinical picture has largely supplanted the necessity of biopsy in PML.

Other clinical entities produced by JCV that depend on various genomic mutations to produce varied proclivity within the central nervous system include granule cell neuronopathy (JCVGCN) and JC virus encephalopathy (JCVE). Both entities are extremely rare. The pathology underlying these entities is distinct from PML. In JCVGCN, the white matter appears spared and oligodendrocytes are not infected. Instead, there is selective depletion of cerebellar granule cells, Purkinje cell sparing, and enlarged, hyperchromatic, atypical nuclei in surviving granule cells; immunohistochemistry reveals evidence of JCV infection restricted to cerebellar granule cell neurons (Koralnik et al. 2005). JCVE demonstrates laminar necrosis at the gray/white junction and bizarre, multinucleated astrocytes. Immunohistochemical staining reveals JCV infection of pyramidal neurons and astrocytes but overall sparing of oligodendrocytes (Wuthrich et al. 2009).



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 7 (a) Luxol fast blue (LFB) stain for myelin and periodic acid-Schiff (PAS) stain for glycogen from a PML lesion at 100× magnification show myelin

loss on the left hand side of the photo. (b) 400× magnification of lesion from (a) shows highlights macrophages (arrows) containing PAS+ and small amount of LFB+ debris (Photos courtesy of Rebecca D. Folkerth, MD)



Comorbidity: Progressive Multifocal Leukoencephalopathy, Fig. 8 (a) Gross coronal section of the right posterior frontal lobe, showing punctate and larger confluent plaques in subcortical white matter (*arrowheads*) abutting the *gray/white* junction. (b) Hematoxylin and eosin (H&E) stain of a plaque at 400 \times demonstrates

infiltrate of macrophages (*black arrows*), glial cells with “smudgy” nuclear inclusions (*arrowheads*), and enlarged, bizarre astrocytes (*white arrows*). Inset: immunohistochemistry for polyomavirus is positive in glial nuclei and corresponds to the “smudgy” changes seen on H&E (Photos courtesy of Rebecca D. Folkerth, MD)

PML-IRIS is yet another pathological entity related to JCV; the difference between PML and PML-IRIS is the fact that the reconstituting immune system causes an inflammatory parenchymal brain reaction in addition to typical PML pathology. This phenomenon has not been described for JCVGCN or JCVE. In these lesions, there is accentuated PML pathology with massive demyelination and macrophage reaction, and additionally there is a perivascular CD3+ cell infiltrate predominated by CD8+ cells (Vendrey et al. 2005).

Diagnosis

Diagnostics for PML have improved considerably since its initial description. Whereas the gold standard initially for diagnosing PML was based on histopathological demonstration of the characteristic triad of demyelination, bizarre astrocytes, and oligodendroglial nuclear inclusions, a recent consensus statement from the American Academy of Neurology proposed alternative means to reach a definitive diagnosis (Berger et al. 2013). Given the accuracy of characteristic neuroimaging and detection of JCV DNA in CSF, in a symptomatic patient with immune suppression or on immune modulatory therapy, these findings obviate the

need for biopsy to diagnose PML. If the clinical and neuroimaging pictures fit the diagnosis but the JCV PCR from CSF is negative, repeat testing should be performed along with work-up for other diagnoses. If there still remains diagnostic uncertainty, biopsy should be sought.

PML-Immune Reconstitution Inflammatory Syndrome

The immune reconstitution inflammatory syndrome is seen when a previously suppressed immune system recovers. It is neither specific to PML nor HIV infection. The events that occur basically amount to an expansion of CD4+ cells followed by a rapid expansion of CD8+ cells once the immune system is free to manufacture these cells. Antigen recognition activates the cytotoxic T cells, which in the setting of immune restoration can result in generation of a massive antigen-specific inflammatory reaction. This can occur when antigens are already present in sufficient quantities, meaning that the disease was overt prior to the ability of the immune system to recognize it; this is termed “paradoxical IRIS” because the previous disease clinically worsens once the immune system that predisposed to the disease is ramped up. Alternatively, if there is only

a minute amount of antigen around and/or the disease was not clinically manifested prior to the onset of IRIS, the situation is termed “unmasking IRIS” because the underlying disease was not known prior to the inflammatory reaction to it. In HIV, risks for developing IRIS are mostly related to rapidity of immune restoration, with precipitous drop in plasma HIV RNA levels and/or marked increase in CD4 T-cell counts. Given the amount of time for the cytotoxic cells to expand, this entity typically develops about 4–8 weeks after initiation of cART in the setting of HIV.

Treatment and Prognosis

Therapeutic strategies for PML have involved immune function restoration and the use of antiviral agents. The only unassailable favorable clinical responses have come from the former approach. In HIV, successful initiation and maintenance of combination antiretroviral therapy (cART) has changed the epidemiology of PML from a rapidly fatal disease to a potentially survivable one. The dismal survival rate of less than 6 months for most prior to cART with only 10% surviving past 1 year (Berger et al. 1998; Hall et al. 1998) has improved significantly. A study from 2003 found a cumulative survival beyond 1 year to be 58% in those who initiated cART at diagnosis of PML, who had been naïve prior to, and who had no other AIDS-defining illnesses, compared to 0% of those failing to receive antiretroviral therapy. Additionally, those with higher CD4 counts to begin with had a higher likelihood of survival (Antinori et al. 2003b); conversely, those with higher JCV DNA levels in CSF have been shown to have worse outcomes (Koralnik et al. 1999). The authors (JL and JB) continue to follow patients who suffered PML years ago but who have survived with variable disability after initiation of cART. It has also been mentioned previously that cART initiation to prevent immune suppression has also decreased the incidence of PML. As such it should go without saying that prevention is the best strategy. IL-2 (which stimulates T cells) and adoptive cytotoxic T-cell infusions of patients with T-cell deficiencies

unrelated to HIV have anecdotally been successful in patients battling PML (Przepiorka et al. 1997; Buckanovich et al. 2002; Balduzzi et al. 2011). These are notably case reports, making generalizability difficult, despite having conceptual logic. In PML associated with iatrogenic immune suppression, removal of the offending agent along with supportive care is the current recommendation, and survival rates are around 80% (Aksamit 2012). Reversal of natalizumab immune suppression by means of plasmapheresis has been frequently associated with an IRIS phenomenon that can be severe, although the relationship of plasma exchange to IRIS is not well understood, as nearly all patients in this scenario develop IRIS regardless (Tan et al. 2011).

Specific treatments for PML have also been studied, albeit not rigorously. These strategies have included targeting JCV replication and/or cell entry. For the most part, the supportive evidence for these strategies is derived from case reports or small series evaluated without standardized outcome assessments, which again limits generalizability and future clinical applicability of the information difficult. That said, there have been several concepts tested that have advanced the experience with the disease and may be able to help guide future work in the field.

Since JCV uses the 5HT_{2A} receptor for entry into cells, drugs that act to bind up the receptor ostensibly would prevent viral spread and have been tried as treatment. Indeed, ziprasidone, olanzapine, and risperidone have shown in vitro inhibition of JCV infection (Altschuler and Kast 2005). Such drugs have been tried alone or in conjunction with viral replication inhibitors with varying results, but unfortunately no appropriate large-scale trials have been undertaken. Chlorpromazine, a serotonin agonist, combined with the replication inhibitor cidofovir in a single patient was unsuccessful (Pohlmann et al. 2007). Several reports on mirtazapine have demonstrated promising results, with early initiation of treatment proposed as a positive prognosticator (Cettomai and McArthur 2009; Lanzafame et al. 2009). A report on the combination of mirtazapine with the nucleoside analog cytosine arabinoside also described a favorable outcome

(Vulliemoz et al. 2006). This all provides only a small amount of anecdotal evidence, however. What has recently been shown and what could explain the variable results of 5HT2A blockade are that JCV can infect cells independently of the 5HT2A receptor (Marshall and Major 2010). This fact may mean that successful treatment of PML may require medications that interrupt multiple sites along the pathway of productive infection.

Inhibition of viral replication using nucleoside analogs has also been attempted with varying results. Although there has been one trial for this concept by the AIDS Clinical Trial Group, the data is outdated (Hall et al. 1998). Most of the data for the use of viral replication inhibition again comes from anecdotal evidence. Agents that have been tried include acyclovir, cidofovir, cytarabine, zidovudine, and vidarabine. Toxicities with each of these medications can be devastating, though, and so their use is not without deleterious effect regardless of efficacy. However, several reports have demonstrated efficacy in AIDS-associated PML (Marshall and Major 2010). These are frequently not combined, but adding cytarabine and cidofovir has given favorable outcomes (Terrier et al. 2007). The only clinical trial of intrathecal or intravenous cytarabine added to antiretroviral therapy compared to antiretrovirals alone for the treatment of PML was a failure (Hall et al. 1998). The most notable point about the trial is that it was conducted before widespread use of cART. As such, the underlying antiretroviral therapeutic strategies used are now considered well antiquated, as NRTI therapy with zidovudine and didanosine was administered as the preferred therapy. Given what is now known about PML survival based on immune restoration, this regimen falls well short of what is currently accepted. Additionally, as cytarabine is not specific for JC virus, further immune inhibition without adequate design to reconstitute the HIV-infected immune system may very well have contributed to the failure. Finally, as has been argued above, the strategy of using combination therapy for cell entry plus viral replication inhibition may prove more successful than use of solitary agents.

Other inhibitors of viral replication have been identified. A drug screening study to identify inhibitors of JCV replication turned up diclofenac, mefenamic acid, flunixin meglumine, isotretinoin, and mefloquine (Brickelmaier et al. 2009; Marshall and Major 2010). As only mefloquine has activity beyond the blood-brain barrier, it has been studied for clinical utility in PML (Clifford et al. 2013). Notably, its mechanism of action is unknown, and in the study, it was not used in combination with any of the above anti-JCV medications. The trial was halted early due to interim data lacking a benefit to the treatment group.

Treatment for PML-IRIS is occasionally with judicious administration of high-dose corticosteroids followed by a prolonged taper. The CCR5 antagonist, maraviroc, has been advocated as a treatment of PML-IRIS (Giacomini et al. 2014). Importantly, there have been no controlled trials for the treatment of PML-IRIS, and the suggested therapies carry a potential pernicious effect on controlling JCV in the brain (Berger 2009). In severe IRIS, there is sometimes consideration of stronger immune suppression, although this practice would also pose a serious threat to the delicate immune system, especially if there is potential for other underlying infections. Notably, and particularly in PML whereby the only treatment known to be of utility is immune restoration, cessation of cART in IRIS, even temporarily, is largely not advised.

Risk Mitigation Strategies

Given that initial infection with JCV typically predates the situations in which patients develop risk for PML and that the virus is ubiquitous, prevention and surveillance strategies for primary infection are of low yield, at least in adults. Additionally, employing experimental agents such as mefloquine, mirtazapine, or cytarabine as prophylactic measures has not been evaluated, although in treatment studies these have failed to show convincing benefit.

In HIV, PML is strongly associated with immune suppression and typically with a CD4

count of <200 cells/mL. Therefore, prevention of immune suppression is paramount in reducing risk of PML in this population. At this time, the general practice in developed countries is to consider initiation of antiretroviral therapy at the time of HIV diagnosis regardless of CD4 count. The World Health Organization guidelines from 2013 prioritize treatment for the profoundly immune suppressed but recommend expanded access to all individuals with HIV at a CD4 count of 500 cells/mL or less.

For other populations at risk for PML, several strategies have been employed to mitigate risk. In multiple sclerosis patients treated with natalizumab, JCV antibody seropositivity, duration of exposure to the medication, and prior use of immunosuppressive therapy impart a much higher risk than the lack of these clinical factors (Bloomgren et al. 2012). Therefore, careful selection of the patient population in which to use the implicated medications is a prudent measure to lower risk. Those who are seronegative for JCV antibody and remain as such have a decreased risk, although as mentioned above, there have been false negatives in patients who developed PML (Major et al. 2013). Additionally, serial magnetic resonance imaging has been used as a surveillance tool for early or asymptomatic lesions that could be due to PML. Additionally, predictive biomarkers could be useful in forestalling disease; a recent study on I-selectin, an adhesion molecule expressed on the surface of CD4 cells, demonstrated significantly lower levels in MS patients treated with natalizumab who developed PML. For patients treated with dimethyl fumarate who developed PML, low lymphocyte counts have predated disease (Ermis et al. 2013). Prompt discontinuation of the offending agent once PML does develop is critical, although aggressive removal of drug via plasma exchange may precipitate an IRIS phenomenon, which can be fatal.

Risk mitigation strategies for PML-IRIS are not described. Given the teleology for JCV entry into cells utilizing the 5HT_{2A} receptor, an argument could be made for prophylaxis once starting cART at least in individuals with known

PML. However, since it is unknown how well these agents actually work to combat the virus, this argument is entirely speculative. Additionally, viral replication inhibitors, especially those with high rate of toxicities, would have to be validated in treatment before theorizing on their utility prophylactically.

Conclusion

PML is the clinical manifestation of the reactivation of the polyomavirus JC, which initially infects the majority of individuals around the world at a young age. Primary infection is asymptomatic, but the virus enters a dormant state and essentially lies in wait of barriers to PML development to be overcome. These include breakdown of immune function, specifically CD4 T-cell physical or functional loss; genetic rearrangements in the noncoding control region that permit neurovirulence; and passage from their site of latency to the brain parenchyma. Productive infection primarily of oligodendrocytes is established, and massive demyelination ensues, producing a clinical syndrome dominated by motor weakness, ataxia, and cognitive dysfunction. Diagnosis is now primarily made by characteristic neuroimaging combined with detection of JCV DNA from CSF, with histopathology becoming important in confusing cases. Treatment primarily lies in restoration of immune function, although if return is too rapid, a paradoxical, potentially fatal clinical worsening known as the immune reconstitution inflammatory syndrome can occur, requiring delicate orchestration of immune modulation to temper immune overactivity all the while continuing to allow for immune system rebuilding. This has changed the epidemiology of PML, which prior to cART was a primarily HIV-associated condition, to, increasingly, a complication of iatrogenic immune suppression for treatment of autoimmune disease and cancer. Although specific targets along the JCV infection cycle could elicit therapeutic options, to date none has proven efficacious.

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Conjunctival Carcinoma

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Definition

Conjunctival carcinoma encompasses several eye neoplasms that include squamous cell carcinoma (SCC) and malignant melanoma. In both cases the

patients tend to be older males, often with a significant history of chronic sun exposure. Ocular surface squamous neoplasia (OSSN) is a term used to describe conjunctival or corneal neoplastic growth; it encompasses a range of conditions from simple dysplasia to conjunctival intraepithelial neoplasia (CIN) and invasive SCC. There is evidence that conjunctival squamous cell carcinoma (CSCC) is becoming more common, more aggressive, and affecting more young people, especially women. Immunocompromised patients, including those infected with HIV, are predisposed to developing these neoplasms. There is mounting evidence that one or more infectious agents, especially types of human papillomavirus (▶ HPV), may be involved in the pathogenesis of CSCC, but there is still some uncertainty about the etiologic role of these agents and additional research is needed.

Introduction

Squamous cell carcinoma (SCC) is among the most common neoplasm of the conjunctiva (Kestelyn et al. 1990). The etiology of cancer of the conjunctiva appears to be multifactorial. Several risk factors have been identified or suggested, such as smoke, human immunodeficiency virus (HIV) infection, ultraviolet (UV) light, history of pterygium, and human papillomavirus infection (▶ HPV) (Newton et al. 1996). It is most often seen in sub-Saharan Africa and other regions with a high prevalence of HIV and intense sunlight. HPV has been proposed as an etiologic agent, but its role remains uncertain.

While there has been considerable interest in HPV as a cause of conjunctival squamous cell carcinoma (CSCC), the heterogeneous prevalence of high-risk HPV types in different studies suggests that only a subset of cases of CSCC can be attributed to these viruses (McDonnell et al. 1986). Differences in detection methods, populations, or geographical distribution could contribute to the variation in HPV infection rates in CSCC (McDonnell et al. 1986). HPV is considered the main etiologic agent in proliferative ocular surface, lacrimal sac lesions, and



Conjunctival Carcinoma, Fig. 1 (a) Representative patient with OSSN and a closer lateral view (b) and (c) H&E staining showing features of in situ carcinoma

characterized by full thickness changes in nuclear to cytoplasm ratio, nuclear pleomorphism, dyskeratotic cells, and presence of koilocytes (Simbiri et al. 2010)

pterygium worldwide, and it has been suggested that HPV types 16 and 18 may play a critical role in the oncogenesis of conjunctival cancers (Hirst et al. 2009; Palazzi et al. 2000). HPV is a group of host-specific DNA viruses with oncogenic subtypes that have also been shown to act as carcinogenic agents in the development of ► cervical cancer, anal carcinoma (► Anal Cancer), penile carcinoma, and oropharyngeal carcinoma (Hirst et al. 2009; Simbiri et al. 2010). The E6 oncoprotein, encoded by HPV16 or HPV18, is known to interact with the well-known tumor suppressor p53 to promote its degradation, while the E7 oncoprotein interacts with the retinoblastoma protein (pRB) and results in E7-induced pRB inactivation. The E5 virus protein cooperates with E7 to transform cells and enhances the ability of E7 to induce proliferation and, with E6, to immortalize cells (Helt and Galloway 2003; Scott et al. 2002; Song et al. 1998; Peralta-Zaragoza et al. 2006).

Clinical Presentation of CSCC and Related Conditions

Ocular surface squamous neoplasia (OSSN) is a term used to describe a conjunctival or corneal neoplastic growth; this term encompasses a spectrum of conditions, ranging from simple dysplasia to conjunctival intraepithelial neoplasia (CIN) to invasive CSCC (Simbiri et al. 2010). CSCC is typically a “low-grade” malignancy with relatively little potential for invasion and metastasis

(Chisi et al. 2006). Given its location on the eye, it can cause substantial morbidity, patient distress, and disability even when small. Also, similar to cancer of the uterine cervix, CSCC has a high rate of recurrence after treatment and may in some cases metastasize. Moreover, CSCC often shows rapid onset and progression, and in the early stages, a mild dysplasia, often resembling a pinguecula, pterygium, or even a phlyctenule, may occur. Progressive growth of the lesion results in an elevated, fleshy mass of pink tissue that develops in the interpalpebral space. These irregularly shaped neoplasms develop a rich intrinsic blood supply from the surrounding conjunctiva and episclera, with large-diameter “feeder vessels” emanating from the mass. Thus, conjunctival neoplasms may initially pose cosmetic concern for patients on routine ocular examination. Larger lesions may interfere with lid function, causing dry eye complaints and possibly dellen formation on the adjacent cornea. Advanced, invasive lesions may compromise the episclera, sclera, cornea, or angle structures. Pain may be a significant factor in later stages due to associated keratitis, uveitis, or rise in intraocular pressure. A representative HIV-1-infected OSSN patient from Botswana is shown in Fig. 1.

Other Conjunctival Tumors

Melanomas can develop in the conjunctivae, although these tumors are relatively rare. The lesions may present as flat or nodular pigmented

area, although amelanotic conjunctival melanomas have been documented. Like squamous cell carcinoma, they typically develop at the limbal margin. As with the carcinomas, vascularization takes place, with numerous feeder vessels supplying the lesion. Unlike carcinomas, however, conjunctival melanomas tend to become multicentric if not promptly diagnosed and managed. Melanomas tend to progress more rapidly and are more invasive into underlying ocular tissues. Hence, it is not uncommon to see associated uveitis or secondary glaucoma if the lesion invades the anterior chamber.

Prior to the AIDS epidemic, ocular involvement of Kaposi sarcoma (► **KS**) was an extremely rare phenomenon, with fewer than 30 cases reported before 1982. However, KS is one of the most common AIDS-related tumors, and up to one in five patients with AIDS-related KS may have some form of ocular involvement (conjunctiva, eye lids, or orbit) (Grulich et al. 2001; Mikropoulos et al. 2011). Conjunctival Kaposi sarcoma has been reported as the initial sole manifestation of HIV infection in a number of reports.

Lymphoma (► **Lymphoma**) can also involve the conjunctivae. In particular, mucosa-associated lymphoid tumor (MALT) can arise in the conjunctivae, appearing as a salmon-colored patch. This tumor is relatively benign and, like gastric MALT, has been hypothesized to be related to local infection with *Helicobacter pylori* (*H. pylori*).

Epidemiology of CSCC and Association with HIV Infection

The incidence of CSCC varies geographically (declining with greater distances from the equator), with Uganda having 1.2 cases per 100,000 persons per year, compared to the UK with fewer than 0.02 cases per 100,000 per year (Ateenyi-Agaba 1995). In the USA, it is a rare disorder, with an incidence of 0.03 per 100,000 persons per year. It has been noted that the relatively high incidence in Kampala, Uganda, is in part due to the high prevalence of HIV infection. Multiple case series have shown a higher prevalence in

male patients and the elderly, with the most frequently reported location being the limbus (Ateenyi-Agaba 1995).

CSCC has for some time been suspected as having an infectious origin. One reason for this is the sharp increase in the incidence of CSCC among patients infected with HIV (Restelli et al. 2005; Chisi et al. 2006), as well as in patients with other forms of immunodeficiency, such as transplant recipients (Macarez et al. 1999; Shelil et al. 2003). CSCC has emerged as an HIV-associated cancer that is increasing in incidence in Uganda and other sub-Saharan African countries over the past two decades, and this increase has been particularly striking in the young and in women (Chisi et al. 2006; Porges and Groisman 2003). The striking association of CSCC with HIV infection and its geographic localization in certain regions of Africa, where there is a high prevalence of several oncogenic viruses, are consistent with, although not proof of, an infectious etiology.

Prior to the HIV pandemic, OSSN was noted to occur predominantly in the elderly, for whom it is the third most common oculo-orbital neoplastic condition after melanoma and lymphoma. In addition to advanced age and male sex preponderance, other risk factors linked to its pathogenesis have included ultraviolet light B, immunosuppression in organ transplant recipients, cigarette smoking, and in some settings HPV (Simbiri et al. 2010). In Africa it is becoming more common, more aggressive, and more likely to affect young people, especially females. Africa also has a high prevalence of HPV infection with prevalence of about 25% in women of age 15–74 years. OSSN is currently the most common ocular neoplastic condition overall among adults in Africa. Findings demonstrated a strong association between OSSN and HIV-1 infection applying to all tumor stages. In a study in Uganda, HIV-positive participants were often markedly immunosuppressed at the time of diagnosis, and their early mortality was high. Almost all of the tumors were in the interpalpebral area of the conjunctiva, which supports the concept of UV radiation as a cofactor in the etiology of OSSN (Simbiri et al. 2010). In a high percentage of HIV-infected patients

presenting with OSSN, the conjunctival tumor was the first sign of HIV, and, in the majority of cases, OSSN was also the only detectable manifestation of HIV (Spitzer et al. 2008).

The increasing incidence and prevalence of OSSN and its association with the HIV pandemic have been described in many sub-Saharan African countries. The role of HIV pathogenesis in CSCC is hypothesized to be through immunosuppression, which then permits activation of oncogenic viruses such as HPV and other herpesviruses, as well as the growth of other infectious pathogens in the conjunctiva (Simbiri and Robertson 2012). Moore and colleagues using a digital transcriptome subtraction (DTS) method did not detect viral sequences from 3 conjunctival carcinoma tissues from Uganda (Feng et al. 2007). As noted, CSCC is also on the rise in developing nations, particularly in sub-Saharan Africa. The increased use of highly active antiretroviral therapy (HAART) in some of the sub-Saharan countries is beginning to show some impact on the risk of HIV-associated tumors, as has been seen in developed countries, although data are scanty at this time. It is hoped that these HIV therapies will also lower the rates of conjunctival cancers, although it is also possible that rates will increase as patients live longer with HIV infection (Spitzer et al. 2008).

Pathogenesis and Possible Role of Infectious Agents

It has been established that infectious agents play an important role in the etiology of certain human malignancies and are thought to be responsible for around 18% of the worldwide cancer burden (less than 10% in developed nations and up to 27% in developing nations). Much of the burden of cancer incidence, morbidity, and mortality occurs in the developing world, with infectious agents attributing to most malignancies of the cervix and vulva (HPV), stomach (*H. pylori*) (Fox et al. 2000; Ferreri et al. 2006a), and liver (hepatitis B and C viruses) (Ferreri et al. 2006c). Other important tumors caused by infectious agents include Kaposi sarcoma (Kaposi-sarcoma-

associated herpesvirus, also called human herpesvirus-8 [KSHV/HHV-8]) and most types of non-Hodgkin lymphoma (Epstein-Barr virus (▶ EBV) and for certain types also KSHV/HHV-8).

Infectious agents display diverse geographic variation in their prevalence and patterns of disease, including oncogenesis. There is evidence that several microorganisms may play a role in different conjunctival malignancies, including HPV in conjunctival papilloma and CSCC, human immunodeficiency virus in CSCC and Kaposi sarcoma, KSHV/HHV-8 in conjunctival Kaposi sarcoma, and *H. pylori*, *chlamydia*, and hepatitis C virus in ocular adnexal mucosa-associated lymphoid tissue (MALT) lymphomas. Unlike cervical cancer, where a predominantly single infectious agent HPV was found in greater than 99% of lesions, multiple organisms may play a role in the etiology of certain ocular neoplasms by acting through various mechanisms of oncogenesis, including chronic antigenic stimulation and expression of oncoproteins by the infectious agents. Similar to a number of other human malignancies, the role of infectious agents in conjunctival carcinomas is most likely a cofactor to genetic and environmental risk factors (Mikropoulos et al. 2011).

Oncogenes are genes that have the potential to cause cancer through mechanisms including disruption of cell cycle mechanisms or interference with apoptosis. They can either develop from a mutated or dysregulated cellular gene, called a proto-oncogene, or can be encoded by infectious agents. High-risk variants HPV16 and HPV18 may drive carcinogenesis by inactivating tumor suppressor gene products p53 and pRb. HPV-E6 and E7 oncoproteins also increase transforming growth factor beta (TGF- β) promoter activity (Peralta-Zaragoza et al. 2006). Notably, TGF- β controls proliferation, differentiation, and other functions in most cell types. Compared to the high-risk HPV type 16 and type 18, the E6 and E7 oncoproteins of HPV type 6 are seemingly less effective in transforming epithelial cells in vitro (Halbert et al. 1992). This finding correlates with the fact that HPV types 6 and 11 are most frequently associated with the benign conjunctival papilloma, whereas the high-risk HPV types

16 and 18 are most frequently associated with CIN and CSCC (Song et al. 1998).

An additional mechanism whereby infectious agents may be involved in the etiology of conjunctival or other systemic neoplasms is by inducing a state of chronic antigenic stimulation. A systemic example is illustrated by the linkage between infection with *H. pylori* and chronic atrophic gastritis, an inflammatory precursor of gastric adenocarcinoma (Mikropoulos et al. 2011). Also, regression of gastric MALT after eradication of *H. pylori* infection with antibiotics is consistent with this hypothesis (Mikropoulos et al. 2011). Similar regression of disease has been reported in ocular adnexal MALT lymphoma after treatment with antibiotics against *Chlamydomphila psittaci* (Ferreri et al. 2004).

H. pylori is a spiral-shaped gram-negative bacteria that infects a large proportion of the population worldwide (Mikropoulos et al. 2011). In developing countries, up to 70–90% of the population is infected (primarily during childhood), whereas in developed countries the prevalence of infection is lower, at 25–50% (Mikropoulos et al. 2011). In most cases the organism causes no symptoms in the host, yet it has been recognized as the cause of duodenal ulcers and gastric adenocarcinoma and can be found in greater than 90% of gastric mucosa-associated lymphoid tissue (MALT) lymphomas. *H. pylori* has also been identified in conjunctival carcinoma tissues (Ferreri et al. 2004). *H. pylori* evades the host adaptive and innate responses by frequent antigenic variation and host antigen mimicry. Even so, the host immune and inflammatory responses to *H. pylori* can increase cellular damage and turnover, thereby promoting carcinogenesis. Whereas eradication of *H. pylori* leads to regression of early gastric MALT lymphoma in up to 80% of cases, its eradication has not been reported to have a similar effect on conjunctival MALT lymphoma lesions. The only study assessing treatment against this organism on ocular lesions was conducted by Ferreri et al. in Italy to assess rates of *H. pylori* gastric infection in patients with ocular adnexal lymphoma (Ferreri et al. 2006b). Of note, the study did not in fact examine *H. pylori* or

its DNA in conjunctival MALT lymphoma lesions. Out of the 31 patients with ocular adnexal MALT lymphoma, 10 (32%) had gastric *H. pylori*, and 4 were treated solely with *H. pylori*-eradicating antibiotics (erythromycin 500 mg twice a day, omeprazole 20 mg twice a day, and tinidazole 500 mg twice a day, for 7 days) (Ferreri et al. 2006b). The ocular adnexal MALT lymphoma in these patients showed no response. Six additional patients received *H. pylori*-eradicating antibiotics concurrently with other therapies (doxycycline, rituximab, or orbit irradiation), achieving lymphoma regression in all cases. Interestingly, three of the patients who were positive for gastric *H. pylori* infection had *C. psittaci*-positive conjunctival MALT lymphomas. Treatment with *H. pylori*-eradicating antibiotics led to no measurable conjunctival lymphoma regression in these three patients (Ferreri et al. 2006b). Although this may raise doubts about the putative role of *H. pylori* in sustaining the growth of this MALT lymphoma, it may also simply highlight the sensitivity of organisms to the specific antibiotics used. Furthermore, clearance of gastric *H. pylori* may not amount to clearance of conjunctival *H. pylori*, and it is also that previous infection with *H. pylori* may have lingering effects promoting MALT lymphoma of the conjunctiva. Other pathogens that may contribute to various conjunctival tumors include chlamydia and hepatitis C virus (HCV). HCV is a major etiologic agent of hepatocellular carcinoma (► [Hepatocellular Carcinoma in HIV](#)) but has also been linked to B-cell lymphomas. It is possible that different infectious agents in different geographical locations contribute to oncogenesis, with host genetic makeup also playing a role.

Of interest, an increase in OSSN has been observed in the genetic disease xeroderma pigmentosum (XP). OSSN occurs predominantly in the elderly, but in patients with XP, as in patients with HIV infection, it tends to occur at a younger age (6–22 years) (Gupta et al. 2011). OSSN appears to be more aggressive than usual in patients with XP (recurrence rate 64.3%). Patients with XP are unable to repair the DNA

that is damaged by ultraviolet rays. This can lead to somatic mutations and the resulting development of oncogenic cells and cancer. This inherent defect accounts for the increased susceptibility to OSSN and the younger age at presentation (Gupta et al. 2011). Some reports have noted a younger age in intraepithelial cases compared with invasive squamous cell carcinoma (Palazzi et al. 2000). Bilateral involvement is encountered very frequently in patients with XP (Gupta et al. 2011).

In patients with XP, simple excision of conjunctival intraepithelial or invasive neoplasia was reported to be associated with a 24–50% recurrence rate (Gupta et al. 2011). Excision with intraoperative control of the surgical margins and adjunctive cryotherapy have been reported to reduce recurrence rates to 12% (Song et al. 1998; Peralta-Zaragoza et al. 2006). A high overall recurrence rate of 64.3% has been observed; the rate of new or recurrent tumors was 25% for intraepithelial squamous carcinoma and 83% for invasive squamous cell carcinoma. Up to four recurrences were noted in a single patient. There is increased tendency of XP patients with OSSN to develop fresh or recurrent lesions. A majority of these patients develop recurrence of the tumor despite a meticulous tumor excision and adjunctive cryotherapy (Gupta et al. 2011).

Prevention and Treatment

While there is evidence that infectious agents may play a role in CSCC, the use of anti-infectious approaches to prevent or treat this condition has not been extensively studied. An effective vaccine for HPV has now been approved, and it will be interesting to see if the use of this vaccine is associated with a decrease in CSCC; if so, this would support an important role for HPV (Spitzer et al. 2008).

Immunomodulatory agents such as topical interferon alpha and the gastric acid inhibitor oral cimetidine have led to conjunctival papilloma regression. In addition to its apoptotic effect on

tumor cells, interferon alpha exerts its antiviral action by preventing the replication of latent virus in the tissues. While there is evidence that HPV plays some role in a subset of conjunctival papillomas, host immune status likely plays a role in pathogenesis and will likely affect the response to treatment (Manns et al. 2001; Midena et al. 2000; Morgenstern et al. 2003).

Even though conjunctival papilloma may persist for extended periods of time, with reported recurrence rates of 6–27%, spontaneous regression can be seen. Also, cure is possible with current therapies. In cases where large lesions cause symptoms or cosmetic defects and periodic observation would be futile, surgery remains the treatment of choice with double freeze-thaw cryotherapy to the remaining conjunctiva to prevent tumor recurrence. Additionally, topical interferon alpha-2b and mitomycin C have been employed in the treatment of recurrent conjunctival papillomas (Manns et al. 2001; Morgenstern et al. 2003).

A high recurrence rate of CSCC has been seen even after cryotherapy, radiation, and chemotherapeutics. Several treatments have been utilized in an attempt to reduce recurrence rates, including topical mitomycin C, 5-fluorouracil, and interferon alpha (Manns et al. 2001; Morgenstern et al. 2003). There is a need for improved therapies, especially those that can be utilized in resource-limited regions.

Conclusions

CSCC and its precursor lesions are most commonly seen in regions with high sunlight exposure, and their increased incidence with HIV infection suggests an infectious etiology. It is relatively uncommon in the USA and Europe and substantially more common in sub-Saharan Africa, especially in regions near the equator. There is some evidence that certain types of HPV may be involved in the pathogenesis of these tumors, but the evidence is not as robust as for other infection-associated tumors, and more research is needed.

Cross-References

- ▶ Anal Cancer
- ▶ Antiretroviral Therapy: When to Start
- ▶ Burkitt and Burkitt-Like Lymphoma
- ▶ Cervical Cancer and HIV
- ▶ Chronic Immune Activation in HIV
- ▶ Diffuse Large B-Cell Lymphoma
- ▶ Epidemiology of HIV-2 Infection in West Africa
- ▶ Epstein-Barr Virus (EBV)
- ▶ Hepatocellular Carcinoma in HIV-Positive Patients
- ▶ HIV Cancers in Resource-Limited Regions
- ▶ HIV-Associated Cancers
- ▶ HIV-Associated Immune Exhaustion
- ▶ Hodgkin Lymphoma in Patients with HIV Infection
- ▶ Host Genetics and Genomics
- ▶ Human Papillomavirus (HPV)
- ▶ Immune Activation and HIV Transmission
- ▶ Immunological Responses to Antiretroviral Therapy
- ▶ Immunopathogenesis of HIV Coinfections
- ▶ Inflammatory Cytokines
- ▶ Kaposi Sarcoma-Associated Herpesvirus (KSHV) or Human Herpesvirus 8 (HHV-8)
- ▶ Lung Cancer
- ▶ Lymphocyte Apoptosis
- ▶ Macrophages in HIV Immunopathogenesis
- ▶ Multicentric Castleman Disease
- ▶ NKT Cells: Bridging Innate and Adaptive Immunity
- ▶ Other HPV-Associated Cancers (Oropharyngeal and Penile)
- ▶ Overview: Immunopathogenesis
- ▶ Primary Effusion Lymphoma
- ▶ T-Cell Homeostasis

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Counteraction of SAMHD1 by Vpx

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Definition

Host cellular proteins that interact with HIV play critical roles to regulate viral infection and pathogenesis. Cellular proteins can either positively or negatively impact different stages of HIV replication. Restriction factors are host proteins that inhibit virus infections and are considered an integral part of the host's intrinsic cellular defense system. Structural and functional studies of restriction factors and their interactions with viral proteins have not only revealed fundamental mechanisms of HIV replication but have also facilitated the generation of better animal virus models for therapeutic and vaccine development. SAM domain- and HD domain-containing protein 1 (SAMHD1) is the first deoxynucleoside triphosphate triphosphohydrolase (dNTPase) identified in mammalian cells. SAMHD1 acts as an HIV-1 restriction factor in nondividing cells and is counteracted by the retroviral protein X (Vpx). Here the latest advances in understanding the mechanisms of SAMHD1-mediated retroviral restriction and its counteraction by Vpx are summarized. The implications and future directions of SAMHD1 studies are also discussed.

Introduction

Intrinsic cellular immunity against invading pathogens is paramount for the survival of any species.

In the past decade, an elite group of cellular proteins, termed restriction factors, have been identified to autonomously block HIV replication and are now considered part of the cell's intrinsic immune system. Coincidentally, viral countermeasures have also coevolved to antagonize such restriction factors in an ongoing conflict between virus and host. SAMHD1 acts as a potent restriction factor of HIV-1 in nondividing myeloid cells and resting CD4⁺ T cells by starving the virus of cellular deoxynucleotide triphosphates (dNTPs) that are needed for efficient conversion of the viral RNA genome into DNA. However, only HIV-2 has uniquely adapted to overcome SAMHD1-mediated viral restriction by encoding a viral antagonist, Vpx. Intriguingly, HIV-1 does not encode Vpx and thereby lacks the capacity to counteract SAMHD1 but is more pathogenic than HIV-2. Thus, the question arises, does the counteraction of SAMHD1 by Vpx serve to promote or hinder disease pathogenesis? In this entry, the mechanistic details of how Vpx counteracts this novel restriction factor, and our current understanding on the implications it has on HIV-1 that lacks a viral countermeasure against SAMHD1, are discussed.

Vpx and SAMHD1: Genes and Proteins

Vpx Gene and Protein

The *vpx* gene has been identified in HIV-2 and simian immunodeficiency viruses (SIVs) from rhesus macaque (SIVmac), sooty mangabey (SIVsmm), mandrill-2 (SIVmnd-2), red-capped mangabey (SIVrcm), and drill (SIVdrl). It is believed that the *vpx* gene of HIV-2 and SIV evolved from its paralogue, *vpr*, as a result of a gene duplication event that occurred during SIV evolution (Fregoso et al. 2013). The *vpx* gene is 342 base pairs in length and encodes a Vpx protein (12–16 kDa) that is packaged into virion particles similar to Vpr. Its presence in the virion suggested that it played a role during the initial stages of HIV-2 and SIV infection. Early studies revealed that delivery of Vpx via virus-like particles to monocyte-derived dendritic cells was sufficient to relieve the

resistance to HIV-1 infection in myeloid cells. Furthermore, Vpx was found to interact with DNA damage-binding protein 1-and-Cullin-4 associated factor 1 (DCAF1) within a CUL4A-DDB1 E3 ubiquitin ligase complex, which suggested that Vpx might target an unknown host restriction factor in myeloid cells for proteasomal degradation to enhance HIV-2 and SIV infection. The search for the unknown cellular protein targeted by Vpx ended with the identification of SAM domain- and HD domain-containing protein 1 (SAMHD1) as a novel host restriction factor present in myeloid cells capable of potently restricting HIV-1 (Hrecka et al. 2011; Laguetta et al. 2011).

Samhd1 Gene and Its Regulation

Human *samhd1* gene was initially identified in a human dendritic cell complementary DNA (cDNA) library as an orthologue of a mouse interferon gamma (IFN- γ)-induced gene. It is located on chromosome 20 and spans 59.5 kilobases in length and encompasses 16 exons. Mutations in *samhd1* gene are associated in different autoimmune diseases, such as Aicardi-Goutieres syndrome (AGS) and Chilblain lupus. At least 16 mutations in *samhd1* have been identified in people with AGS syndrome that leads to immune system abnormalities and inflammatory damage to the brain and skin. Chilblain lupus 2 is associated with heterozygous mutation in the *samhd1* gene. These findings suggest that SAMHD1 is involved in innate immunity and inflammation. Alternative splicing of *samhd1* gives rise to two splice variants that lack exons 8–9 and 14, respectively, but due to their inherent instability in the cell and lack of dNTPase activity, their physiological roles have not been identified (Welbourn et al. 2012). Different epigenetic mechanisms regulate *samhd1* gene expression. For example, gene promoter methylation, histone deacetylation (de Silva et al. 2013), and microRNA-181 (Jin et al. 2014) can downregulate SAMHD1 expression in certain cell lines.

SAMHD1 Protein

SAMHD1 protein is expressed in human myeloid cells, such as monocytes, dendritic cells, and

macrophages, and also in B and T lymphocytes. SAMHD1 is a 626 amino acid (aa) protein with a molecular weight of 72.2 kDa and is comprised of an N-terminal nuclear localization signal domain (¹¹KRPR¹⁴), a sterile alpha motif (SAM) domain (residues 45–110), a histidine-aspartate (HD) domain (residues 167–311), and a C-terminal variable domain. The nuclear localization signal plays an important role in the nuclear localization of SAMHD1 protein. The SAM domain is one of the most common protein-protein interaction modules and is involved in the interaction with a variety of signaling molecules including kinases, scaffolding proteins, RNA-binding proteins, transcription factors, and GTPases. However, its contribution to HIV restriction remains under scrutiny. The HD domain of SAMHD1 is responsible for the deoxyguanosine triphosphate (dGTP)-regulated dNTPase activity that decreases the cellular levels of dNTPs and is important for the restriction of different viruses. The HD domain is also necessary for SAMHD1 to oligomerize and bind to RNA. The catalytic activity of SAMHD1, residing in the central HD domain, is essential for its ability to inhibit HIV infection in nondividing cells (Laguette et al. 2011).

SAMHD1 protein exists in a dGTP-induced tetrameric form in the cell and the tetramerization is modulated by elements flanking the catalytic core domain within the C-terminal region of the protein. The tetrameric state of SAMHD1 is critical for its catalytic and anti-HIV activities (Yan et al. 2013; Ji et al. 2014). SAMHD1 protein has dGTP-stimulated dNTP triphosphohydrolase activity and nuclease activity against single-stranded DNA and RNA which is associated with its HD domain. This HD domain is responsible for the reverse transcription block to HIV-1 infection observed in myeloid or resting CD4⁺ T cells by limiting the intracellular pool of dNTPs. Phosphorylation of SAMHD1 at threonine 592 (T592) negatively regulates its HIV restriction function but not its dNTPase activity (Cribier et al. 2013, White et al. 2013b), suggesting a dNTPase-independent mechanism of SAMHD1-mediated HIV-1 inhibition.

Physiological Functions of SAMHD1

Regulator of the Cellular dNTP Pool

While the physiological role of SAMHD1 is still being unraveled, mutations in the *SAMHD1* gene have been found to cause AGS in humans, a rare autosomal recessive disorder that mimics congenital viral infection (Rice et al. 2009). Mutations in other genes such as the three prime repair exonuclease 1 (*TREX*) and ribonuclease H2 (*RNaseH2*) also lead to AGS. The common underlying function of these proteins, including SAMHD1, is their ability to prevent the accumulation of abnormal self-nucleic acids in the cytoplasm via their exonuclease or dNTPase activity. AGS is a pro-inflammatory autoimmune disease that is characterized by elevated levels of IFN α in the cerebral spinal fluid. Certain mutations in SAMHD1 associated with AGS patients render the protein defective in its ability to degrade dNTPs. Thus, it is speculated that SAMHD1 functions to suppress type I IFN production in the cell by regulating the overall level of dNTPs and preventing the buildup of nucleic acids in the cytoplasm as a consequence of active endogenous retroelements. SAMHD1 protein levels and its dNTPase activity are regulated during the different phases of the cell cycle where it is maximally expressed during quiescence (G₀) and active during the G₀ and gap 1 (G₁) phases (Franzolin et al. 2013). Conversely, SAMHD1 expression and activity decreases during the synthesis (S) phase of the cell cycle, allowing for high levels of dNTPs to be available to meet the demand for cellular DNA replication to proceed in preparation for mitosis. Hence, SAMHD1 plays a major role as a catabolic enzyme in regulating cellular nucleotide metabolism and works in conjunction with ribonucleotide reductase, which is the key cellular enzyme involved in *de novo* dNTP synthesis, to maintain dNTP homeostasis in the cell.

Maintaining Genomic Stability

Maintaining genomic stability in the cell is critical for proper cellular function and preservation of genetic integrity. Seventeen percent of the human genome is comprised of long interspersed elements 1 (LINE-1) – the only class of active autonomous

retrotransposons that can alter the genomic landscape of the cell unless properly controlled. Through its ability to regulate dNTP levels, SAMHD1 is able to regulate LINE-1 and LINE-1-mediated Alu/SVA retrotransposition in cells by serving as a blockade to the reverse transcription stage of the transposition process (Zhao et al. 2013). Thus, SAMHD1 acts as a cell intrinsic regulator of endogenous retroelements in mammals as a means to preserve genomic integrity. Moreover, somatic mutations of the *samhd1* gene have been reported in chronic lymphocytic leukemia (Clifford et al. 2014). SAMHD1 is associated with DNA repair proteins at sites of DNA damage, suggesting its potential role in DNA double-stranded break repair (Clifford et al. 2014). Given its role in regulating cellular dNTP levels, it is speculated that mutations in *SAMHD1* may play a role in the development and progression of certain types of cancers.

Retroviral Restriction and Regulation of the Innate Immune Response

Another major physiological role of SAMHD1 is to block retroviral infection in nondividing cells, such as myeloid lineage cells and quiescent CD4⁺ T cells, and thereby regulate the innate immune response to retroviral infection. For instance, a hallmark of HIV-1 infection is the ability of the virus to evade immune recognition and clearance. One of the main reasons HIV-1 is able to accomplish such a task is in part due to its reduced capacity to infect antigen-presenting cells, such as dendritic cells and macrophages, that would normally trigger a robust type I IFN response upon pathogen recognition and antigen presentation. If such an innate immune response was mounted, it would lead to an antiviral state and clearance of HIV-1 by a subsequent adaptive immune response. However, due to the restrictive functions of SAMHD1, HIV-1 infection of antigen-presenting cells is blocked, which leads to a lack or attenuated innate and adaptive immune response. Thus, while SAMHD1 limits productive HIV-1 infection of myeloid cells, it inadvertently prevents antigen presentation and immune detection, which enables efficient HIV-1 transmission to CD4⁺ T cells and establishes persistent viral infection.

Mechanisms of SAMHD1-Mediated Retroviral Restriction

Biochemical Basis of HIV-1 Restriction by SAMHD1

Post-entry block to HIV-1 infection in terminally differentiated myeloid lineage cells occurs at the level of reverse transcription, which leads to incomplete synthesis of viral cDNA. Vpx is able to relieve this block to infection by permitting reverse transcription to proceed although the underlying mechanism remained unknown. The identification of SAMHD1 as the cellular protein counteracted by Vpx suggested that SAMHD1 was able to potently block HIV reverse transcription in nondividing cells such as myeloid lineage cells and quiescent CD4⁺ T cells (Hrecka et al. 2011; Laguette et al. 2011; Baldauf et al. 2012). Subsequent studies have characterized SAMHD1 as a dNTPase that is capable of hydrolyzing dNTPs to their constituent deoxynucleosides and inorganic triphosphate in a dGTP-dependent manner (Goldstone et al. 2011). The dNTPase activity of SAMHD1 in nondividing cells reduces the intracellular dNTP pool to levels below that which is required by HIV-1 reverse transcriptase for efficient conversion of viral RNA into complementary DNA (Baldauf et al. 2012; Lahouassa et al. 2012; St Gelais et al. 2012). Mutation of key residues in the catalytic HD domain of SAMHD1 abrogates its restriction activity and ability to hydrolyse dNTPs, suggesting that dNTP regulation is a key mechanism in SAMHD1-mediated retroviral restriction (Fig. 1).

It is likely that the structure of SAMHD1 regulates its biological activity, including its dNTPase function, which could have consequences for retroviral restriction. The crystal structure of recombinant SAMHD1 has revealed that binding of dGTP or GTP to an allosteric site causes SAMHD1 to form catalytically active tetramers (Ji et al. 2014). Mutations of the dGTP binding residues affect tetramer assembly, dNTPase activity, and HIV-1 restriction (Zhu et al. 2013). The C-terminus of SAMHD1 regulates tetramerization of SAMHD1, which is critical for its dNTPase activity and HIV-1 restriction

(Yan et al. 2013; Zhu et al. 2013). Controversially, a separate study suggested that oligomerization and tetramer formation were not required for efficient HIV-1 restriction (Brandariz-Nunez et al. 2013). It is currently unclear whether phosphorylation of SAMHD1 at T592 affects its tetrameric structure and thereby regulates its HIV-1 restriction function.

Mechanisms Independent of the dNTPase Activity of SAMHD1

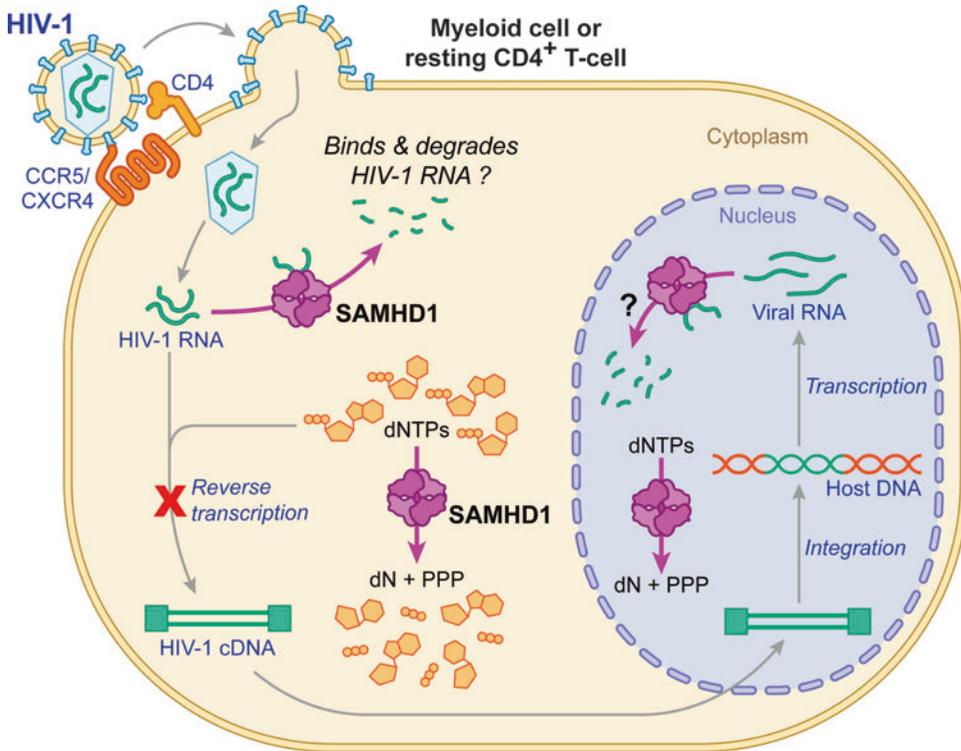
Although there is a strong correlation between SAMHD1's dNTPase function and HIV-1 restriction, the addition of exogenous deoxynucleotides to cells cannot fully rescue HIV-1 infection. This suggests that additional mechanisms, independent of its dNTPase activity, might be involved in SAMHD1-mediated retroviral restriction. Furthermore, the possibility of an alternative mechanism of HIV-1 restriction by SAMHD1 was demonstrated when studies showed that phosphorylation of SAMHD1 at T592 negatively regulates its HIV-1 restriction function. SAMHD1 is phosphorylated in many nonrestrictive cell types but is not phosphorylated in restrictive myeloid or resting CD4⁺ T cells (Cribier et al. 2013; White et al. 2013b). SAMHD1 phosphorylation is mediated through the interaction of SAMHD1 with cellular host factors cyclin A2 and cyclin-dependent kinases 1/2 (CDK1/2), in a cell-type-dependent manner (St Gelais et al. 2014). SAMHD1 functions as a dNTPase independent of the phosphorylation status of the protein, whereas restriction of HIV-1 is abrogated when SAMHD1 is phosphorylated, providing evidence that the dNTPase function of SAMHD1 may not be the sole mechanism of retroviral restriction (White et al. 2013b).

Apart from its role as a dNTPase, new studies have suggested that SAMHD1 may have additional cellular functions that could impact its retroviral restriction and role in innate immune signaling. SAMHD1 has been identified as a nucleic acid binding protein that has a preference for RNA over DNA (Goncalves et al. 2012). The RNA-binding ability of SAMHD1 has been mapped to the HD domain and C-terminus of SAMHD1 (White et al. 2013a). A separate study

also showed that SAMHD1 possesses 3'-5' exonuclease activity against single-stranded DNAs and RNAs in vitro. Further, SAMHD1 is able to bind structured HIV-1 *gag* and *tat* RNA, correlating nucleic acid binding and exonuclease activity with the SAM domain (Beloglazova et al. 2013). Recently, SAMHD1 was found to possess RNase activity in the cell, enabling it to degrade incoming genomic viral RNA during the early stages of infection (Ryoo et al. 2014). Mutational analysis of the allosteric site of SAMHD1 identified mutants that could specifically abrogate either the dNTPase function (D137N) or the RNase function (Q548A). Interestingly, only the RNase-defective mutant, but not the dNTPase-defective mutant, lost its ability to restrict HIV-1, suggesting that the RNase activity of SAMHD1 was more important than the dNTPase activity for HIV restriction. Furthermore, phosphorylation of T592 appears to negatively regulate the RNase activity of SAMHD1 (Ryoo et al. 2014). These results indicate that multiple mechanisms of restriction may occur and vary depending on the target cell type and infecting virus (Fig. 1). How the phosphorylation of SAMHD1 affects its RNase activity and retroviral restriction has not been defined. It remains unknown if SAMHD1 has a broad RNase specificity to restrict other RNA viruses or if this is unique to HIV-1. Moreover, it is also unknown whether SAMHD1 may have RNase activity to degrade newly transcribed HIV-1 mRNA or genomic RNA.

Restriction of Other Viruses by Human SAMHD1

SAMHD1 is able to restrict other retroviruses including feline immunodeficiency virus, bovine immunodeficiency virus, N- and B-tropic murine leukemia virus, and equine infectious anemia virus, through its ability to maintain a low dNTP pool concentration which blocks efficient completion of reverse transcription (White et al. 2013a). Prototype foamy virus (PFV) and human T-cell leukemia virus type 1 (HTLV-1) are not affected by SAMHD1-mediated restriction. PFV reverse transcribes its RNA genome prior to target cell entry and, thus, is not dependent on the intracellular dNTP level in the target cell. HTLV-1, which



Counteraction of SAMHD1 by Vpx, Fig. 1 Mechanisms of SAMHD1-mediated HIV-1 restriction. The figure illustrates the molecular and cellular mechanism underlying SAMHD1-mediated HIV-1 restriction in non-dividing myeloid cells or resting CD4⁺ T cells. SAMHD1 protein localizes in the nucleus and cytoplasm of these cells. Acting as a cellular dNTPase, SAMHD1 decreases

intracellular dNTP levels and thereby limits HIV-1 DNA synthesis during reverse transcription. SAMHD1 has also been proposed to function as an RNase to degrade HIV-1 RNA in the infected cell, which remains to be confirmed. The authors thank Mr. Tim Vojt for the illustration (Reproduced by permission of The Ohio State University)

still undergoes reverse transcription in the cytosol, may have adapted an unknown mechanism to antagonize SAMHD1-mediated restriction (Gramberg et al. 2013). In contrast, abortive HTLV-1 infection in monocytes is linked to SAMHD1 restriction through apoptosis triggered by innate antiviral responses (Sze et al. 2013).

The repertoire of viruses that SAMHD1 can restrict has been further expanded with recent studies finding that DNA viruses, which do not undergo reverse transcription but are dependent on intracellular dNTPs, can also be inhibited by SAMHD1. For example, herpes simplex virus 1 (HSV-1) is restricted in nondividing macrophage cell lines (Kim et al. 2013), vaccinia virus and HSV-1 in nondividing cells (Hollenbaugh et al. 2013), and hepatitis B virus in liver cell lines (Chen et al. 2014).

Counteraction of SAMHD1 by Vpx and Its Implications

Evolutionary Origins of SAMHD1 Counteraction by Vpx

Given its ability to potentially block HIV-1 infection in myeloid cells and quiescent CD4⁺ T cells, it is surprising that HIV-1 lacks Vpx and has not evolved a strategy to counteract SAMHD1. Only two out of the eight primate lentivirus lineages, namely, HIV-2 and SIVsm-derived lineages (SIVsm and SIVrcm), encode the *vpx* gene. Only Vpx in HIV-2 SIVsm, but not SIVrcm, is able to counteract SAMHD1, indicating the species-specific nature of SAMHD1 antagonism by Vpx. HIV-1 and HIV-2 that infect humans have arisen from cross-species transmission of SIV that infects chimpanzees (SIVcpz) and sooty

mangabeys (SIVsm), respectively. One of the key genomic features that distinguish HIV-1 from HIV-2 is its lack of the *vpx* gene, which is also absent in its ancestor, SIVcpz. It is intriguing that HIV-1 and SIVcpz would lack such an important antiviral countermeasure that would enable it to efficiently replicate in myeloid cells and quiescent CD4⁺ T cells and thus increase its pathogenicity. This begs the question whether the lack of *vpx* in HIV-1 is a consequence of the evolutionary selection process and whether it has enabled HIV-1 to be more pathogenic than HIV-2?

Evolutionary analyses have revealed that SAMHD1 underwent a period of strong positive selection during primate evolution where not only Vpx but also Vpr from certain primate lentivirus lineages were able to antagonize SAMHD1 during lentiviral infection (Laguette et al. 2012; Lim et al. 2012). During a genetic conflict between cellular and retroviral genes such as SAMHD1 and Vpx, positive selection occurs in both genes where certain gene mutations that alter the amino acid sequence are rapidly selected in order for one of the genes to overcome the other in the form of an evolutionary arms race. Phylogenetic and functional evidence shows that Vpr was the first lentiviral accessory protein that was able to degrade SAMHD1, which was prior to the “birth” of *vpx* (Lim et al. 2012). It was such an antagonism that initiated an evolutionary arms race between SAMHD1 and Vpr that continued with the advent of Vpx in subsequent lentivirus lineages. It is believed that the birth of *vpx*, which occurred via a gene duplication event of *vpr*, was driven by the need to counteract the restriction imposed by SAMHD1 in certain primate species. Interestingly, as primate lentiviruses evolved under the selective pressure of SAMHD1 restriction, it appears that the *vpx* gene was not inherited by the co-ancestor of SIVcpz and HIV-1 (Zhang et al. 2012), which still enabled these lentiviruses to be pathogenic in their respective hosts. When comparing the pathogenicity of HIV-1 to that of HIV-2, it appears that the lack of Vpx and thereby its inability to counteract SAMHD1 might be one of the reasons why HIV-1 is more pathogenic than HIV-2. However, direct evidence is still lacking to confirm this hypothesis.

Mechanism of SAMHD1 Counteraction by Vpx
HIV-2 and some strains of SIV encode Vpx that is capable of targeting SAMHD1 for proteasomal degradation. This action by Vpx is sufficient to relieve the retroviral restriction posed by SAMHD1 in nondividing cells. Consequently, lentivirus strains that encode Vpx are able to replicate more efficiently in cells that are typically resistant to HIV-1 infection. Virion-incorporated Vpx is released into the cytosol of the infected cell upon viral entry and is able to hijack cellular processes and target cellular SAMHD1 for degradation. The cellular machinery hijacked by Vpx is the DCAF1-E3 ubiquitin ligase complex, which is responsible for the polyubiquitination of cellular proteins. Structure-based analysis of the complex of Vpx with DCAF1 and the C-terminal region of SAMHD1 has provided a molecular mechanism of how Vpx is able to subvert the cell’s normal protein degradation pathway to inactivate the intrinsic immunity against retroviral infection (Schwefel et al. 2014). The precise molecular determinants for the Vpx-SAMHD1 interaction are species dependent, and Vpx from the HIV-2 and SIVmacaque lineages has been mapped to the C-terminus of SAMHD1. The N-terminus of Vpx, which contains a highly conserved motif corresponding to amino acids 24–39 and is present in diverse Vpx and Vpr proteins of primate lentiviruses, interacts with the C-terminus of SAMHD1 and directs it to the DCAF1-E3 ubiquitin ligase complex that targets SAMHD1 for proteasomal degradation (Ahn et al. 2012; Wei et al. 2012). However, the N-terminus of SAMHD1 modulates SAMHD1 interactions with Vpx from SIVmnd-2 and SIVrcm. These molecular variations in the interaction between SAMHD1 and Vpx are not completely elucidated and are likely to have arisen throughout lentiviral evolution and adaptation to the host (Fregoso et al. 2013).

Degradation of SAMHD1 by Vpx occurs in the nucleus (Berger et al. 2012), and Vpx cannot degrade cytoplasmic variants of SAMHD1. Although cytoplasmic SAMHD1 is able to interact with Vpx in the cytoplasm, this complex remains retained in the cytoplasm; thus it is likely that initiation of degradation of SAMHD1

requires nuclear localization of both SAMHD1 and Vpx. Interestingly, although not susceptible to Vpx degradation, cytoplasmic SAMHD1 mutants are capable of effectively restricting lentiviral infection (Brandariz-Nunez et al. 2012; Hofmann et al. 2012).

Counteraction of SAMHD1 by Vpr from SIVs that Lack Vpx

The primate lentiviral protein Vpx counteracts SAMHD1. However, Vpx is unique to only two primate lentivirus lineages, namely, SIVsm and SIVmac. All primate lentiviruses, as well as HIV-2, encode Vpr, a homologue of the HIV-1 Vpr protein. Vpx probably evolved via duplication of its paralogue primate *vpr* gene. Some Vpr proteins from Vpx-lacking lentiviruses also can potentially degrade SAMHD1, often with species specificity. For example, Vpr from HIV-1 and SIV that infects chimpanzee and Old World monkeys cannot degrade human SAMHD1. However, Vpr from SIV that infects De Brazza's monkey can degrade SAMHD1 from all primate species, including human SAMHD1. Similarly, Vpr from SIV that infects mustached monkeys, which is in the same genus as SIV infecting De Brazza's monkey, has broad specificity against primate SAMHD1 proteins. It is likely that positive selection of interacting residues has affected the specificity and sensitivity of binding of Vpx/Vpr protein and degradation of SAMHD1. Evolutionary analysis suggests that *vpr* gained the SAMHD1 degradation function during evolution and indicates that divergent Vpr proteins can functionally degrade SAMHD1 (Lim et al. 2012).

Implications for HIV-1 Due to Lack of Vpx

The presence of Vpx has given HIV-2 an advantage over HIV-1 in the fact that the former is able to replicate more efficiently in myeloid cells that are important for innate immunity. However, incongruously HIV-2 is less pathogenic than HIV-1 and it is speculated that overcoming SAMHD1-mediated restriction in myeloid cells through Vpx triggers an innate immune response by HIV-2 which limits viral replication. Conversely, HIV-1 that lacks such a viral countermeasure against SAMHD1 fails to trigger an innate

immune response due to the lack of productive infection of myeloid cells and is able to evade detection by the immune system. Recent experimental evidence has demonstrated that if SAMHD1-mediated restriction can be overcome in myeloid cells, HIV-1 is then able to trigger an innate immune response and activate myeloid cells, which further confirms the role SAMHD1 plays during HIV-1 infection and in regulating the innate immune response.

Conclusion

The discovery of SAMHD1 as an HIV-1 restriction factor in 2011 and the rapid progress in studying its functions have generated rich information about this novel dNTPase. The focus has been on dissecting the molecular and cellular mechanisms by which SAMHD1 blocks retroviral infections and interacts with the lentiviral protein Vpx and other host proteins. It is clear that SAMHD1 acts as an HIV-1 restriction factor only in nondividing cell types, such as myeloid cells and resting CD4⁺ T cells. However, the molecular basis of the cell-type-specific HIV-1 restriction is not fully understood. Low levels of intracellular dNTPs and expression of unphosphorylated SAMHD1 in these cell types are clearly correlated with HIV-1 restriction. Several cell-cycle regulatory host proteins, including cyclin A2 and CDK1/2, phosphorylate SAMHD1 in cells and may affect cellular dNTP hemostasis. Additional mechanisms, such as RNase activity of SAMHD1, may also contribute to SAMHD1-mediated HIV-1 restriction in nondividing cells.

The future directions of SAMHD1 studies may include at least three major aspects. First is to clearly define the molecular mechanisms underlying SAMHD1-mediated retroviral restriction in primary human myeloid cells and resting CD4⁺ T cells. It is important to dissect the connections between HIV-1 restriction function of SAMHD1 and the regulation of the cell cycle. Second is to translate basic knowledge of SAMHD1 into development of new therapeutic interventions against HIV-1. The key question is how to balance HIV-1 inhibition and nonspecific dNTP

degradation by SAMHD1 in seeking a potential anti-HIV strategy. Third is to explore its physiological functions *in vivo* with a focus of its functions in regulating the innate immunity during viral infections. The success of these three aspects would depend upon further structural and functional analyses of SAMHD1 and its interactions with Vpx and host cofactors.

Cross-References

- ▶ [APOBEC3F/G and Vif: Action and Counteractions](#)
- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [Cellular Cofactors of HIV as Drug Targets](#)
- ▶ [Interactions Between HIV-2 and Host Restriction Factors](#)

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CRM1

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Definition

CRM1, chromosome *region maintenance* 1 protein also known as exportin-1 (XPO1), mediates the export out of the nucleus of cellular proteins with a leucine-rich nuclear export signal (NES, a short 8–15 amino acid hydrophobic motif), such as the HIV-1 Rev and the HTLV-1 Rex proteins, and the transport of small RNA cargoes. The gene for CRM1 is located on chromosome 2p15. CRM1 is widely conserved and is found in species ranging from candida, yeast, drosophila, xenopus, and mammals.

Organization of CRM1 Protein

The CRM1 protein is 1071 amino acids in length (<http://www.uniprot.org/uniprot/O14980>) (Kudo et al. 1997). It is a member of the karyopherin β family (Moroiianu 1998). This family of proteins is involved in many nucleocytoplasmic transport events; the karyopherin proteins are termed either importins or exportins, depending on the direction that they ferry their cargoes. Human CRM1 shares an N-terminal domain (amino acids 46–112) with homology to karyopherin β 1, β 2, β 3, and β 4 (Fornerod et al. 1997), and it has 10 HEAT repeats (amino acids 217–1039). The HEAT-repeat domain was originally identified in the four proteins that give rise to the name [Huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and yeast kinase TOR1]; the HEAT repeats form a rodlike helical structure that provides a broad surface accessible for interaction with multiple ligands such as GTPase proteins and the FG (phenylalanine-

glycine) sequence repeats, commonly found in many nuclear pore proteins (Denning et al. 2003). Mutagenesis analyses of the CRM1 protein have shown that the region spanning amino acids 1–679 are important for supporting HTLV-1 Rex function while amino acids 327–450 interact with the Ran (*Ras*-related nuclear protein) GTP-binding protein, and amino acids 800–820 bind the HIV-1 Rev protein. Structures have been obtained of CRM1 bound to NES peptides (Guttler et al. 2010); from the structure, it can be deduced that Leptomycin B, a potent antifungal antibiotic, blocks the action of CRM1 via its formation of a covalent bond with the Cys528 residue of human CRM1, which is located in its NES-binding groove (Dong et al. 2009a) Dong et al. 2009b). Leptomycin B, thus, prevents NES proteins from CRM1 binding.

Cargo Transport Functions of CRM1

Karyopherin β proteins are involved in the import or the export of cargoes into or out of the nucleus. The direction of movement is governed by a RanGTP gradient. Thus karyopherin β importins bind their import substrates at low concentrations of RanGTP in the cytoplasm and release them in the high-RanGTP environment in the nucleus (Rexach and Blobel 1995; Jakel and Gorlich 1998) Conversely, karyopherin β exportins bind their export substrates at high-RanGTP concentrations in the nucleus and then move into the cytoplasm (Fornerod et al. 1997) where the cargo is disassembled upon GTP hydrolysis by RanGAP (Ran GTPase-activating protein) and RanBP1 (Ran-binding protein) or RanBP2 (Bischoff and Gorlich 1997; Kutay et al. 1997). The exportin proteins can be re-imported into the nucleus to provide a next round of service.

A major function of CRM1 is its export of NES-containing proteins. A recent survey identified 221 human NES proteins that are substrates of CRM1 (Xu et al. 2012). This list of proteins, which includes several biological important factors such as BRCA1, Cyclin B1, hTERT, I κ B α ,

and NANOG, is available at the <http://prodata.swmed.edu/LRNes> website. Besides its role in the export of NES proteins, CRM1 is also well described to function in the nuclear export of U-rich snRNAs (small nuclear RNAs) and in the intranuclear transport of snoRNAs (small nucleolar RNAs). The U snRNAs (e.g., U1, U2, U4, and U5) act in RNA splicing and are transcribed in the nucleus where they are monomethyl m^7G -capped. Interestingly, m^7G -capped U snRNA complexed with a phosphorylated adaptor for RNA export (PHAX, Ohno et al. (2000)) is then exported into the cytoplasm by CRM1 where it associates with survival of motor neuron (SMN) proteins (Massenet et al. 2002) and becomes hypermethylated (trimethylated) on the G cap by the PIMT protein (see ► [PIMT/TGS1](#)) (Yedavalli and Jeang 2010) to form a mature snRNP (small nuclear ribonucleoprotein) complex. Trimethylated-G-capped snRNPs are then re-imported into the nucleus to participate in mRNA splicing. Another activity of CRM1 is the intranuclear transport of small snoRNAs (e.g., U3, U8, and U13) into and between nuclear compartments such as speckles, Cajal bodies, and nucleoli (Boulon et al. 2002). Interestingly, snoRNAs, like mature snRNAs, have a trimethylated G cap, and it has been raised that this N-terminal trimethylated G cap might in part contribute to the specificity of snRNA and snoRNA recognition by CRM1 (Yedavalli and Jeang 2011). CRM1 has also been suggested to be one of several nuclear export receptors for the 60S ribosomal subunit in yeast (Hung et al. 2008).

Rev Is an Export Substrate of CRM1

The HIV-1 Rev (see ► [HIV-1 Rev Expression and Functions](#)) and the HTLV-1 Rex proteins are now known to be two of the 221 NES-containing proteins that are exported from the nucleus by CRM1 (Xu et al. 2012). Historically, the initial observations were made that the HIV-1 Rev protein competed for nuclear export in a pathway employed by 5S rRNA and U snRNAs (Fischer et al. 1995). Subsequent observations from model experiments

performed in xenopus (Fornerod et al. 1997) and yeast (Neville et al. 1997) cells verified that the export of Rev from the nucleus was dependent on the interaction between Rev's leucine-rich NES signal and the CRM1 nuclear receptor. Rev is a 116 amino acid protein that binds to a ~350 nucleotide stem-loop-rich RRE RNA motif positioned at nucleotides 7709–8063 in the HIV-1 genome. The RRE motif is present in 9 kb (unspliced) and 4 kb (singly spliced), but not 1.8 kb (multiply spliced), viral transcripts. Currently, it is understood that Rev is first imported into the nucleus via interaction between its arginine-rich nuclear localization sequence (NLS) with importin β , and it exits the nucleus via NES engagement with the CRM1 protein. Rev binds RRE-containing HIV-1 RNAs and is instrumental in the egress of unspliced and singly spliced, but not multiply spliced, HIV-1 RNAs from the nucleus (see ► [DDX3, Cofactors, and RNA Export](#); Harris and Hope 2000). Parallel studies have also shown that CRM1 is also the nuclear export receptor for the HTLV-1 Rex protein (Hakata et al. 1998).

Conclusion

Studies on CRM1 have enriched the understanding of pathways employed for the nuclear export of important biomolecules. Moving forward, an emerging area of interest lies with the development of CRM1-specific inhibitory drugs that are potentially useful for blocking protein mislocation (see ► [Cellular Cofactors of HIV as Drug Targets](#)). In cancer cells, tumor suppressor proteins are frequently mislocated from the nucleus into the cytoplasm, rendering them functionally effete. Blocking the nuclear export function of CRM1 in some instances has demonstrated utility in cancer therapy (Turner and Sullivan 2008). An increasingly large number of new CRM1 inhibitors are being developed (Wach et al. 2010; Bonazzi et al. 2010; Monovich et al. 2009; Sakakibara et al. 2011; Ranganathan et al. 2012). They hold promise for future investigation of physiological and/or therapy of pathological CRM1-mediated processes.

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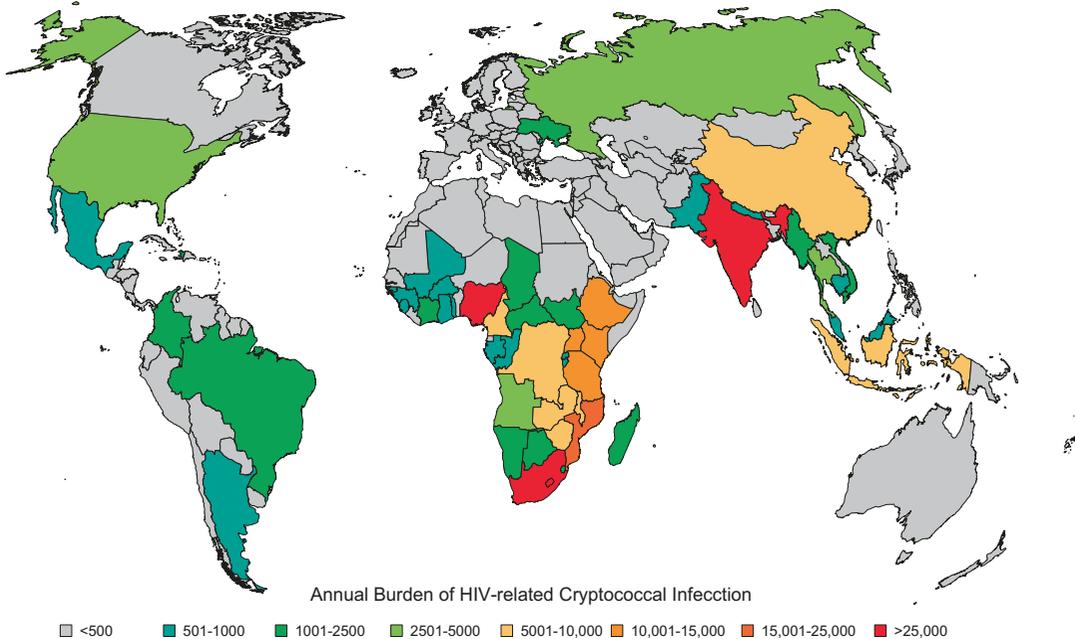
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Cryptococcosis and HIV

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Definition

Cryptococcus neoformans and *Cryptococcus gattii* are encapsulated yeasts found in the environment. *Cryptococcus neoformans* is classically isolated from the soil and from bird guano.



Cryptococcosis and HIV, Fig. 1 Biology of *Cryptococcus*. *Cryptococcus* lives in the environment (1), usually in association with certain trees or soil around trees. Humans and animals can become infected after inhaling airborne, dehydrated yeast cells or spores (2), which travel through the respiratory tract and enter the lungs of the host (3). The small size of the yeast and/or spores allows them to become lodged deep in the lung tissue. The environment inside the host body signals *Cryptococcus* to transform into its yeast

form, and the cells grow thick capsules to protect themselves (4). The yeasts then divide and multiply by budding. In persons with weaker immune systems, after infecting the lungs, *Cryptococcus* yeasts can travel through the bloodstream (5) – either on their own or within macrophage cells – to infect other areas of the body, typically the central nervous system (6) (Modified from Centers for Disease Control and Prevention www.cdc.gov/fungal/diseases/cryptococcosis-neoformans)

Cryptococcus gattii has been isolated from plants and trees, classically eucalyptus trees. This fungus infrequently causes infection in healthy persons but is also an opportunistic pathogen in persons with defects in cell-mediated immunity, such as HIV. In HIV-infected persons, those with a $CD4 < 100$ cell/ μ L are at particular risk of developing cryptococcal infection. *Cryptococcus* yeasts are acquired through inhalation, with ubiquitous environmental exposure (Fig. 1). As the immune system weakens, from conditions such as HIV infection, organ transplantation, cirrhosis, or corticosteroid use, the fungus can disseminate from the lungs throughout the body, including the brain, skin, and other organs.

Cryptococcus gattii

Although *C. neoformans* causes the predominant burden of disease, *C. gattii* has been identified as

causing disease in immunocompetent persons. Historically, *C. gattii* has been endemic in Australia, with reservoirs in the soil, plants, and trees. The majority of those exposed to *Cryptococcus* remain asymptomatic. If symptoms develop, they are typically mild pulmonary symptoms, and spread to the brain is uncommon but possible. However, in 1999 there was a *Cryptococcus gattii* outbreak in Vancouver Island, British Columbia, Canada, that eventually spread to the United States' Pacific Northwest by 2004. In British Columbia alone, 338 cases were described between 1999 and 2012 (Phillips et al. 2015) and another 100 cases in the Pacific Northwest between 2004 and 2013 (Harris et al. 2013). With respect to this specific epidemic, risk factors for infection include oral steroids, underlying lung conditions, smoking, HIV infection, and underlying malignancy (Phillips et al. 2015). During this

outbreak, only ~20% of persons had an underlying immunocompromising condition. Presentation has typically been with nonsevere pulmonary illness, though approximately one third of persons presented with central nervous system (CNS) disease (Phillips et al. 2015; Smith et al. 2014). During this outbreak, ~25% of persons died within 12 months (Phillips et al. 2015).

Clinical Manifestations

In HIV-infected persons, cryptococcosis typically manifests as meningitis in persons with $CD4 < 100$ cells/ μ L. Other manifestations include pneumonitis and extrapulmonary infections such as cryptococcosis of the skin, eye, or prostate.

Cryptococcal Meningitis

Although the dissemination is widespread, the spread to the brain causes a subacute meningoencephalitis with symptoms developing over several weeks. Typical symptoms include fevers, headache, stiff neck, nausea, vomiting, visual changes, altered mental status, and/or seizures. Cryptococcal meningitis is the most common CNS infection among persons living with AIDS.

Pulmonary Manifestations

As mentioned previously, cryptococcal infection is acquired by inhalation and can colonize the airway without manifestation of symptoms. However, cryptococcosis can cause a range of pulmonary manifestations including pneumonia, pulmonary nodules, cavitary lesions, and hilar adenopathy. In those severely immunosuppressed with a $CD4 < 100$ cells/ μ L, persons are more likely to have symptoms such as fever, cough, and shortness of breath, and there is often quick progression to meningitis. Infectious differential for pneumonia in this patient population includes *Pneumocystis*, bacterial pneumonia, *Nocardia*, mycobacterial infection, and Kaposi sarcoma.

Other Manifestations: Skin, Prostate, and Eye

Skin manifestations are the third most common presentation of cryptococcal infection and can

signify disseminated infection. In HIV-infected persons, cutaneous cryptococcosis resembles molluscum contagiosum, though in immunocompromised hosts it can also present as a cellulitis, ulcers, or abscess. Given the variety in presentations diagnosis is confirmed by a skin biopsy histopathology. Cryptococcal infection of the prostate gland is often asymptomatic and identified during urologic surgery for other reasons. During surgery, infection can spread to the bloodstream. Ocular manifestations of cryptococcal infection are often related to elevated intracranial pressure and compression of the ophthalmic artery. Thus, on ophthalmologic exam, one may find papilledema or cranial nerve palsies. Management of elevated intracranial pressure can prevent blindness in this context. Ocular involvement may manifest during paradoxical immune reconstitution inflammatory syndrome (IRIS) (Boulware et al. 2010a).

Epidemiology

Before the extensive roll out of antiretroviral therapy (ART), the burden of cryptococcal meningitis was estimated in 2008 to be almost 1 million cases annually, causing 624,700 deaths annually (Park et al. 2009). The vast majority of cases (70%) occur in sub-Saharan Africa, where *Cryptococcus* is the most common cause of meningitis in adults (Siddiqi et al. 2014; Durski et al. 2013). Typically, 95% of those with cryptococcal meningitis have a $CD4 < 100$ cells/ μ L in the absence of ART. One multinational study of 501 patients with cryptococcal meningitis observed a median $CD4$ count of 24 cells/ μ L (IQR 10–50 cells/ μ L) (Jarvis et al. 2014). In settings with excellent healthcare access to early ART with retention-to-care, cryptococcal meningitis is relatively rare. However, persons still present late to care worldwide, and in the USA, at least 2400 recognized hospitalizations for HIV-related cryptococcal meningitis occur annually (Pyrgos et al. 2013). The overall incidence rate in the USA is 1.1 per 100,000 total population, making cryptococcal meningitis more common than all causes of bacterial meningitis combined at 0.73 hospitalizations per 100,000

population (Pyrgos et al. 2013; Castelblanco et al. 2014). In resource-limited settings, with limited ART coverage, challenges with retention in care, and lack of virologic monitoring, people still present with opportunistic infections such as cryptococcal meningitis. An increasing proportion (40–50%) of cryptococcal meningitis is occurring among persons on ART, being a mixture of unmasking disease in the first few months of ART or late virologic failure with AIDS progression. In low- and middle-income countries, cryptococcal meningitis accounts for 15–20% of AIDS-related deaths (Park et al. 2009; Liechty et al. 2007).

Diagnosis

Classically, the gold standard for the diagnosis of cryptococcal meningitis is through growing *Cryptococcus* yeasts on CSF culture. While indisputable, this often takes 5–14 days to culture. Practically speaking, given that cryptococcal meningitis is a life-threatening infection that is uniformly fatal without therapy, treatment must be initiated immediately, and more timely diagnosis is essential to avoid starting potentially toxic therapy if not necessary. As a result, surrogate tests such as India ink and especially cryptococcal antigen (CrAg) are often used for presumptive diagnosis while awaiting final culture results.

India ink essentially uses black ink as a negative stain of CSF on a microscope slide. With a light microscope, the thick polysaccharide capsule of the organism remains unstained and lights up over a dark background. This quick test is ~85% sensitive and is often the first test performed in resource-limited settings (Boulware et al. 2014a). Unfortunately, among HIV-infected persons in sub-Saharan Africa, the most common meningitis diagnosis among CSF India ink negative persons often is still cryptococcal meningitis (Boulware et al. 2014a), since for persons with access to medical care presenting early with low burden of infection, the India ink sensitivity is poor (Boulware et al. 2014a; Williams et al. 2015).

A more sensitive diagnostic technique is to detect the CrAg which is the polysaccharide sugar of the capsule of the yeast. CrAg can be detected by enzyme immunoassay, latex agglutination, or lateral flow assay. CrAg assays in the CSF and serum are highly sensitive and specific (>99%). CrAg latex agglutination has been used for many decades; however, the process of latex agglutination requires refrigeration of reagents, heat inactivation of the specimen, lab infrastructure for agglutination testing (e.g., rotator, heat block, electricity), and laboratory labor which although simple is relatively labor intensive (Rajasingham et al. 2012a).

In July 2011, a lateral flow assay was US Food and Drug Administration (FDA) approved and CE marked in Europe for rapid, point-of-care detection of CrAg (Immy Inc. Norman, Oklahoma, USA). This dipstick test requires a drop of CSF, serum, plasma, or urine and produces positive or negative results within 10 min. This is more practical in most settings as it can be done outside of the lab, without additional equipment, and at room temperature. This test has been validated to have a sensitivity and specificity of >99% in CSF and serum and in fact has been found to be more sensitive than culture (Boulware et al. 2014a). Real world end-user costs in low/middle income countries are approximately \$3–4/test, including assay cost, shipping, importation fees, distribution, laboratory supplies, and labor.

The CrAg lateral flow assay has changed the landscape of cryptococcal diagnostics. As a point-of-care test, the test can be used at the bedside at the time of lumbar puncture to test CSF. However, a more useful application, particularly in resource-limited settings, is point-of-care CrAg testing prior to performing a lumbar puncture. The CrAg lateral flow assay can also be used with whole blood, serum, or plasma. If CrAg is positive in blood or by fingerstick testing (Williams et al.), then a manometer can be used during lumbar puncture to measure opening pressure. If no manometer is available, then a large volume (20 mL) lumbar puncture can be performed (Rolfes et al. 2014). The point-of-care nature of this test allows healthcare workers to triage

patients to further intervention after early identification of this fatal illness.

Positive CrAg in blood amongst persons who are symptomatic should always prompt a lumbar puncture for evaluation for cryptococcal meningitis and control of increased intracranial pressure.

Serum CRAG may be a good screening tool for HIV-infected persons with suspected pulmonary cryptococcal infection. However, if positive, one tries to confirm with fungal cultures of the sputum. Given the broad differential for an HIV-infected person with a CD4 < 100 cells/ μ L with pulmonary infiltrates, bronchoscopy is often performed to evaluate for other causes of infection. If a nodule is present, biopsy and histopathology can distinguish cryptococcal infection from malignancy.

Treatment

Treatment of cryptococcal meningitis requires antifungal therapy along with management of elevated intracranial pressure. Intracranial pressure management is an equally important aspect of treatment which is often neglected. Antifungal therapy is generally divided into induction, consolidation, and maintenance therapies. Induction therapy is initiated soon after the diagnosis is made. The preferred induction therapy is 14 days of intravenous amphotericin B 0.7–1.0 mg/kg/day in combination with flucytosine 100 mg/kg/day (Table 1) (Perfect et al. 2010; World Health Organization 2011; Masur et al. 2014). This regimen has been proven to be superior to amphotericin alone with improved survival and sterilization of

Cryptococcosis and HIV, Table 1 Treatment of cryptococcal meningitis

Medication and Daily Dose	~2 weeks ^b	8 weeks	52 weeks
Amphotericin B deoxycholate 0.7 to 1.0 mg/kg + second agent ^a	Continue until CSF is known sterile		
Fluconazole 800-1200mg			
Fluconazole 400mg			
Fluconazole 200mg			
Treatment Phase	Induction	Consolidation	Secondary ^d Prophylaxis

^aFlucytosine (5FC) 100 mg/kg/day preferred where available (Day et al. 2013), otherwise fluconazole at 800–1200 mg/day in divided doses. KCl 40–60 mEq/day should be given with amphotericin (Bahr et al. 2014); Flucytosine is dose reduced with kidney dysfunction with 50% dose reduction at creatinine clearance of <50 mL/min and 25% of dose at <25 mL/min (Perfect et al. 2010; Masur et al. 2014)

^bOptimal duration of initial induction therapy is unknown and likely dependent on the fungal burden of the individual patient. Traditional induction therapy duration is 14 days (Perfect et al. 2010; World Health Organization 2011; Masur et al. 2014). In resource-limited regions, the cost-benefit is likely maximal for 1 week induction with amphotericin B deoxycholate at 1 mg/kg/day coupled with 4–6 weeks of fluconazole 1200 mg/kg/day (Rajasingham et al. 2012b). In settings where electrolytes cannot be actively monitored and managed, no more than 1 week of amphotericin should be given (World Health Organization 2011)

^cContinue fluconazole at 800–1200 mg/day until the CSF culture result is known to be sterile and ART has been initiated. Consider longer duration of enhanced consolidation therapy if CSF culture is positive at 2 weeks. Reverting to 400 mg/day of fluconazole when culture positive is a risk factor for 10-week mortality and paradoxical IRIS (van der Horst et al. 1997; Jarvis et al. 2014; Chang et al. 2013). Continuing 800 mg/day of fluconazole ameliorates this excess risk of 2 week CSF culture positivity (Rolfes et al. 2015)

^dSecondary prophylaxis with fluconazole 200 mg/day should be continued for at least 1 year. IDSA guidelines recommend until CD4 > 100 cells/ μ L and viral suppression for >3 months (Perfect et al. 2010). In the absence of virologic monitoring, WHO recommends continuation for at least 1 year and until two CD4 > 200 cells/ μ L measurements at least 6 months apart (World Health Organization 2011)

CSF cultures (Day et al. 2013; van der Horst et al. 1997). In resource-rich settings liposomal amphotericin is often used due to better tolerability, fewer side effects, and decreased risk of nephrotoxicity.

Side effects from amphotericin are many, most importantly causing renal wasting of potassium and magnesium which can cause life-threatening hypokalemia (Bahr et al. 2014). Up to 30% of persons will have a transient significant rise in their creatinine (Jarvis et al. 2014); however, overt acute kidney failure is uncommon with appropriate care. In older populations and those with preexisting kidney disease, amphotericin-related nephrotoxicity can be expected. Patients receiving amphotericin require at least 1 L of intravenous normal saline prior to each dose and frequent monitoring of serum electrolytes. The WHO now recommends preemptive electrolyte supplementation with potassium and magnesium during amphotericin therapy to prevent life-threatening electrolyte disturbances (World Health Organization 2011; Bahr et al. 2014). Other side effects include phlebitis and infusion reactions including rigors, chills, and fevers secondary to the medication. The vast majority of clinical trials have used amphotericin B deoxycholate. Liposomal amphotericin does not have better efficacy but does have fewer side effects.

Flucytosine is used in combination with amphotericin, at a dose of 25 mg/kg every 6 h in those with normal renal function. Strict dosage adjustment is required in those with reduced renal function, which is common especially when given in combination with amphotericin. Dose reduction is proportional to the decreased creatinine clearance with 50% of dose at creatinine clearance of <50 mL/min and 25% of dose at creatinine clearance of 10–25 mL/min. Side effects include bone marrow toxicity and hepatotoxicity, so drug levels are monitored two hours after dosing, typically after 3–5 days to keep peak levels under 100 µg/mL. Flucytosine is not available in most locales due to its current cost (~\$2000/day in the USA) but is available in Europe. Thus, the majority of patients globally do not receive this medication. Instead, adjunctive

fluconazole is used as an alternative (Perfect et al. 2010; World Health Organization 2011). High dose fluconazole (800–1200 mg daily) is more fungicidal than 400 mg/day without appreciable side effects (Longley et al. 2008). Fluconazole has good penetration into the parenchyma of the brain, whereas amphotericin penetration is more limited. As fluconazole is 90% eliminated by the kidneys, dose adjustments are recommended with declining renal function.

Consolidation therapy, as recommended by the IDSA guidelines, consists of fluconazole at 400–800 mg daily for at least 8 weeks. As the day 14 CSF culture status is typically not known for another 10–14 days, many experts will continue an initial *enhanced* consolidation therapy of fluconazole at 800–1200 mg/day until the CSF is known to be sterile and ART has been started (Rolfes et al. 2015). With doses of fluconazole ≥ 800 mg/day, dividing the dose can decrease nausea. Persons with positive cultures at 2 weeks should have longer consolidation therapy of 10–12 weeks.

Maintenance therapy for secondary prophylaxis is with fluconazole 200 mg daily. IDSA guidelines recommend discontinuing this maintenance after 1 year of antifungal therapy once CD4 > 100 cells/µL and the patient has an undetectable HIV VL for >3 months to prevent cryptococcal relapse. For many individuals, ART is initiated 4–5 weeks after the initial infection with *Cryptococcus*, and the CD4 should have reconstituted by the time the maintenance phase is reached. However, if ART is unavailable, or the patient remains on a failing regimen, fluconazole should be continued while CD4 < 200 cells/µL. In the absence of virologic monitoring, WHO recommends continuation of secondary prophylaxis for at least 1 year and until the CD4 is >200 cells/µL for more than 6 months (World Health Organization 2011).

Management of Intracranial Pressure

A critical part of treatment success in the management of cryptococcal meningitis is controlling intracranial pressure (Rolfes et al. 2014).

Cryptococcal meningitis is unique from other types of meningitis in that the large cryptococcal capsule obstructs the outflow of CSF in the brain's arachnoid villi. This obstruction results in elevated intracranial pressure. An increased pressure reduces local tissue perfusion and function of the brain. Symptoms of elevated intracranial pressure include headache, nausea, vomiting, and vision changes. Signs on exam may include papilledema, cranial nerve palsies, meningismus, acute onset blindness, or reduced level of consciousness. Elevated intracranial pressure in cryptococcal meningitis has been associated with morbidity and mortality when uncontrolled, and lumbar punctures are recommended to therapeutically reduce pressure. Normal CSF opening pressure is <20 cm H₂O when measured with a manometer in the lateral decubitus position. Lumbar punctures to control intracranial pressure are associated with improved survival, regardless of initial intracranial pressure (Rolfes et al. 2014), suggesting potential benefit for all patients with cryptococcal meningitis. Infectious Disease Society of America guidelines recommend when the CSF opening pressure is >25 cm H₂O, CSF is drained until opening pressure is reduced by 50% or to a normal pressure <20 cm H₂O (Perfect et al. 2010). Thereafter, repeat lumbar punctures should be performed daily to remove CSF until pressures are <20 cm H₂O (Perfect et al. 2010). Unfortunately, in resource-constrained settings, where the majority of cryptococcal meningitis occurs, manometers to measure ICP are unavailable. In this situation, one can use a three-way stopcock, IV tubing, and a meter stick to measure opening pressure (Meda et al. 2014). Alternatively, for a diagnostic lumbar puncture in a person with known cryptococcosis (e.g., CrAg positive in blood), empiric removal of 25 mL of CSF is reasonable, as this is the median amount removed in many African cohorts (Rolfes et al. 2014).

If initial lumbar puncture does not demonstrate elevated opening pressure, and daily assessments are negative for classic signs of elevated ICP, a repeat lumbar puncture is still recommended at the end of induction therapy to document sterilization of CSF. Approximately 20% of persons with

normal intracranial pressure at diagnosis will develop increased intracranial within 7 days (Rolfes et al. 2014), thus there should be a low threshold for repeat therapeutic lumbar punctures.

Neither steroids nor acetazolamide are effective at reducing intracranial pressure and both are not recommended (Perfect et al. 2010; Masur et al. 2014). Serial therapeutic lumbar punctures are the management of choice or neurosurgical placement of shunts for those with persistent problems with increased pressure.

Timing of ART Initiation

Earlier ART initiation is generally preferred for other AIDS-related opportunistic infections, which do not involve the central nervous system (CNS) (Zolopa et al. 2009; Blanc et al. 2011). Although earlier immune recovery decreases the risk for developing other new opportunistic infections, early ART also increases the risk of paradoxical immune reconstitution inflammatory syndrome (IRIS) (Blanc et al. 2011). The vast majority of paradoxical IRIS reactions can cause transient morbidity, being severe at times, but rarely mortality. The exception is IRIS events involving the CNS. IRIS within the CNS initiates local inflammation in a confined space within intracranial structures that are neither permissive to inflammation nor to compression. Cryptococcal-related IRIS can result in death, thus earlier ART may have substantial risk. A multicenter randomized clinical trial tested whether earlier ART (1–2 weeks) vs. delayed ART (4–6 weeks after diagnosis) was the optimal strategy. This Cryptococcal Optimal ART Timing (COAT) trial was terminated early due to 15% worse 26-week mortality with earlier ART. Notably, earlier ART had markedly worse survival among those lacking CSF pleocytosis (i.e., <5 WBC/ μ L CSF) (Boulware et al. 2014b), and this subgroup previously has been shown to be at high risk of paradoxical IRIS (Boulware et al. 2010b; Chang et al. 2013). Additionally, those with ongoing altered mental status at 1 week of antifungal treatment also did worse with earlier ART (Boulware et al. 2014b). There

was no subgroup in whom earlier ART was clearly preferred. This trial definitively proved that starting earlier ART during induction therapy does not improve survival. The COAT trial did not prove that the optimal time to initiate ART is at precisely 5 weeks, and physician judgment is necessary. Aiming for a 4–5 week window is reasonable; however, one first needs to verify that the 2-week CSF culture is sterile before decreasing fluconazole dosing and initiating ART.

Immune Reconstitution Inflammatory Syndrome (IRIS)

IRIS occurs after initiating ART with rapid, excessive inflammation in the context of immune restoration in response to alive or dead organisms within the body that may have gone unrecognized prior to initiation of ART. IRIS can occur with any opportunistic infection, but IRIS is more common for infections where antigen is slowly cleared or persists, such as tuberculosis and *Cryptococcus*. There are two distinct immune reconstitution syndrome scenarios, unmasking IRIS and paradoxical IRIS. These are separate, distinct clinical scenarios but often confusingly grouped together simply as IRIS.

Cryptococcal-related **paradoxical IRIS** occurs in 10–30% of persons with cryptococcal meningitis who survive to initiate ART, at a median time of 6–10 weeks after starting ART but can also occur within the first days to weeks of ART (Boulware et al. 2010a; Chang et al. 2013; Bahr et al. 2013). Paradoxical IRIS occurs after a patient with cryptococcal meningitis has been treated with antifungals, clinically improves, starts ART, and then deteriorates with recurrent meningitis symptoms in the setting of successful microbiologic treatment and sterile cultures. Paradoxical IRIS is primarily an immunologic disease where a previously anergic immune system can now generate an effective immune response against persistent antigen from dead, nonviable organisms. The major risk factors for paradoxical IRIS include initial anergy, poor clearance of antigen, and residual CSF fungal burden (Boulware et al. 2010a, b; Jarvis et al. 2014; Chang

et al. 2013). Readily available clinical risk factors include: (1) low initial CSF white cell count at diagnosis (i.e., anergy); (2) persistently positive CSF cultures when beginning fluconazole 400 mg/day consolidation therapy; (3) high pre-ART serum CrAg ($\geq 1:512$ titer); (4) high pre-ART serum C-reactive protein (CRP) (>32 mg/L) indicative of immune dysregulation and/or smoldering ongoing low-grade infection (Boulware et al. 2010a, b; Jarvis et al. 2014; Chang et al. 2013).

Distinguishing between paradoxical IRIS and culture-positive relapse remains clinically impossible, as symptoms are identical. CSF analysis is critical for distinguishing between IRIS and relapse (Haddow et al. 2010). CSF culture ultimately determines whether there is viable *Cryptococcus* growing suggesting relapse. Sterile CSF cultures are typical of IRIS. In the absence of culture data, IRIS typically presents with more inflammation in the CSF, whereas relapse frequently has more of a paucity of CSF inflammation. There is overlap of CSF parameters, and culture remains essential.

The management of IRIS includes controlling intracranial pressure as a first step. While CSF cultures are pending, increasing antifungal therapy with higher dose fluconazole (800–1200 mg/day) and/or short course amphotericin (i.e., 7 days) is also recommended. For severe cases, with signs of cerebral edema, steroids can be given, although no clinical trials have evaluated the benefit of steroids specifically in cryptococcal IRIS. In TB-IRIS, steroids are effective (Meintjes et al. 2010); however, IRIS may commonly recur with tapering of steroids. In most cases, continuation of ART is recommended (Haddow et al. 2010); however, in severe, life-threatening IRIS, ART may be temporarily discontinued. Intraparenchymal inflammation in the brain can commonly cause new onset seizures as a presenting feature of IRIS. Magnetic resonance imaging (MRI) or head computerized tomography (CT) can often demonstrate variable degrees of intraparenchymal inflammation associated with IRIS.

Cryptococcal unmasking IRIS occurs when a person starts ART with unrecognized, subclinical cryptococcal infection. Due to

immunosuppression, early disseminated cryptococcal infection often has minimal or vague symptoms but is detectable by the presence of CrAg in peripheral blood. If untreated, this will progress to overt symptomatic cryptococcal meningitis over weeks to months (French et al. 2002; Letang et al. 2015). With ART-mediated reconstitution of the immune system, the immune system can respond to the cryptococcal organisms in the brain and/or throughout the body. Clinical manifestations of unmasking disease on ART are more diverse than traditional cryptococcosis in ART-naïve persons. Often symptom onset can be more rapid among persons receiving ART. Additionally, the spectrum of clinical manifestations can be more varied with presentations of meningitis, seizures, focal neurologic deficits, pneumonitis, lymphadenopathy, or sepsis. Within the first 6 months of ART among persons with CD4 nadir of <150 cells/ μL , any new onset CNS symptoms occurring should prompt immediate CrAg testing as well as consideration in any ill-defined, unexplained disease process.

Cryptococcal Relapse

Cryptococcal relapse occurs in approximately 5% of persons who survive cryptococcal meningitis and then have a second episode of cryptococcal infection with viable organisms cultured from the CSF. Relapse can occur years later (Katchanov et al. 2015). Primary causes for relapse include: lack of secondary prophylaxis with fluconazole 200 mg/day or interval development of fluconazole resistance (Jarvis et al. 2010).

Persons with cryptococcal meningitis relapse often present with recurrent signs and symptoms such as fever, headache, possible visual changes, and signs of elevated intracranial pressure. Clinically, if the patient has recently started ART, it is difficult to distinguish from paradoxical cryptococcal IRIS. The ultimate diagnosis can only be made by lumbar puncture and CSF culture. If there is *Cryptococcus* growing in CSF culture, this would indicate relapsed cryptococcal infection. If there is inflammation but sterile CSF cultures, this would suggest cryptococcal IRIS. CrAg

in the CSF or in the serum is not helpful, as the CrAg often takes years to fully clear from the body and is qualitatively positive in both IRIS and cryptococcal relapse. India ink may also be positive in both scenarios. Distinguishing between these two complications of cryptococcal meningitis is critical, as management approaches differ. However, IRIS and relapse may not always be distinct and can coexist (Musubire et al. 2013).

Cryptococcal relapse is distinct from initial treatment failure. In treatment failure, the CSF is never sterilized after the initial episode of meningitis, whereas in relapse, the CSF is sterilized and then subsequently re-infected. Treatment failure suggests an inadequate treatment regimen or fluconazole resistance. Optimal induction therapy for cryptococcal meningitis consists of an amphotericin-based regimen with flucytosine or fluconazole if flucytosine is unavailable. Many resource-limited settings use fluconazole monotherapy, and treatment failure is mainly seen in this context. There is also the possibility of fluconazole resistance if low dose fluconazole is used alone. Amphotericin resistance has not been described with respect to cryptococcal infection. IDSA guidelines recommend induction therapy for 4–10 weeks, using high dose amphotericin at 1 mg/kg/day.

Management of relapse is similar to management of the initial cryptococcal meningitis episode. That is, induction therapy with amphotericin (0.7–1 mg/kg/day) with flucytosine, or fluconazole if flucytosine is unavailable, along with management of elevated intracranial pressure with serial lumbar punctures. Thereafter, one should reinstitute consolidation therapy with high dose fluconazole. If ART has already been initiated, it is important to ensure that persons with relapse are on a fully active regimen, and in the process of reconstituting the CD4 cells, as this will ultimately prevent future infection.

Prevention and Preemptive Therapy

Given the substantial morbidity and mortality from cryptococcal meningitis, especially in low income countries with a high burden of AIDS, an

effective prevention strategy would be highly valuable to health systems, not to mention life saving. Universal fluconazole prophylaxis for all those with a CD4 < 200 cells/ μ L has been evaluated in a randomized controlled trial (Parkes-Ratanshi et al. 2011). Although fluconazole prophylaxis decreased cryptococcal meningitis, overall mortality did not differ between those who received fluconazole prophylaxis and those who did not. Thus, universal prophylaxis has not been recommended, as persons living with AIDS require prompt ART initiation, whereby immune recovery is the most effective long-term prevention.

Screening for cryptococcal infection using serum or plasma CrAg testing prior to initiation of ART among persons with CD4 < 100 cells/ μ L is a cost-effective method to identify subclinical infection that can reduce morbidity and mortality (Mfinanga et al. 2015; Meya et al. 2010). Prevention via screening is possible as CrAg can be detected in the blood weeks to months prior to the development of fulminant meningitis, when people are asymptomatic. CrAg + prevalence amongst asymptomatic HIV-infected persons has been evaluated in multiple settings across sub-Saharan Africa and Asia with a prevalence averaging 7% amongst persons with a CD4 < 100 cells/ μ L (Rajasingham et al. 2012a). Those with detectable CrAg in their blood, despite not having meningitis, have a higher mortality, compared to those who are CrAg negative (Liechty et al. 2007; Mfinanga et al. 2015; Meya et al. 2010). Being CrAg + is 100% sensitive for predicting the development of meningitis within 1 year of ART initiation (Jarvis et al. 2009). Conversely, if one is CrAg negative when initiating ART, the risk of developing cryptococcal meningitis is near zero (Jarvis et al. 2009; Pongsai et al. 2010). Subclinical cryptococcal infection also occurs among persons with CD4 100–200 cells/ μ L; however, the prevalence is lower (approximately 2%). Most of these persons will have an uneventful immune recovery with ART; and the cost-effectiveness is less favorable (Rajasingham et al. 2012a). How best to conduct the screening may vary by structure of the healthcare system; however, many sites are using reflex CrAg lateral flow assay testing of the

leftover plasma sample whenever the CD4 < 100 cells/ μ L.

Those who are asymptomatic but CrAg + derive significant benefit from fluconazole preemptive therapy (Mfinanga et al. 2015; Meya et al. 2010). In 2011, the WHO recommended CrAg screening of those HIV infected with a CD4 < 100 cells/ μ L and preemptive therapy for those CrAg+ with high dose fluconazole (800 mg/day for 2 weeks, followed by 400 mg/day for 8 weeks) (World Health Organization 2011). This may not be optimal preemptive therapy, as the mortality rate is still 30% among CrAg + persons preemptively treated (Mfinanga et al. 2015). Studies are underway evaluating optimal dose and duration of preemptive therapy in this population. The benefit of secondary prophylaxis with fluconazole 200 mg/day in this CrAg + population is also unclear. Coupled with the point-of-care, affordable CrAg lateral flow assay, pre-ART CrAg screening is now recommended in an increasing number of national HIV program guidelines, with various degrees of implementation.

Conclusion

In summary, cryptococcal meningitis is the most common cause of adult meningitis in sub-Saharan Africa, affecting HIV-infected persons predominantly with a CD4 < 100 cells/ μ L. Due to challenges in accessing and retaining persons in HIV care, cryptococcal meningitis continues to occur among marginalized populations and accounts for approximately 20% of AIDS-related deaths in some areas. Cryptococcal meningitis remains a neglected disease with poor outcomes even among optimally treated persons. A novel point-of-care CrAg lateral flow assay has changed the landscape of cryptococcal prevention and diagnostics. Treatment requires management of elevated intracranial pressure by serial lumbar punctures coupled with antifungals. Optimal antifungal therapy of amphotericin and flucytosine is rarely available in resource-limited settings, where the majority of cryptococcal infection occurs. Initiation of ART is unique from other

opportunistic infections as in those with cryptococcal meningitis waiting for approximately 4–5 weeks after diagnosis and treatment of meningitis results in improved outcomes. Paradoxical IRIS can occur after ART initiation, and IRIS can be fatal in cryptococcal infection. Finally, prevention with pre-ART CrAg screening and preemptive treatment of CrAg + persons is a promising, cost-effective public health intervention to decrease the development of cryptococcal meningitis once persons enter into HIV care.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Immunological Responses to Antiretroviral Therapy](#)

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Cryptosporidiosis

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Definition

Cryptosporidiosis is a gastrointestinal infection presenting as diarrhea due to infection with

various species of *Cryptosporidium*, an Apicomplexan parasite.

Introduction

Cryptosporidium is an intestinal parasite of the phylum Apicomplexa, which causes diarrheal disease in humans and animals worldwide (Tzipori and Ward 2002). Initially thought to be a coccidian (subclass Coccidiasina), recent phylogenetic studies indicate that *Cryptosporidium* is more closely related to gregarines (subclass Gregarinasina). Although *Cryptosporidium* was discovered in 1907, it was not until 1976 that the first human cases of cryptosporidiosis were reported and not until the onset of the AIDS epidemic in the 1980s that this parasite was recognized as a significant human pathogen (O'Connor et al. 2011). Cryptosporidiosis was one of the original AIDS-defining illnesses and was associated with an increased risk of death compared to other AIDS-defining illnesses (Shikani and Weiss 2014).

In addition to AIDS patients, other immune-compromised populations at risk for cryptosporidiosis include those on immunosuppressive therapy such as transplant recipients, patients with severe combined immunodeficiency, and X-linked hyperimmunoglobulin M syndrome. Children under the age of 5 years in resource-limited settings, particularly those who are malnourished, are also vulnerable to moderate to severe diarrhea caused by *Cryptosporidium*. In these young children, immaturity of the immune system combined with malnutrition may contribute to immune compromise.

Species and Life Cycle

Twenty-seven species of *Cryptosporidium* have been described (Leav et al. 2003). However, two major species most commonly infect humans; *C. parvum* infects humans and animals while *C. hominis* almost exclusively infects humans. Other species including *C. meleagridis*, *C. muris*, *C. canis*, *C. felis*, *C. suis*, *C. muris*, and *C. andersoni* are also known to infect humans. *Cryptosporidium* exists in several

intracellular and extracellular developmental stages, and the life cycle is completed within a single host. The infective stage of the parasite is the environmentally resistant oocyst which can survive in water for months. The infective dose of oocysts is extremely low but varies by the infecting species or isolate. As few as 10 oocysts have been reported to be sufficient to cause infection.

Infection is initiated by ingestion of oocysts which excyst in the upper small intestine to release four motile sporozoites. Sporozoites attach to and invade the brush border membrane of intestinal epithelial cells and are engulfed by the microvilli. Intracellular replication occurs within a parasitophorous vacuole, in a unique intracellular but extracytoplasmic niche. Small villi-like folds of the parasite cytoplasm extend into the host cell to form a structure called the feeder organelle which is thought to serve as a route of transport between the parasite and host. Within the parasitophorous vacuole, *Cryptosporidium* replicates via asexual and sexual cycles. During the asexual cycle, sporozoites develop into trophozoites which differentiate into Type I meronts. Merozoites, which are morphologically indistinguishable from sporozoites, are released from meronts into the lumen and rapidly invade adjacent cells where they perpetuate the asexual cycle or differentiate into Type II meronts. Merozoites from these meronts initiate the sexual cycle by forming microgamonts and macrogamonts. Microgametes released from microgamonts fertilize macrogamonts and form zygotes which mature into either thick-walled oocysts that are released into the external environment via the feces or thin-walled oocysts that rupture in the intestinal lumen releasing sporozoites that initiate a new round of replication. These thin-walled auto-infective oocysts are thought to contribute to perpetuation of the infection in patients with HIV/AIDS.

Epidemiology

Cryptosporidiosis is found worldwide, but infection prevalence varies widely among different regions and populations at risk (Collinet-Adler and Ward 2010; Checkley et al. 2015). Prevalence rates range from as low as 1% in industrialized

countries to as high as 30% in developing countries. In the USA, the incidence of *Cryptosporidium* was reported to be 2.5–2.7/100,000 for 2009 and 2010 respectively. Seroprevalence rates are higher than those estimated by microscopic examination of stools and range from 30% to 89% depending on geographic region. The widespread use of combination antiretroviral therapy (cART) for HIV/AIDS in developed countries has drastically reduced the prevalence of cryptosporidiosis in these countries. Although cART is available in some developing countries it is not widely available or affordable in some areas where the burden of HIV/AIDS is high. In these countries, *Cryptosporidium spp.* remain a major cause of diarrheal disease in untreated AIDS patients, with prevalence rates as high as 80% reported.

In the general population those at increased risk of acquiring cryptosporidiosis include children in day care centers and their caregivers, travelers to endemic areas, backpackers, campers or hikers who drink unfiltered water from streams or rivers, those who drink unfiltered water from wells, and those who handle infected dairy or farm animals. The risk of acquiring cryptosporidiosis in patients with HIV/AIDS is associated with the degree of immunosuppression as measured by CD4 T cell counts. Other risk factors for cryptosporidiosis in these patients include male gender, younger age, and sexual practices involving oral-anal contact.

Cryptosporidium spp. are one of the most frequent causes of waterborne disease outbreaks. In the USA and other developed countries, most infections are caused by waterborne outbreaks, mostly in drinking water but more recently in recreational water sources such as swimming pools or water parks. The largest ever documented outbreak of waterborne disease in the USA was caused by *Cryptosporidium* in Milwaukee, WI, in the USA and affected an estimated 400,000 people with over 100 deaths in immune-compromised and elderly individuals.

Clinical Manifestations

The clinical features of cryptosporidiosis vary widely depending on the immune status of the

host (Shikani and Weiss 2014). In immune-competent individuals most *Cryptosporidium* infections are asymptomatic. The incubation period for symptomatic cryptosporidiosis is 1–2 weeks. Young children and the elderly are at greater risk for symptomatic infections. Watery diarrhea is the commonest symptom, but abdominal cramps or pain, nausea, vomiting, fever, anorexia, and weight loss may also occur. Diarrhea generally lasts for 1–3 weeks but may persist for a month. In immune-competent individuals, infection is self-limited, and spontaneous recovery is the rule. Recurrence of symptoms may also occur. For example, 30% of those infected during the Milwaukee outbreak reported recurrence of symptoms.

Oocyst shedding may occur in the absence of symptoms. In symptomatic infections, oocyst shedding occurs for 1–2 weeks after symptoms subside but may occasionally continue for up to 2 months. The intensity and duration of oocyst shedding may also depend on the infecting species. The oocyst burden in stools is reported to be greater and oocyst shedding longer in *C. hominis* than in *C. parvum* infections.

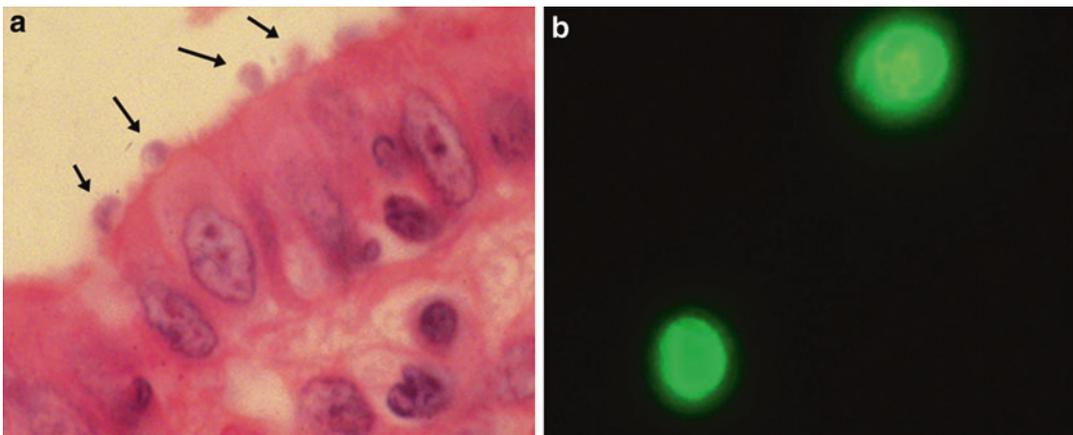
In immune-compromised hosts such as AIDS patients, severity of clinical symptoms is generally related to the degree of immune compromise, with those with CD4 T cell counts less than 200 cells/mm³ being at risk for more severe

clinical disease. Most patients with fulminant cryptosporidiosis have CD4 T cell counts less than 50 cells/mm³. However, some patients with CD4 T cell counts less than 200 cells/mm³ are reported to have asymptomatic infection or mild illness, while others experience severe, debilitating, and chronic diarrhea and wasting which may be ultimately be fatal. The reason for this variability is unknown but may be related to coinfection with other enteric pathogens which exacerbate or suppress symptoms of cryptosporidiosis.

In immune-competent individuals, infection is generally limited to the small intestine (Fig. 1a). However, in AIDS patients, particularly those with low CD4+ T cell counts, other parts of the gastrointestinal tract may be involved including the pharynx, esophagus, stomach, colon, and rectum. Extraintestinal infection involving the gallbladder, biliary tract, pancreatic duct, and respiratory tract have also been reported. Cryptosporidiosis has also been associated with autoimmune conditions such as reactive arthritis and Reiter's syndrome.

Diagnosis

Diagnosis is typically made by microscopic examination of stool samples using acid fast staining or immunofluorescence with



Cryptosporidiosis, Fig. 1 (a) Intestinal biopsy stained with hematoxylin and eosin showing intracellular stages of *Cryptosporidium* (arrows). (b) Immunofluorescence assay

of *Cryptosporidium* oocysts with a monoclonal antibody to the oocyst wall

Cryptosporidium-specific antibodies (Fig. 1b) (Checkley et al. 2015). Acid fast staining has a ~70% sensitivity compared to immunofluorescence. Microscopy with immunofluorescence staining is currently considered the gold standard for clinical diagnosis. Antigen detection methods by ELISA or immunochromatography have higher throughput, but sensitivities vary from 70% to 100%.

Molecular methods including conventional and real-time PCR targeting the 18S ribosomal RNA gene or other genes are much more sensitive than microscopy or antigen detection but are mainly used for research purposes. Real-time PCR quantitation can be multiplexed for detection and quantitation of multiple pathogens and has been adapted to the Luminex platform. Targeting the 18S rRNA gene detects both major species as well as other species that infect humans. Serological assays are used for epidemiological studies and can be indicative of past or current infection. *Cryptosporidium*-specific fecal IgA is associated with recent infection (within 1 month), whereas serum IgG can persist for months to years.

Treatment and Prevention

Management of dehydration caused by diarrhea, particularly in AIDS patients and young children in developing countries, is the mainstay of treatment for cryptosporidiosis and includes oral or intravenous replacement of fluid and electrolytes (Checkley et al. 2015; Leav et al. 2003). Antimotility agents such as opiates or loperamide may also be used. Tincture of opium may be more effective than loperamide. Total parenteral nutrition might be indicated in some patients. Since cryptosporidiosis may be associated with lactase deficiency which may persist into the recovery phase, milk products should be restricted and/or lactase used.

In AIDS patients the mainstay of treatment is restoration of immune function. Antiretroviral (cART) therapy significantly improves diarrheal symptoms in AIDS patients with

cryptosporidiosis. Unfortunately, in spite of extensive testing of various agents, there is no consistently effective antiparasitic drug for cryptosporidiosis in immune-compromised patients (Checkley et al. 2015; Shikani and Weiss 2014). Nitazoxanide and Paromomycin have shown limited efficacy in some trials. Nitazoxanide, a broad spectrum thiazole-containing antiparasitic agent, has been approved by the US Food and Drug Administration for treatment of cryptosporidiosis in immune-competent children and adults. This drug has demonstrated efficacy in reducing the severity and duration of diarrhea in these individuals. However, regardless of dosage or duration of treatment, Nitazoxanide has not been shown to be very effective as primary therapy in immune-compromised patients. In these patients, particularly those with advanced AIDS, Nitazoxanide (500–1,000 mg twice daily for 14 days) or Paromomycin (500 mg four times a day for 14–21 days) in conjunction with cART (but never without cART) may be used.

HIV-infected individuals should be counseled about the different modes of transmission of *Cryptosporidium* and advised to practice scrupulous hand washing after potential contact with human feces, to avoid drinking water from lakes or streams, and to avoid swimming in water that may be contaminated.

Conclusion

Cryptosporidiosis is an important cause of diarrhea in AIDS patients and can be associated with chronic infections as an AIDS defining illness. Infection is associated with higher mortality rates. There are currently no highly effective anti-cryptosporidial drugs for patients with immune dysfunction. Immune restoration and supportive care are the cornerstones of current treatment. Research into effective therapy has been limited due to the absence of long-term culture system, the absence of genetic systems for experimental studies, and the unique cellular niche in which this organism lives.

Cross-References

- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [Immunopathogenesis of HIV Coinfections](#)
- ▶ [Mucosal Immunity to HIV-1](#)

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CXCR4, Coreceptors

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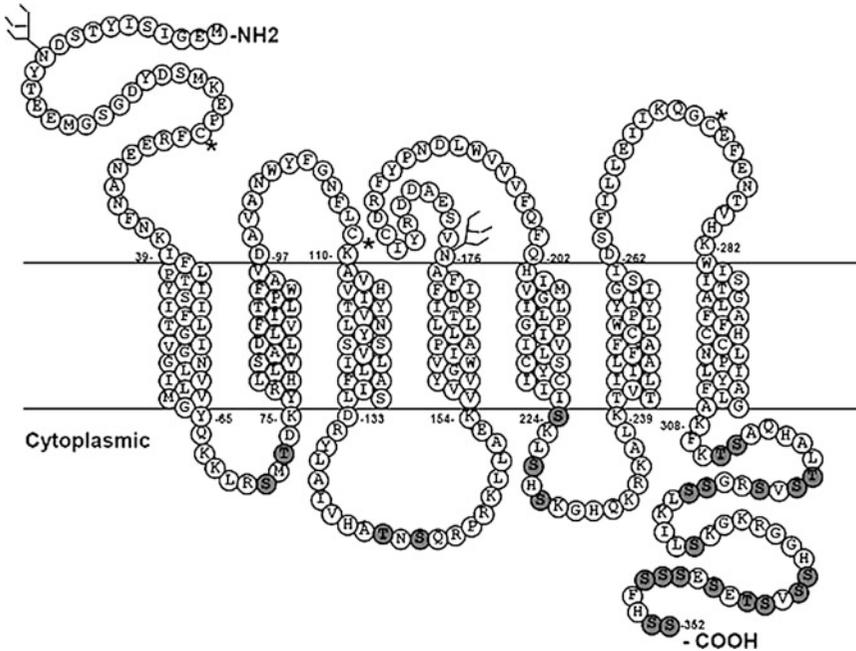
Definition

C-X-C chemokine receptor type 4 (CXCR4) is a G-protein-coupled receptor (GPCR) for the

chemoattractant cytokine (chemokine) CXCL12 (or stromal cell-derived factor-1alpha, SDF-1 α). Along with the CC chemokine receptor 5 (CCR5), CXCR4 is a major coreceptor for the human immunodeficiency virus (HIV) on the surface of CD4⁺ cells. Attachment of the HIV envelope glycoprotein to CXCR4 allows for efficient fusion, entry, and replication of HIV (Su et al. 1999). Amino acid changes in the HIV gp120 glycoprotein, particularly in the V3 loop region, induce HIV to switch coreceptors from CCR5 to CXCR4 which is often associated to a late stage of disease progression or as a mechanism of virus resistance to drugs targeting CCR5. Drugs that block CXCR4 are potent inhibitors of CXCR4-using HIV strains in cell culture and in pilot drug efficacy studies. However, development of such agents has been halted due to adverse drug effects. The CXCR4 antagonist plerixafor (AMD3100, Mozobil[®]) has been approved for clinical use as a hematopoietic stem cell mobilizer as it potently blocks the CXCL12-induced homing of stem cell to the bone marrow. New generation inhibitors and new gene therapy approaches are being developed to block CXCR4 function as a HIV coreceptor.

History of CXCR4 as HIV Coreceptor

CXCR4 (previously known as leukocyte-derived seven-transmembrane domain receptor, LESTR) was first identified as an orphan receptor after isolation of a cDNA clone encoding a 352 amino acid protein (Fig. 1) with roughly 93% homology with that of bovine neuropeptide Y (NPY) receptor and similar to the receptor for interleukin-8 (IL-8) known in the early 1980s to activate and attract leukocytes toward the interleukin gradient (chemotaxis) (Vaddi et al. 1997). However, LESTR expressed in cells after mRNA transfection did not bind or react to other identified chemokines, including NPY, IL-8, NAP-2, GRO alpha, PF4, IP10, MCP-1, MCP-3, MIP-1 alpha, HC14, I309, RANTES, C3a, or LTB4. The close identity of LESTR to the IL-8 receptor was indicative of its function as a chemokine receptor. LESTR, however, was an orphan receptor.



CXCR4, Coreceptors, Fig. 1 Amino acid sequence of CXCR4. Membrane orientation of the seven-transmembrane segments of the receptor and the N- and C-terminal regions in the extracellular and intracellular

regions, respectively. (*) marks the cysteine residues involved in disulfide bonding. Shaded residues represent potential phosphorylation sites. N-glycosylation sites are also indicated

In 1996, the group of Edward Berger discovered fusin, a G-protein-coupled receptor with seven-transmembrane segments that allowed efficient HIV entry when recombinant fusin was transfected into CD4-expressing nonhuman cell types. Antibodies directed to fusin blocked HIV-1-induced cell fusion and infection with normal CD4-positive human target cells and fusin messenger RNA levels correlated with HIV-1 permissiveness in diverse human cell types (Feng et al. 1996).

The CC chemokines MIP-1 alpha and MIP-1 beta and RANTES (renamed CCL3, CCL4, and CCL5, respectively) had been shown to prevent infection with primary, monocyte-tropic viruses but were inactive against T-cell-tropic HIV. These findings were a clear indication that chemokines and their receptors played a major role in the initial steps of HIV infection and set the stage for the identification of stromal cell-derived factor-1alpha (SDF-1 α) or CXCR4 ligand 12 (CXCL12, as it is known now), as the ligand for fusin/LESTR/CXCR4. In 1996, two independent

groups showed that CXCL12 activated nonhuman cells transfected with CXCR4 cDNA as well as blood leukocytes and lymphocytes. In cell lines expressing both CXCR4 and CD4 and in blood lymphocytes, CXCL12 was shown to be a powerful inhibitor of infection by lymphocyte-tropic HIV-1 strains.

Function and Regulation of CXCR4

CXCR4 is one of 19 known human chemokine receptors, belongs to the superfamily of seven-transmembrane G-protein-coupled receptors, and is activated exclusively by the isoforms of chemokine CXCL12. Chemokine binding to CXCR4 induces a change in conformation of the receptor that is transmitted to the cytoplasm domains of the protein, enabling it to couple with an intracellular heterotrimeric G protein that acts as an intracellular signal (Alkhatib 2009).

CXCR4 is functionally expressed on the cell surface of human blood monocytes, neutrophils,

lymphocytes, and phytohemagglutinin (PHA)-activated T-cell lymphoblasts. CXCR4 is also expressed in various cancer cells and plays a role in cell proliferation and migration of these cells, being associated with more than 23 types of cancer, where it promotes metastasis, angiogenesis, and tumor growth or survival. Deletion of CXCR4 or CXCL12 in mice confers embryonic lethality and leads to defects in vascular and central nervous system development, hematopoiesis, and cardiogenesis, indicating a monogamous relation between CXCL12 and CXCR4. The CXCR4-CXCL12 axis is functional in evolutionarily distant organisms such as zebrafish and mice, in which CXCR4 expression is a prerequisite for germ cell migration to CXCL12-expressing gonads during development (Este and Lederman 2009).

CXCL12 induces internalization of CXCR4 molecules leading to downregulation of receptor sites at the cell surface, desensitizing cells from further chemokine stimulus and contributing to the chemokine-mediated inhibition of HIV-1 entry by making the cell invisible to CXCR4-using virus. Downregulated receptors are endocytosed and either recycled back to the plasma membrane or sorted into the degradative pathway. For CXCR4, this process is controlled by a protein called E3 ubiquitin ligase AIP4 that is required for degradation of CXCR4 and a cytokine-independent survival kinase (CISK) that blocks AIP4 activity, inhibits endosomal sorting, and favors recycling of the receptor (Alkhatib 2009).

The Structure of CXCR4

The limited crystallography data on chemokine receptors was mainly due to the fact that such proteins are highly hydrophobic and cannot be readily purified. However, in 2010, the crystal structures of human CXCR4 were resolved in complex with a small-molecule antagonist (IT1t, Fig. 4) at 2.5 Å resolution and with a cyclic peptide inhibitor at 2.9 Å resolution. The final model included 293 residues (27–319) of the 352 residues of CXCR4 (Wu et al. 2010). The main fold of CXCR4 consists of the canonical

bundle of seven-transmembrane helices (Fig. 2), which shows about the same level of structural divergence from seven-transmembrane helical bundles of previously solved GPCR structures as the rhodopsin receptor, the β_2 -adrenergic receptor, and the adenosine receptor A_{2A} .

The extracellular interface of CXCR4 consists of 34 N-terminal residues, extracellular loop 1 (ECL1, residues 100–104) linking the transmembranes helices II and III, ECL2 (residues 174–192) linking helices IV and V, and ECL3 (residues 267–273) linking helices VI and VII (Fig. 2). The intracellular side of CXCR4 contains the intracellular loop 1 (ICL1, residues 65–71) linking helices I and II, ICL2 (residues 140–144) linking helices III and IV, ICL3 (residues 225–230) linking helices V and VI, and the C terminus. There are two disulfide links in CXCR4 formed between cysteine (Cys) residues 28 and 274 and 109 and 186, both in the extracellular part of the receptor. The disulfide link between Cys109 and Cys186 is critical for ligand binding and functions by constraining ECL2 and the N-terminal segment (residues 27–34), which shapes the entrance to the ligand-binding pocket (Wu et al. 2010).

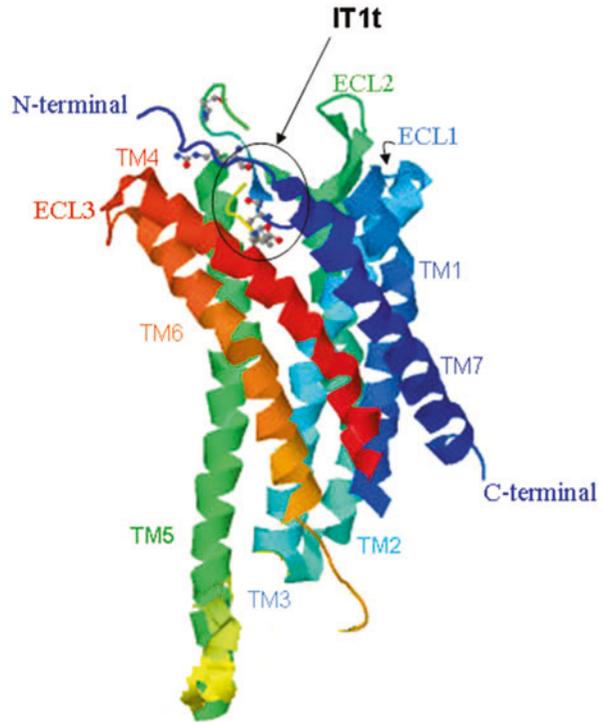
CXCR4 has been shown to form homo- and heterodimers, constitutively and upon binding to CXCL12. Receptor dimerization is important for *in vivo* pharmacological effects. For example, warts, hypogammaglobulinemia, infections, and myelokathexis syndrome (WHIM syndrome) has been linked to mutations in the C terminus of CXCR4 and results in truncated variants that exhibit enhanced signaling and fail to desensitize and internalize upon CXCL12 stimulation.

CXCR4 Interaction with HIV-1

HIV binds to the cell surface of CD4-positive cells through the virus envelope complex formed by two glycoproteins: gp120 and gp41. HIV-1 gp120 mediates the initial contact with the cell surface, binds the CD4 receptor, and interacts with the coreceptors CCR5 and CXCR4. The viral surface protein gp120 is characterized by a high degree of genetic variability among HIV-1

CXCR4, Coreceptors,

Fig. 2 Crystal structure of CXCR4 bound to the small molecular weight inhibitor IT1t. The helices that form each of the seven-transmembrane (TM) segments are depicted, linked by three extracellular (ECL) regions, two intracellular regions (not shown), and the extracellular N-terminal and intracellular C-terminal regions. Note that only one subunit is shown in each case – the receptor normally functions as a dimer of two subunits. The *circle* highlights the site of interaction of IT1t



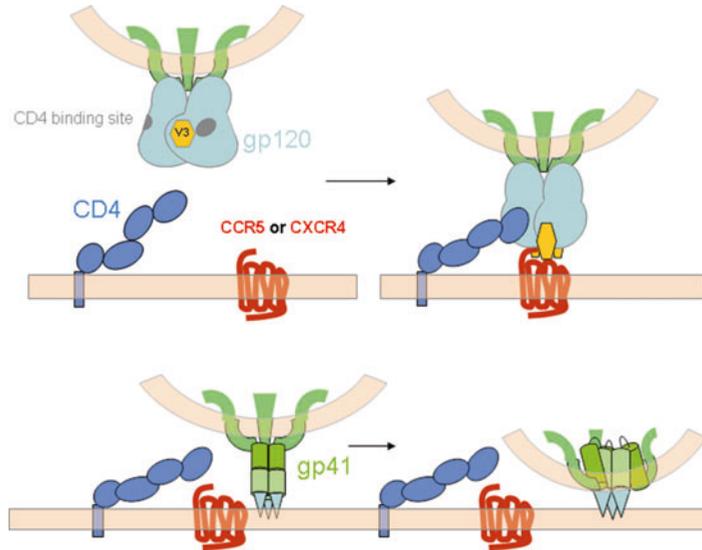
isolates. Evaluation of the amino acid sequence of different HIV-1 strains led to the identification of five variable (V1–V5) and 4 constant (C1–C4) regions in gp120. The region of gp120 that binds the coreceptor has been revealed by the crystal structure of gp120. The residues involved are located within the conserved stem of the V1/V2 structure, near the base of the V3 loop, and in other regions folded into proximity. The V3 sequence usually contains 34–36 amino acids arranged in a disulfide loop involving cysteine residues at position 296 and 331. This domain plays an important role in governing several biological properties of the virus including cell tropism, cytopathicity, fusogenicity, and coreceptor use. Although the actual sequence between the two cysteines seems not to be of importance for processing and binding to CD4, it is important for the CXCR4-using property of some virus strains.

The sulfotyrosine-containing N-terminal region of CXCR4 serves as the initial site of interaction with the V3 loop, analogous to CXCL12 recognition. This interaction results in a conformational change of the V3 loop, the

subsequent interactions of the rearranged V3 loop with the CXCR4 extracellular loops, and the ligand-binding pocket of the coreceptor. The structural details of CXCR4 revealed in Wu et al. (2010) and its implications for the binding of CXCL12 and gp120 of HIV-1 to CXCR4 are of great value for understanding the signal transduction via the receptor and for the design of new strategies targeting the viral fusion of HIV-1.

The HIV Entry Process and Coreceptor Use

The simplest model of coreceptor use suggests that CCR5 and CXCR4 are the main coreceptors used by HIV (Fig. 3). The expression of CCR5 or CXCR4 on different CD4+ target cells determines their permissiveness to infection by the corresponding CCR5-using (R5) or CXCR4-using (X4) HIV-1 strain. In addition, there are HIV-1 strains that may use both CCR5 and CXCR4, referred to as dual tropic (D) R5/X4 strains, and there are patients that have mixtures



CXCR4, Coreceptors, Fig. 3 HIV entry into target cells is a sequential process that begins with attachment of the virus to host cell surface, followed by binding of the envelope gp120 (glycoprotein) to the CD4 receptor. This binding promotes conformational changes that expose the gp120 coreceptor-binding site. Then, the CD4-envelope

glycoprotein complex interacts with the appropriate chemokine, mainly CCR5 (R5 virus) or CXCR4 (X4 virus), used as a coreceptor. In response to such coreceptor binding, there are additional conformational changes that allow gp41-mediated fusion of virus and cell membranes

(M) of R5 and X4 HIV-1 isolates. D or M isolates are indistinguishable in HIV coreceptor phenotype assays (Este and Telenti 2007).

After CD4 virus attachment and coreceptor engagement, a series of conformational changes in gp120 allow gp41 to reorient parallel to the viral and cellular membranes and promote the events leading to virus and cell membrane fusion.

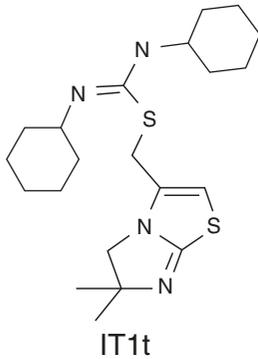
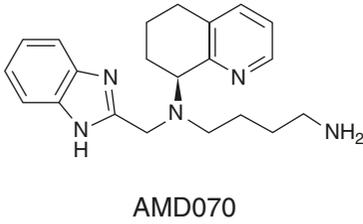
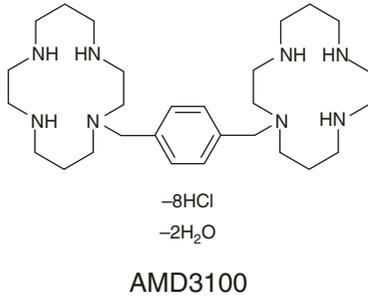
R5 viruses are preferentially transmitted over X4 viruses and are almost exclusively associated with acute infection, irrespective of the route of transmission. The basis of this preferential selection is unclear but multiple barriers to infection by X4 viruses have been proposed.

R5 viruses also predominate during most of the chronic stage of the disease. Eventually, X4 variants emerge in 40–60% of HIV-1-positive individuals. X4 variants have been associated to expanded cell tropism, increased virus replication rate, and a faster disease progression. However, the emergence of X4 virus is not a prerequisite for the development of AIDS. Whether emergence of CXCR4-using strains is a marker for disease progression rather than the cause is not known.

Inhibition of CXCR4 as an Antiviral Strategy

The various steps in virus entry have long been considered a relevant target for anti-HIV intervention, and a large number of agents have been tested and are developed as promising anti-HIV drugs. At least one agent targeting the CCR5 coreceptor (maraviroc, Selzentry[®]) has been approved for the treatment of HIV infection.

Although no CXCR4 antagonists are in active clinical trials for the treatment of HIV-1 infection, several are in development and have demonstrated potent inhibitory effects on X4-tropic strains (Tilton and Doms 2010). The first low molecular weight anti-HIV agents targeting a coreceptor was the bicyclam AMD3100 (Fig. 4), a potent antagonist of CXCR4. Since then, a number of agents have been tested, and some have advanced to clinical trials, but most of them have failed. The latest AMD11070 was evaluated in a pilot monotherapy study (eight and two patients receiving 200 and 100 mg b.i.d., respectively) in patients with X4-tropic or dual/mixed-tropic



CXCR4, Coreceptors, Fig. 4 Chemical structure of CXCR4 antagonists

HIV. No significant changes in HIV-1 viral load or CD4 cell count were noted. Four of nine patients evaluated achieved a reduction in X4 virus population. The median change in X4 virus population at the end of treatment was $-0.22 \log_{10}$ relative units. Three of four patients who responded to therapy showed a tropism shift from dual/mixed-tropic viruses to exclusively R5 virus by day 10. These results demonstrate the activity of AMD11070. However, development of AMD11070 was suspended because of liver histology changes and animal hepatotoxicity (Este 2010).

Despite the apparent lack of success of CXCR4 antagonists as anti-HIV agents, the clinical

evaluation of AMD3100 helped to recognize these agents as potent hematopoietic stem cell mobilizers. AMD3100 (plerixafor, Mozobil[®]) is now indicated in combination with granulocyte colony-stimulating factor (G-CSF) to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma.

Several polypeptide mimics of CXCL12 have been evaluated. These compounds, including T-22 and its analogues T-134, T-140, and ALX40-4C, act by binding specifically to the CXCR4 receptor and preventing gp120 binding. ALX40-4C was tested in humans prior to the identification of CXCR4 as a coreceptor for HIV and despite being well tolerated did not have a significant effect on reduction of HIV viral loads. However, the majority of patients in this study were later found to have R5 strains of HIV.

Other Approaches to Inhibiting CXCR4 Use

Alternative methods have been evaluated to block CXCR4. Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations. The *CCR5* gene was efficiently disrupted through ZFNs in human CD4⁺ T cells or hematopoietic stem cells and found to confer protection in vitro and in humanized mice to infection by HIV-1 isolates that require CCR5 (but not CXCR4) providing an important proof of concept. ZFNs targeting *CXCR4* have also been employed rendering human CD4⁺ T cells permanently resistant to X4 HIV-1 strains. It has been demonstrated that *CXCR4* can be safely and efficiently disrupted in CD4⁺ T cells obtained from CCR5D32 homozygotes resulting in cells resistant to all strains of HIV-1 tested. This suggests that combined treatment of mature CD4⁺ T cells with X4-ZFNs and R5-ZFNs can provide permanent protection against HIV-1 infection (Wilens et al. 2011).

Resistance to CXCR4 Inhibitors

The driving force behind the numerous HIV variants is the combination of an error-prone reverse transcriptase, the viral enzyme transcribing the viral RNA genome into DNA on the one hand, and the human immune system on the other hand. This puts a constant selection pressure on the HIV population leading to the emergence of escape mutants.

The development of resistance to agents targeting CXCR4 has mostly been studied in cell culture experiments. An AMD3100-resistant strain derived from HIV-1 NL4-3 was obtained after long-term passaging in lymphoid cells in the presence of progressively increasing concentrations of the compound. The AMD3100-resistant strain proved 300-fold resistant to AMD3100 and cross-resistant to other bicyclam analogues with similar or lower anti-HIV activity indicating that all bicyclam analogues share a common mode of action. This AMD3100-resistant strain is cross-resistant to CXCR4 agents such as CXCL12 or peptidic CXCR4 antagonists such as Allelix-40-4C. However, T134, an analogue of the CXCR4 antagonist T22, has been shown active against the AMD3100-resistant strain.

If CXCR4 agents can effectively block CXCR4 for use as HIV coreceptor, then HIV resistance may emerge only by two possible alternatives: HIV-1 can utilize the drug-bound conformation of CXCR4 by the antagonist or it may change coreceptor use. The selection of the AMD3100-resistant strain was done in the lymphoid cell MT-4. This cell line can be easily infected by X4, laboratory adapted strains of HIV, and express CXCR4; but, MT-4 cells cannot be effectively infected with R5 strains such as HIV-1 BaL and do not express CCR5. Therefore, HIV-1 in the absence of coreceptors other than CXCR4 and under selective pressure by a CXCR4 antagonist will develop into a strain that continues to use CXCR4 differently and retains the X4 phenotype. Conversely, selection of resistant variants using clinical isolates of HIV-1 leads to coreceptor switch from CXCR4 to CCR5. Within a patient, the circulating virus population is composed of various mixtures of closely related

but genetically distinct viruses that may comprise a pure population sharing the same coreceptor use or a mixed population. Therefore, selective antiviral activity toward the X4 component leads to the emergence of minority R5 strains and the switch in coreceptor phenotype.

CXCR4 Use as a Mechanism of CCR5 Drug Resistance

Similar to CXCR4 agents, there are two possible pathways of viral escape from CCR5 antagonists: the selection of viruses that can use CXCR4 as their entry coreceptor and the selection of viruses that continue to use CCR5 but can recognize the drug bound for of the coreceptor. Maraviroc, the only approved antiretroviral drug targeting a coreceptor, demonstrated potent and selective antiviral activity against all CCR5-tropic HIV-1 viruses tested, including primary isolates and virus resistant to other existing drug classes. However, screening for clinical trials with maraviroc demonstrated that roughly 60% of the patients for whom at least two antiretroviral regimens failed were infected with R5 HIV-1 only. That is, the majority of patients failing treatment with maraviroc had a change in tropism to dual tropic/mix (R5/X4) or X4 at time of failure, drawing attention to the propensity of virus isolates from patients to switch coreceptor preference. Therefore, HIV-1 may escape from CCR5 drug treatment by utilizing CXCR4.

Methods for Identifying CXCR4-Using HIV Variants

Numerous studies have associated different regions in the HIV-1 envelope as determinants of CCR5 and CXCR4 tropism. For example, the X4 phenotype seems to be determined by the presence of a positively charged V3 region of the envelope protein gp120, particularly amino acids in positions 11 and/or 25 in the 35 amino acid coding sequence. On the other hand, regions outside the V3 loop have been shown to be involved with R5, X4, or R5/X4 phenotypes. Amino acid substitutions in other env hypervariable regions

(V1, V2, V4, and V5), the entire C1-V4 region, and even the gp41 transmembrane unit may also play a role in HIV-1 tropism. As a consequence, HIV-1 coreceptor usage and its implication on disease progression, antiretroviral therapy, and even on vaccine strategies have led to the development of multiple tools aimed at determining the ability of HIV-1 to use coreceptors to infect target cells (Rose et al. 2009). Importantly, evaluation of virus coreceptor use is required prior to the initiation of antiretroviral therapy containing a CCR5 inhibitor.

Viral tropism can be assessed with either genotypic or phenotypic approaches. Coreceptor genotype testing is based on amplification and population sequence analysis of patient-derived V3 region. Two different sequencing approaches, population-based and pyrosequencing, have been used, the latter with improved sensitivity to detect minority X4 variants. Then, bioinformatic interpretation techniques are used to predict coreceptor use from a consensus sequence. Phenotypic tests include the coculture of patient-derived cells with lymphoid cells expressing CXCR4 (MT-2 cells) or infection of reporter cell lines with recombinant or pseudoviruses containing *env* genes derived from the virus population in a given patient (Vandekerckhove et al. 2011). A widely used phenotypic assay, Trofile™, is a single-cycle, recombinant virus assay. The entire patient-derived *env* gene is amplified by polymerase chain reaction (PCR) and inserted into an expression vector containing a luciferase reporter gene that is transfected into cells to produce a pseudovirus population subsequently used to infect CD4⁺ cells expressing either the X4 or R5 receptor on their surface. Trofile™ has been utilized in the characterization and clinical development of several HIV-1 CCR5 inhibitors, and it is currently used to identify patients with X4 viruses as exclusion criteria for treatment with CCR5-targeting strategies.

Conclusion

CXCR4 is one of the two major coreceptors used by HIV to enter cells. Viruses that use CCR5 are commonly transmitted and persist throughout the

course of infection, but CXCR4-using viruses may emerge at later stages and dominate the virus population. Importantly, CXCR4 use is strongly associated to treatment failure of CCR5 drugs, with CD4 T-cell decline and the onset of AIDS. There are no drugs targeting CXCR4 currently approved for the treatment of HIV infection, but novel strategies are envisioned to simultaneously block CCR5 and CXCR4 to prevent HIV infection.

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Cyclophilin A and HIV-1 Replication

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Definition

Cyclophilin A (CypA) is the founding member of the large cyclophilin family of proteins. It is encoded by the 5 exon *PPIA* gene on chromosome 7p13. CypA is a globular protein with a hydrophobic pocket that is expressed constitutively at high level. There are roughly 20 genes that encode proteins with a CypA domain, as well as many nonfunctional CypA pseudogenes. The owl monkey TRIM5-CypA fusion gene is a textbook example of how new exons are generated by LINE-1-catalyzed retrotransposition of abundant cDNAs, in this case the CypA cDNA. Orthologues of CypA are found in most species, from eubacteria through mammals. The only species that do not have orthologues are extremophile archaeobacteria. The hydrophobic pocket of CypA binds to proline-containing peptides, as best exemplified by proline 90 on the external face of the HIV-1 capsid. CypA is thought to carry out a global function in protein folding by catalyzing the *cis-trans* interconversion of peptide bonds that contain proline.

Introduction: Retrovirus *gag*-Encoded Proteins as Multitaskers

The *gag* gene of HIV-1 and other retroviruses encodes a polyprotein that is sufficient to direct

the assembly of virions (► [Life Cycle Overview](#); ► [Virus Assembly](#); Franke et al. 1994). The Gag polyprotein is targeted to the plasma membrane where it buds out of the cell. Concurrent with virion budding, the viral protease is activated. This cleaves the Gag polyprotein into at least six mature, *gag*-encoded, virion-associated proteins, among which is the matrix protein (MA), which associates with the inner face of the virion membrane, and the highly basic, zinc-finger-containing nucleocapsid protein (NC), which coats the viral genomic RNA. The capsid protein (CA) undergoes a large conformational transformation upon proteolytic cleavage and collapses to form the protein lattice that encases the viral genome at the core of the virion (► [HIV-1 Virion Structure](#)).

Following membrane fusion with a susceptible target cell, the CA core is released into the cytoplasm where it regulates ► [reverse transcription](#) (Braaten et al. 1996a). What happens to the CA core lattice at this stage of the virus replication cycle is frankly unclear. It may crumble into monomers of CA or small CA multimers, or it might disassemble in a more complex, directed fashion. Some believe that the CA lattice core persists in the cytoplasm and docks to the nuclear pore – perhaps via interaction with discrete nucleoporins like Nup358 (► [Nuclear Import: HIV-1 Goes NUPs](#)), while others have provided evidence that CA multimers traffic to the nucleus with the preintegration complex (► [Integration](#)).

Discovery of CA Interaction with Cyclophilin A

The wide-ranging functions of the *gag*-encoded proteins suggest that multiple host factors (► [Identification and Validation of Cofactors](#)) assist the structural transformations and functional changes described above. Host factors are undoubtedly also required to propel CA on its wide-ranging journey through the various subcellular compartments. Additionally, host cells elaborate restriction factors that hinder retrovirus progression through the replication cycle.

As a step toward the identification of host factors of functional relevance for *gag*-encoded

proteins, host proteins were sought that interact with the Gag polyprotein or with each of the Gag proteolytic products (Luban et al. 1993). cDNAs encoding HIV-1 Gag-interacting proteins were screened in the yeast two-hybrid system. Cyclophilin A came out of these initial screens with the HIV-1 Gag polyprotein, as well as in subsequent screens with CA (Luban et al. 1993). It is now clear that CypA binds to HIV-1 CA and the CA of a large subset of lentiviruses, including some HIV-2 isolates and FIV, but not the CA of all retroviruses (Braaten et al. 1996a; Franke et al. 1994; Gamble et al. 1996; Goldstone et al. 2010). CypA set the stage for the more extensive functional investigation of *gag*-interacting proteins that followed.

CypA and Protein Folding

The ubiquitous cellular protein cyclophilin A (CypA) was first discovered as a high-affinity cellular binding protein for the immunosuppressive drug cyclosporine (Handschumacher et al. 1984). CypA was then shown to possess an enzymatic activity. It catalyzes the rate of interconversion between *cis* and *trans* isoforms of peptide bonds that possess proline (Fischer et al. 1989). CypA is a small globular protein that has a hydrophobic pocket that binds to proline-containing peptides. It is believed to have subtle yet global effects on protein folding within cells. As a complex with the immunosuppressive drug cyclosporine, it is a potent inhibitor of the CA-dependent phosphatase calcineurin in T cells (Colgan et al. 2005; Liu et al. 1991). Among the specific cellular functions of CypA, it regulates the nuclear export of Zpr1 (zinc finger protein 1) in yeast (Ansari et al. 2002), the kinase activity of Itk (inducible T-cell kinase) in CD4⁺ T cells (Colgan et al. 2004), and the nuclear translocation of apoptosis-inducing factor in neurons after cerebral hypoxia-ischemia (Zhu et al. 2007).

It thus is reasonable to propose that HIV-1 Gag is a catalytic folding substrate for CypA and that the conformational changes necessary for the function of *gag*-encoded proteins would be aided

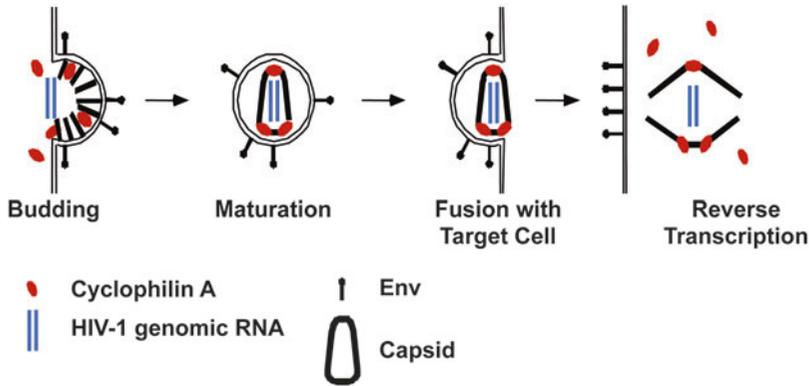
by CypA. HIV-1 CA interaction with CypA involves the insertion of a critical proline residue, P90, into the catalytic pocket of CypA (Franke et al. 1994; Gamble et al. 1996). Binding of CypA indeed catalyzes a conformational change in CA that is detectable by NMR (Bosco et al. 2002). To this date, though, the functional significance of this modest conformational change in CA structure remains undetermined.

CypA and HIV-1 Assembly

CypA interacts with the HIV-1 Gag polyprotein and with the mature HIV-1 CA via the same local contact points, with similar micromolar affinities (Colgan et al. 1996; Gamble et al. 1996; Luban et al. 1993). In some assays using recombinant protein *in vitro* or with viral protein in cells, interaction with the Gag polyprotein is more readily detectable than interaction with CA, perhaps because of greater avidity provided by Gag polyprotein multimerization.

CypA is incorporated into HIV-1 virions in a specific manner (Fig. 1), via interaction with a critical proline and adjacent amino acid residues within the Gag polyprotein (proline residue 222 with respect to the Gag polyprotein – proline 90 with respect to capsid – consensus clade B strain) (Braaten et al. 1996a; Franke et al. 1994). The stoichiometry is on the order of one CypA molecule to five Gag molecules. The CypA protein is protected within the virion membrane envelope from digestion by exogenous protease (Ott et al. 1995).

CypA interaction with the Gag polyprotein can be disrupted by several different means. It has been disrupted by knockout and by knockdown of CypA within virion producer cells (Braaten and Luban 2001; Sokolskaja et al. 2004). Mutation of critical amino acid residues in the Gag polyprotein blocks CypA interaction with Gag and incorporation of CypA into virions (Braaten et al. 1996a; Franke et al. 1994). The Gag-CypA interaction can also be disrupted by cyclosporine or non-immunosuppressive analogues of this drug (Luban et al. 1993; Thali et al. 1994). Each of these experimental tools has been used to examine



Cyclophilin A and HIV-1 Replication, Fig. 1 Cyclophilin A (red ovals) is stoichiometrically incorporated into HIV-1 virions during virion budding, via interaction with Gag polyprotein. Virion-associated cyclophilin A is likely associated with the CA in the core of the virion, where it is protected by the virion membrane from protease digestion.

The function of cyclophilin A during virion assembly is unknown. When a virion fuses with a susceptible target cell, the virion CA protein interacts with the target cell cyclophilin A protein. The latter interaction has functional consequences; it promotes HIV-1 reverse transcription and nuclear entry

CypA function during ► **virion assembly**. None of them disrupt rates of Gag polyprotein synthesis, kinetics of Gag polyprotein cleavage by viral protease, virion assembly kinetics, particle yield, virion infectivity, virion protein content, or virion morphology by electron microscopy or cryo-EM. To date no convincing data has been obtained demonstrating a role for CypA in HIV-1 virion assembly, or within the virion. Given the specific interaction of CypA with the Gag polyprotein, it is surprising that the interaction has no influence on replication. Perhaps the right assay has not been performed. Or, maybe CypA plays a role in virion assembly in vivo that is not detectable in tissue culture. Finally, it might be that CypA interaction with Gag is selected for because it influences how the immune system detects virion-producing cells (Manel et al. 2010).

The Role of CypA During the Early Steps of HIV-1 Replication

In contrast to the lack of effect on the late steps of the HIV-1 replication cycle, CypA has clear effects on the efficiency of HIV-1 transduction (Fig. 1). Disruption of CypA in the target cell by knockdown, knockout, or cyclosporine reduces

the ability of HIV-1 to reverse transcribe within, and infect, a target cell (Braaten et al. 1996a; Franke et al. 1994; Hatzioannou et al. 2005; Sokolskaja et al. 2004). Similar reduction in infectivity is observed with Gag mutants that do not bind CypA. This effect is usually modest, somewhere between two and fivefold. How CypA promotes reverse transcription is not known, but it seems related to effects on CA.

CypA and CA-Dependent Restriction

In some cases, in particular cell lines, for example, CypA has paradoxical effects on HIV-1 replication. While CypA knockdown was associated with a reduction in HIV-1 replication in primary T cells, an increase in HIV-1 infectivity was observed with CypA knockdown in HeLa cells. As compared with wild-type HIV-1 CA, this increase in infectivity is greatly pronounced with particular CA mutants, including A92E and G94D (Braaten et al. 1996b; Yin et al. 1998). Whatever the explanation for this cell-type-specific, CypA-dependent inhibition of HIV-1, heterokaryon experiments have demonstrated that it acts in a dominant fashion (Song and Aiken 2007). The identity of this dominant-acting, CypA-dependent

inhibitor is unknown. Some have reported elevated CypA protein levels in the cell lines in which CypA KD increases HIV-1 infectivity. This suggests that CypA interaction with HIV-1 CA is intrinsically inhibitory of CA function, at least under conditions of heightened CypA expression. Others failed to detect this increase in CypA level.

CypA and TRIM5

Soon after the discovery of HIV-1, it was clear that this virus is unable to infect certain monkey species. Over subsequent years it was shown that HIV-1 CA conferred sensitivity to the block, that the block was dominant, and that the block occurred soon after virus entry into the cell cytoplasm. Ultimately, expression screens identified TRIM5 α in macaques, and the TRIM5-CypA fusion gene in owl monkeys, as responsible for the block (Sayah et al. 2004; Stremlau et al. 2004). In the latter case, the CypA was fused directly to TRIM5, thus targeting the restriction factor to the HIV-1 CA. In the former case, CypA, as a protein acting in trans, is required for TRIM5 α recognition of the HIV-1 CA (Berthoux et al. 2005).

Conclusion

Studies on CypA led to the discovery of TRIM5 as a capsid-specific restriction factor (Sayah et al. 2004; Towers et al. 2003). Further assessment of the effects of CypA on CA recognition may lead to the identification of additional host factors that regulate HIV-1 replication. With improved understanding of the HIV-1 CA lattice structure may come further information about how CypA regulates CA conformation and accessibility of antiviral factors such as TRIM5. It should also be pointed out that cyclophilin A-binding, non-immunosuppressive analogues of cyclosporine have been shown to inhibit HIV-1 in infected people (Flisiak et al. 2008); such drugs (► [Cellular Factors as Drug Targets](#)) may one day have a role in the clinic.

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Cystoisosporiasis

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Definition

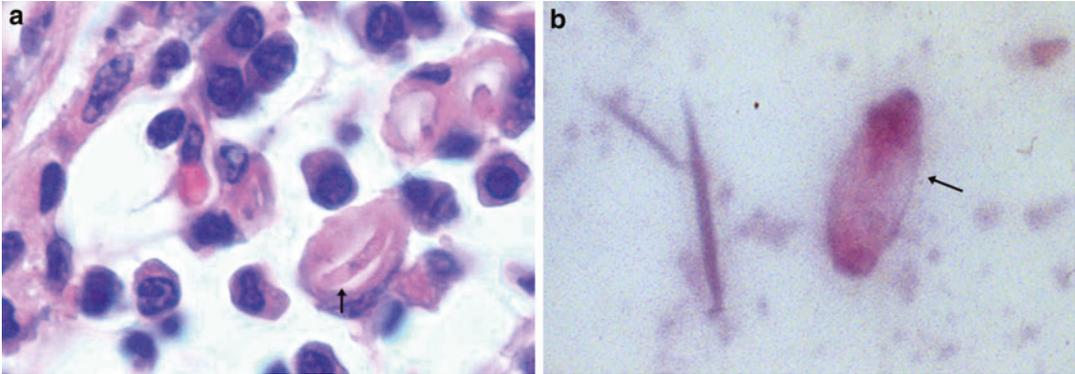
Cystoisosporiasis, also known as isosporidiasis, is caused by *Cystoisospora belli* and is a gastrointestinal infection that presents as diarrhea without associated fever.

Introduction

Cystoisospora belli is a member of the Phylum Apicomplexa in the Subclass Coccidiasina and Family Sarcocystidae (Lindsay and weiss 2015). While previously referred to as *Isospora belli*, and occasionally as *I. hominis* (some of these reported cases may also have been due to misidentified *Sarcocystis* species), both the life cycle of this human pathogen and molecular phylogeny indicate that this organism should be a member of genus *Cystoisospora*, which contains over 200 species.

Species and Life Cycle

Cystoisospora belli causes diarrhea in humans and is the only member of this genus that commonly causes human infection. There are a few reports from South Africa, in the literature prior to 1955, of a second species, *C. natalensis*, causing infections; however, these have not been subsequently confirmed (Lindsay and Weiss 2015). *C. belli* infections accounted for 2–3% of AIDS-



Cystoisosporiasis, Fig. 1 (a) Tissue section of intestine demonstrating tissue cyst of *Cystoisospora belli* (arrow). (b). Acid fast stain of stool demonstrating *Cystoisospora*

belli oocyst in stool (arrow). Charcot leyden crystals are also demonstrated in this specimen

defining conditions in the 1980s (Pape et al. 1989), but with the widespread use of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis for *Pneumocystis pneumonia* this decreased to 0.1% in the late 1990s. In reports of groups of patients with AIDS and chronic diarrhea, prior to widespread use of combination antiretroviral therapy (cART), this infection accounted for up to 25% of cases; however, with cART the prevalence of this infection as a cause of chronic diarrhea has decreased.

Infection with *C. belli* is more commonly found in tropical, subtropical, and warm temperate regions (Pape et al. 1989); however, indigenous infection has been reported from temperate regions. Transmission is due to fecal oral spread, usually by ingestion of sporulated oocysts, and foodborne outbreaks have occurred. Sexual practices that permit fecal oral spread are also associated with infection. It is possible that transmission may also occur by the ingestion of raw or undercooked tissues from paratenic (i.e., transport) hosts, although this mechanism has not been definitively proven to occur. As with most fecal oral pathogens improved sanitation and water quality decreases the risk of transmission.

The life cycle of *C. belli* generally occurs in a single host, with the formation of oocysts occurring 9–17 days following infection which are passed in feces; however, there is evidence that this organism can be facultatively heteroxenous

(use two hosts) and that it forms tissue cysts following the initial infection (Lindsay et al. 1997). In fact, these tissue cysts are probably the source of relapsing infections seen in immunocompromised hosts. Oocysts are generally non-infectious when passed in the feces and complete sporulation within 1–5 days depending upon the environmental conditions. Shedding of oocysts usually lasts for 30–50 days; however, in immune-suppressed patients shedding may be prolonged lasting over 6 months and in such patients recrudescence of oocyst shedding occurs. This prolonged shedding is presumably due to activation of dormant tissue resident monozytic tissue cysts (Fig. 1a). Cysts can be found in a variety of tissues, including lamina propria, mesenteric lymph nodes, liver, and spleen. These cysts are thick walled and measure $12\text{--}22 \times 8\text{--}10 \mu\text{m}$ in size, each containing a single dormant sporozoite/merozoite about $8\text{--}10 \times 5 \mu\text{m}$. These tissue cysts are suggestive that a paratenic host could be involved in the life cycle of *C. belli*, as this has been shown to occur in the *Cystoisospora* species that infect cats and dogs.

Clinical Manifestations

In immune-competent hosts 1 week following the ingestion of oocysts a self-limiting diarrheal illness results that lasts 2–3 weeks and is associated with

malaise, weight loss, cramps, watery diarrhea, steatorrhea, headache, low-grade fever, abdominal pain, vomiting, dehydration, and weight loss (Lindsay and Weiss 2015). Infection can result in a very severe illness and fatalities have occurred. Blood is not usually present in the feces. Eosinophilia is observed in many patients. Oocysts can be present in the feces or biopsies for several months to years. Recurrences occur and have been reported as long as 10 years after initial infection.

In general, clinical disease is more severe in infants, young children, and in immunocompromised host. In the patient with HIV infection with low CD4 counts, infection results in severe watery, secretory-like diarrhea that often leads to dehydration requiring hospitalization. This can be associated with fever and weight loss. In patients with AIDS infection tends to be chronic and recrudescence is common if the CD4 count is under 200. Hemorrhagic colitis as well as extraintestinal infection has been described in this setting. Coinfection with other pathogens can occur. In addition to patients with AIDS, *C. belli* infection has been reported in patients on systemic corticosteroids being treated for eosinophilic gastroenteritis, in patients with renal transplants, liver transplants, Hodgkin's disease, non-Hodgkin's lymphoproliferative disease, human T-cell leukemia virus type I-associated adult T-cell leukemia, acute lymphoblastic leukemia, and human T-cell-leukemia-virus-1-associated T-cell lymphoma. In general, these immune-suppressed hosts respond to TMP-SMX and other anti-*C. belli* treatments. In addition to the intestine, *C. belli* has been found in gallbladder epithelium, endometrial epithelium, and in bile samples. Clinical signs in patients with parasites in these locations are not specific for coccidiosis; however, biliary track infection with cholangitis was the presenting manifestation of this AIDS defining infection in a case report.

Diagnosis

Diagnosis of infection is best accomplished by examination of stool specimens, usually three

specimens collected on different days, for oocysts. Concentration techniques like formalin-ethyl acetate (rarely formalin-ether) sedimentation or sucrose centrifugal floatation are helpful when few oocysts are present. *C. belli* oocysts stain red with the modified Kinyoun's acid-fast stain (Fig. 1b); however, this staining can be variable and some oocysts do not stain. Autofluorescence is seen with *C. belli* oocysts and can be a useful tool for finding this organism in stool samples using UV fluorescent microscopes. There are no commercial monoclonal antibodies available for the detection of this pathogen in stool. There are no FDA-approved molecular techniques for the diagnosis of *C. belli*; however, PCR assays using primers based on ITS and ssRNA have been described and three genotypes of *C. belli* can be identified using PCR and RFLP using MboII digestion; PCR has been able to identify stool negative infections that were confirmed by finding developmental life cycle stages in tissue samples. This organism has not been cultured in vitro and no serological or antigen diagnostic tests are available for this pathogen.

Treatment and Prevention

The drug of choice for the treatment of *C. belli* is trimethoprim-sulfamethoxazole (TMP-SMX) (Pape et al. 1989). A dose of TMP 160 mg-SMX 800 mg two to four times a day for 10–14 days results in rapid (within 2–3 days) clearance of parasites and diarrhea. In patients with HIV-1 infection and CD4+ cell counts less than 200 secondary prophylaxes with TMX 320 mg/SMX 1600 once daily or three times a week prevent relapses. In general, secondary prophylaxis can be stopped if the CD4+ count exceeds 200; however, there are case reports of chronic infection with relapse still occurring despite patients having CD4+ counts above 200 and having received primary therapy with TMP/SMX (Boyles et al. 2012). In patients unable to tolerate sulfonamides due to allergy or intolerance there is no standard treatment. Pyrimethamine at a dose of 50–75 mg/day is an effective alternative treatment

in patients with sulfonamide allergies and secondary prophylaxis can be performed with pyrimethamine 25 mg/d (Weiss et al. 1988). When pyrimethamine is administered it should be given with folic acid (5–10 mg/day) to minimize bone marrow suppression. Ciprofloxacin can be used as an alternative treatment, although it is less effective than either TMP/SMX or pyrimethamine (Verdier et al. 2000). In a randomized study of 22 patients with cystoisosporiasis and HIV infection, 10/10 patients on TMP/SMX had cessation of diarrhea within 2 days and 10/12 on ciprofloxacin (500 mg BID) had a cessation of diarrhea within 4.5 days. All three patients (two with diarrhea and one without) who had persistent *C. belli* oocysts in their stools responded to treatment with TMP/SMX with clearance of the parasite. In patients who responded to ciprofloxacin continued prophylaxis with ciprofloxacin prevented recurrence of disease. Nitazoxanide has also been used to treat *C. belli* infections (Fox and Saravolatz 2005). Two patients on 500 mg nitazoxanide twice daily for 3 days were oocyst negative after treatment; a patient treated with 500 mg nitazoxanide twice daily for 7 days became oocyst negative by day 14 after treatment. However, in another case report treatment failure occurred in a patient with biliary cystoisosporiasis and malabsorption on 2 g nitazoxanide twice daily, perhaps due to poor absorption of this drug as indicated by low serum drug levels. A study of eight AIDS patients with *C. belli* enteritis treated with diclazuril 200 mg/day for 7 days demonstrated resolution of diarrhea in half of the treated patients. A patient with allergy to both TMP/SMX and pyrimethamine was successfully treated with diclazuril 300 mg twice a day; however, when the drug was reduced to once a day the infection recurred. Treatment with metronidazole, tinidazole, quinacrine, or furazolidone is not effective for this pathogen.

Conclusion

Cystoisospora belli causes diarrhea in humans and is found in immune-competent and immune-

deficient patients. This diarrheal syndrome, however, is often chronic in patients with AIDS and more frequently seen in these patients. Infection responds rapidly to therapy with trimethoprim-sulfamethoxazole unlike other causes of chronic diarrhea patients with AIDS. While cystoisosporiasis can be acquired in any country, it is more common in tropical environments.

Cross-References

- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [Immunopathogenesis of HIV Coinfections](#)
- ▶ [Mucosal Immunity to HIV-1](#)

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Cytomegalovirus Infection and HIV

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Definition

Cytomegalovirus (CMV) is a herpesvirus that infects most individuals worldwide. It establishes latency and persists for the life of the individual usually only producing overt disease in individuals with immature immune systems (e.g., fetuses following stem cell transplant) or the immunocompromised (solid organ transplant, AIDS). Detection of active CMV infection in a patient is thus an indicator of functional suppression of cell-mediated immunity (CMI) as well as a harbinger of serious end-organ disease.

Introduction

CMV is first acquired in developing countries early in childhood where seroprevalence in adults may be over 90%. In developed countries, primary infection is delayed into adulthood in many people (adult seroprevalence rates are 50–60% although seroprevalence is higher in those at risk of HIV infection including MSMs and intravenous drug users.) Infection is spread within families through contact with saliva and urine of young children, particularly as viral shedding tends to persist longer in children than in immunocompetent adults. It is also spread in communities through sexual contact (Staras et al. 2008). Additional routes of transmission include in utero acquisition from an infected mother or perinatally acquired infection from maternal secretions or via breast milk, from a donor organ to a recipient during solid organ transplantation, by semen during in vitro fertilization, or by blood transfusion (now rare).

In HIV-positive adults, CMV seroprevalence is typically high. Before the use of highly active

antiretroviral therapy (HAART), CMV end-organ disease was a major cause of morbidity and mortality in HIV-infected patients. With the advent of potent antiretroviral therapy, cases of CMV end-organ disease including retinitis, encephalitis, colitis, and pneumonitis are seen less frequently. Diagnostic difficulties lie in distinguishing whether CMV despite being detectable by PCR is the sole causative agent of disease as it may coexist with other pathogens, e.g., *P. jirovecii*. Management of CMV disease includes the use of HAART and antiviral medication against CMV including ganciclovir, foscarnet, and cidofovir. Several new drugs with activity against CMV are in development.

Pathogenesis

CMV is a member of the *Betaherpesvirinae* subfamily. It contains a double-stranded DNA genome that encodes approximately 165 named proteins. Every protein is recognized by CMI and by humoral immunity which act to keep the virus suppressed into latency in most individuals so that they usually remain asymptomatic. However, reactivation from latency produces active CMV infection that can disseminate within the body and also spread to others. People with active infection can cause primary infection in contacts who are CMV seronegative or reinfect people who are CMV seropositive. Thus, natural immunity against CMV provides substantial protection against disease but is less effective at preventing acquisition of new strains of CMV.

If natural immunity is compromised, CMV replication continues in an individual. Over weeks or months, the viral load in the blood (termed viremia or DNAemia because the quantity of virus is measured by polymerase chain reaction (PCR) amplification of viral DNA) increases. A high viral load is a prerequisite for CMV crossing the blood-organ barrier to cause end-organ disease. In allograft recipients, about 1% of patients with CMV end-organ disease have retinitis, whereas the corresponding figure for AIDS patients is 85%. The reason for this

remarkable difference in localization of CMV to one body site in these two groups of immunocompromised hosts is not known. Other organs affected by viremic spread of CMV include the gastrointestinal tract (about 10%), central nervous system, and lungs. The processes that lead to viremia and an increasing viral load are typically found once HIV has damaged the immune system so that the CD4 count declines below 100/ μ L. Thus, natural immunity, as measured by the CD4 count, has to decline approximately 90% (from 1000 to 100) before CMV becomes a clinical problem. Suppressing HIV replication so that the CD4 count increases back above 100 restores natural immunity to CMV so that viremia is no longer detectable.

As well as causing overt end-organ disease, CMV has the potential to interact with HIV to hasten progression to AIDS and death (Griffiths 1998). Cohort studies report that adults or children with perinatally acquired HIV have increased clinical endpoints of death and morbidity if coinfecting with CMV (Kovacs et al. 1999; Deayton et al. 2004). One nonrandomized clinical intervention study also reports a survival benefit from systemic treatment of CMV in patients with retinitis (Kempen et al. 2003). The mechanisms for this so-called cofactor relationship between CMV and HIV are not well defined, but studies looking at direct interactions between the two viruses have not consistently shown that CMV increases the viral load of HIV. Like HIV, CMV also affects the immune system, so this might explain some of the phenomena. Over decades, CMV increases the proportion of T cells in normal individuals with markers of differentiation (CD57+, CD28-) and thereby contributes to the laboratory definition of immunosenescence. This phenomenon is also seen in AIDS patients, where the abundance of these T cells was reduced by placebo-controlled administration of valganciclovir (Hunt et al. 2011). Finally, now that CMV positive serostatus has been shown to increase the mortality of patients in large cohorts of normal individuals followed long term (Simanek et al. 2011; Gkrania-Klotsas et al. 2013), the cofactor phenomenon might simply be the recognition of this effect in AIDS patients.

Irrespective of the final explanation, these data should encourage physicians to keep HIV suppressed so that it does not allow CMV to reactivate.

Clinical Presentations

Eye Disease

Retinitis, either unilateral or bilateral (up to half of patients), is the major clinical presentation of CMV infection in AIDS patients, particularly in those with CD4 counts less than 50/ μ L. It is the most common cause of retinitis in HIV infection, ahead of toxoplasmosis, cryptococcal retinitis, and varicella zoster virus (VZV) infection. Prior to the use of HAART, CMV retinitis was seen in 30–40% of all patients with AIDS leading to significant decreases in visual acuity and loss of visual field. Development of successful antiretroviral therapy has led to a dramatic decline in the incidence of CMV retinitis of around 80%, and it is now usually limited to those who are not taking antiretroviral therapy or have failed to respond.

The patient may complain of visual disturbance or “floaters,” flashing lights (photopsia), or visual field defects including scotomata. Depending on the area of the retina affected, the retinitis may be asymptomatic and found incidentally on clinical examination. CMV retinitis is described in terms of its stage and the zone of the retina affected (zone 1 closest to the optic nerve and fovea and zone 3 in the periphery). Retinitis may begin in the peripheral retina (zones 2–3) and often progresses slowly over weeks but ultimately has a major effect on vision if left untreated. This may be because the spreading lesion involves the macula and/or because the scarring of the retina impairs attachment to the underlying choroid layer leading to retinal detachment, particularly in those with large peripheral lesions.

Immune Recovery Uveitis

Recovering immune responses to CMV induced by antiretroviral therapy may lead to immune recovery uveitis within weeks after starting antiretroviral therapy. This may occur in patients with

subclinical retinitis who start antiretroviral therapy as unmasking immune reconstitution inflammatory syndrome (IRIS). Therefore, it is important to perform an ophthalmological examination before starting antiretroviral therapy in all patients who are CMV seropositive and have CD4 counts $<100/\mu\text{L}$.

Immune recovery uveitis is also seen in patients who are treated for CMV retinitis and thereafter start antiretroviral therapy (paradoxical IRIS). In these patients, after an initial recovery of retinitis, vision may worsen again. Paradoxically, this inflammatory reaction may affect the patient's vision more severely than did the initial CMV retinitis with the potential for cataract formation, vitreous hemorrhage, and glaucoma. Perseverance with treatment for both HIV and CMV is indicated, because long-term visual acuity reflects the degree of control of progression of the underlying retinitis (Thorne et al. 2011).

The diagnosis of CMV retinitis and its complications is through dilated ophthalmoscopy performed by an experienced ophthalmologist.

Typically, lesions are irregular and yellow-white close to retinal blood vessels with accompanying hemorrhage or present a "brushfire" appearance with a border of advancing retinitis surrounding the atrophic retina. More rarely, retinitis may take on a granular form in the periphery without coexistent hemorrhage. All of these may progress to retinal necrosis if left untreated.

On histological examination, full-thickness retinal necrosis and edema may be present with accompanying vasculitis and choroiditis. Infected cells may show intranuclear or intracytoplasmic "owl's eye" inclusion bodies.

Diagnosis of CMV retinitis is usually based on clinical findings. However, vitreous/aqueous sampling and PCR for CMV DNA may be performed in those cases where the diagnosis remains unclear or retinitis is unresponsive to therapy.

Gastrointestinal Tract

As with retinitis, the incidence of gastrointestinal manifestations of CMV infection in AIDS patient has decreased with the use of HAART. CMV may present with ulcerations in the esophagus, causing odynophagia, dysphagia, and nausea and should

be considered in the differential for esophagitis in HIV infection which also includes candida and herpes simplex virus (HSV) infection. Ulcers are usually grouped near the lower esophageal sphincter but may be more widespread. More commonly, CMV may have similar effects on the colon leading to diarrhea with or without blood in the feces, weight loss, and abdominal pain. CMV colitis may result in massive hemorrhage or perforation. Rarely, ulceration may occur in the mouth or anus.

In both clinical presentations, endoscopy and biopsy are required for diagnosis. Macroscopically, large shallow ulcers are seen in the upper GI tract; in the lower GI tract, appearances range from more superficial erosions to necrotizing colitis. Microscopically, mucosal inflammation and tissue necrosis may be present with intranuclear and intracytoplasmic inclusions.

CMV DNA PCR of the biopsied tissue may be positive but is not definitively diagnostic of end-organ disease and should be interpreted together with clinical findings and the presence of other potential pathogens or inflammatory processes.

Central Nervous System Disease

CMV infection in the CNS results in a variety of clinical presentations.

CMV encephalitis may present with acute ventriculitis, which can include cranial nerve involvement, or subacute encephalitis, which resembles dementia caused by HIV, and CMV DNA may be detectable on cerebrospinal fluid (CSF) PCR.

CMV may cause myelitis, presenting with leg weakness and hyperreflexia or polyradiculopathy where the patient develops urinary retention and/or difficulty in walking. CMV can also affect peripheral nerves resulting in a mononeuritis multiplex picture, involving the peripheral or cranial nerves, in particular the laryngeal nerves.

Diagnostic tools include imaging which may show periventricular or meningeal enhancement in encephalitis or cord enhancement on spinal MRI in CMV myelitis. CMV DNA may be detectable on CSF PCR in cases of encephalitis, myelitis, and polyradiculopathy. In the setting of

Cytomegalovirus Infection and HIV, Table 1 Dosing of antivirals in the treatment of CMV end-organ disease

Medication	Standard treatment dosing in end-organ disease	Standard maintenance dosing
Valganciclovir (oral tablet)	900 mg twice daily	900 mg once daily
Ganciclovir (intravenous)	5 mg/kg twice daily	5 mg/kg daily
Ganciclovir (intravitreal injection – retinitis only)	Single 2 mg injection or 1–4 doses over 7–10 days with systemic therapy If no systemic therapy available, 2 mg twice weekly without systemic therapy	Only if systemic therapy is not available
Foscarnet (intravenous)	60 mg/kg three times daily or 90 mg/kg twice daily	60–120 mg/kg daily
Foscarnet (intravitreal injection – retinitis only)	Single 2.4 mg injection or 1–4 doses over 7–10 days with systemic therapy If no systemic therapy available, 2.4 mg twice weekly	Only if systemic therapy is not available
Cidofovir (intravenous)	5 mg/kg once weekly with prehydration and probenecid for 2 weeks	5 mg/kg every 2 weeks

polyradiculopathy, the CSF contains an abundance of polymorphonuclear leucocytes, whereas cell counts may remain normal in myelitis.

Lung Disease

In advanced HIV infection, CMV may cause very rarely pneumonitis, presenting with a dry cough and shortness of breath on exertion. Auscultation may be unremarkable but imaging may show diffuse interstitial infiltrates. CMV DNA can be detected by PCR on bronchoalveolar lavage samples, and CMV inclusion bodies are found in lung biopsies or cytology.

Positive CMV PCR is not specific for CMV pneumonitis, and CMV DNA is often detectable in bronchoalveolar lavage samples in cases of pneumonia due to other pathogens including *Pneumocystis jirovecii*. Specific treatment against CMV is not required in most of these cases but should be considered if multiple CMV inclusion bodies are found in lung tissue or cytology.

Treatment

The mainstay of treatment is to keep HIV replication under control with HAART so that natural immunity to CMV can, in turn, keep CMV suppressed into latency.

Drugs with activity against CMV include ganciclovir and its oral prodrug valganciclovir,

foscarnet and cidofovir. Standard doses for these medications are shown in Table 1.

Ganciclovir

Ganciclovir is a guanosine analogue which, once activated to its monophosphate by the viral enzyme encoded within the 97th gene of the unique long region (UL 97), is converted to its triphosphate by cellular enzymes. Ganciclovir triphosphate is a potent inhibitor of the CMV-encoded DNA polymerase (UL 54). It is available for intravenous administration, as a topical gel or for intravitreal use (discussed below). The main toxicity of ganciclovir is bone marrow suppression, in particular causing neutropenia, leading to discontinuation or interruption of therapy in around 30% of patients. This effect may be augmented by the coadministration of other myelosuppressive agents including zidovudine. Neutropenia often responds to granulocyte colony-stimulating factor (G-CSF), and these effects may resolve once ganciclovir is stopped. Regular full-blood-count monitoring is therefore advised. Dose adjustment is necessary for those with renal impairment.

Valganciclovir is an oral prodrug of ganciclovir with relatively stable bioavailability of 55–65% as compared to IV ganciclovir. The proposed dosage in Table 1 therefore leads to similar pharmacokinetic curves as in patients with IV ganciclovir (Martin et al. 2002). Side

effects are the same as those with ganciclovir although phlebitis is avoided by oral administration.

Cidofovir

Cidofovir is a phosphonate (structurally equivalent to a monophosphate) which bypasses the UL97 step to be converted by cellular enzymes to its diphosphate form. Cidofovir's main dose-limiting side effect is nephrotoxicity and its use is contraindicated in preexisting renal impairment or with other nephrotoxic agents. This may manifest as a dose-dependent Fanconi-like syndrome. Renal impairment may be seen after only a single dose. It should be given with adequate prehydration and probenecid to reduce tubular secretion. Side effects of probenecid include nausea, fever, and rash. As with ganciclovir, cidofovir may also cause neutropenia, rarely neuropathy. In animal models, cidofovir is teratogenic. Dosing must be reduced or drug discontinued if renal dysfunction or proteinuria develops depending on its severity. Unlike ganciclovir and foscarnet, cidofovir has minimal CSF penetration.

Foscarnet

Foscarnet is different; it does not require anabolism and occupies the pyrophosphate binding site of UL54 to block DNA replication. It is available as a solution for infusion and may be administered IV or via the intravitreal route.

Its predominant toxicity is renal affecting around a third of patients, and dosage must be adjusted for renal function. It may cause proteinuria and nephrogenic diabetes insipidus. It is known to affect electrolyte balance in particular leading to hypokalemia, hypomagnesemia, and hypocalcemia. These effects may be enhanced by the use of other nephrotoxic drugs, e.g., aminoglycosides, amphotericin B, and pentamidine so that close monitoring is required. These effects may be minimized by good hydration.

Direct skin contact with urine containing foscarnet may lead to skin ulceration – a differential for ulceration around the genital area in AIDS patients.

Specific Treatment of Retinitis

In patients who present with first-episode retinitis, immediate treatment with ganciclovir or valganciclovir is indicated. If the ophthalmologist is concerned about a lesion that is immediately sight threatening, he/she may give intravitreal treatment with ganciclovir or foscarnet as well. Note that cidofovir is contraindicated by the intravitreal route.

First-episode lesions usually respond to treatment, but constant vigilance is required to identify when progression occurs and to alter treatment. In some cases, progression is due to resistance to ganciclovir and/or foscarnet. CMV DNA sequenced from the blood of first-episode cases matches that of CMV sequenced from the vitreal fluid. In case of doubt, a vitreal sample (conveniently taken when intravitreal therapy is needed) can be amplified to detect mutations in UL97 and/or UL54.

Close coordination is required between the ophthalmologist and HIV physician to optimize the clinical outcome of patients with retinitis. Specifically, the former may be focused on local treatment for the eye with retinitis plus complications, while the latter will want to monitor for viremia and give systemic treatment to prevent CMV spreading to the currently unaffected eye. Following initial therapy, if the patient's CD4 count remains under 100, maintenance therapy may be considered until the CD4 count has been above 100 for at least 3 months to allow development of a successful lymphocyte response. This decision must be made taking into account the likelihood of treatment adherence (both ART and CMV therapy), the potential for development of resistance or side effects and the risk to sight in both the affected and the unaffected eye.

Management of Immune Recovery Uveitis

Immune recovery uveitis should be managed in coordination with the ophthalmologist.

Treatment is dependent on the site and severity of disease including the presence of complications such as cystoid macular edema and epiretinal membrane formation.

Corticosteroids are often used for treatment and may be administered topically to the eye, by

periocular or intraocular injection (e.g., into the sub-Tenon space) or systemically although evidence from controlled trials for this is lacking. There is no proven benefit of intensification of anti-CMV therapy in IRU.

In severe cases with features such as epiretinal membrane formation, surgery (e.g., vitrectomy) may be warranted.

Three new drugs active against CMV (maribavir, brincidofovir, and letermovir) are currently in phase 3 clinical trials in transplant patients. If licensed, they may provide additional options for managing patients in the near future.

Treatment of Other CMV Manifestations

Colitis

Patients with CMV colitis should receive at least 3 weeks of therapy and until clinical resolution. Failure to respond after 6 weeks of adequate therapy should lead to consideration of an alternative diagnosis. The intravenous route may be preferred, at least initially, unless the disease is mild and there are no concerns over oral absorption. Ganciclovir is the first-line agent but foscarnet may also be used. The efficacy of cidofovir in GI disease is unknown.

CNS Disease

There is an absence of clinical trial data to support specific medications in the treatment of CNS infection. In practice, both ganciclovir and foscarnet are used either alone or as proposed by many experts in combination to achieve an additive inhibition of CMV replication. Despite this combination treatment, the clinical outcome is often poor.

Resistance Development

CMV resistance is sometimes seen in AIDS patients with previous antiviral exposure. Resistance to ganciclovir can occur through mutation in the UL97 kinase or via mutation in the CMV DNA polymerase which may also confer resistance to cidofovir and foscarnet. Genotypic resistance testing should be performed if resistance is suspected.

Prevention

Patients with HIV should be counseled to avoid CMV where possible by using the same safe sex guidelines that are given for HIV. This applies to the uncommon cases who are CMV seronegative, with the intention of preventing primary CMV infection, and to the majority of seropositives, with the intention of preventing reinfection.

Vaccines against CMV have reached phase 2 clinical trials in women of childbearing age and in candidates for solid organ transplantation or stem cell transplantation (Pass et al. 2009; Griffiths et al. 2011; Kharfan-Dabaja et al. 2012). Even if they become licensed and are recommended for universal immunization, it will take decades to bring CMV under control even in developed countries (Griffiths 2012). However, the same vaccines have also shown promise when used immunotherapeutically to boost the immunity of seropositives, so this mode of administration could be evaluated in HIV-positive patients in the future.

Conclusions

CMV is a ubiquitous virus that can act as an opportunist in patients whose CMI is suppressed by HIV. Knowledge of the complex natural history of this virus and the treatments currently available for both HIV and CMV has helped bring about a massive reduction in CMV retinitis and morbidity for AIDS patients.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Chronic Immune Activation in HIV](#)

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D

DDX3, Cofactors, and RNA Export

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Definition

RNA helicases are enzymes that bind to RNA, hydrolyze ATP in an RNA-binding-dependent manner, and separate two annealed RNA duplex strands. Based on sequencing data, there are approximately 85 deduced RNA helicases in the human genome. They have been postulated to have many different functions inside cells. DDX3 is a DEAD (Asp-Glu-Ala-Asp) box RNA helicase protein. There are two forms of the protein. DDX3X is encoded on the X chromosome at position Xp11.3-p11.23 while DDX3Y is located at Yq11. The X-encoded protein and the Y-encoded protein are 91% identical. The DDX3 helicase has attributed roles in pre-mRNA splicing, RNA export, and translation of mRNAs, among other functions.

Organization of DDX3 Protein

There are five superfamilies of helicases, SF1–5 (Kwong et al. 2005). DDX3 is a member of the

SF2 family of helicases. Helicases are operationally defined by whether they can bind single- or double-stranded nucleic acids, unwind RNA or DNA, or both, in either 5' to 3' or 3' to 5' directions. They generally, albeit not invariably, contain certain conserved signature motifs (Kwong et al. 2005). The two major superfamilies of helicases, SF1 and SF2, share at least seven conserved protein motifs. These include domains that specify for nucleic acid binding, ATP hydrolysis, and core helicase activity. The conserved motifs in DDX3 and the demonstration of its RNA unwinding activity have been previously outlined (Yedavalli et al. 2004).

Pleiotropic Functions Attributed to DDX3

Several different functions have been attributed to DDX3. For example, it has been reported that DDX3 has a cell proliferative function through enhancing the translation of cyclin E1 (Lai et al. 2010) and that DDX3 can influence the progression of some cancers through increasing the expressing of the SNAIL transcription factor (Sun et al. 2011). On the other hand, DDX3 has also been reported to act as a tumor suppressor through its transcriptional upregulation of p21waf1/cip1 (Chao et al. 2006), and in settings of environmental insult, DDX3 was reported to inhibit eIF4e (eukaryotic initiation factor 4E), leading to a repression of translation accompanied

by an increase in stress granule formation (Shih et al. 2012). DDX3's inhibitory effect on eIF4e-mediated translation appears to correlate with its described tumor-suppressing function, but interestingly, these activities apparently do not require intact ATPase or helicase functions (Shih et al. 2008). DDX3 also has been reported to play roles in neuronal RNA granules and RNA transport (Kanai et al. 2004), in spliceosomes and RNA splicing (Zhou et al. 2002), in innate antiviral immunity to virus infections (Schroder 2011), as well as in interactions with HCV (Ariumi et al. 2007; Angus et al. 2010) and HIV (Yedavalli et al. 2004; Chen et al. 2012).

Interactions of DDX3 with HIV

Several viruses encode RNA helicases. Herpes virus UL5 and UL9, alphavirus nsP2, rubella virus p70, SARS coronavirus nsp13, hepatitis E virus ORF1, and flavivirus NS3 are some examples of virus-encoded helicases (Jeang and Yedavalli 2006). HIV-1 does not encode an RNA helicase, but there is growing evidence that it interacts with several RNA helicases for replication (Chen et al. 2012). First, cDNA microarray analyses have found that the expression of RNA helicases DHX9, DDX11, DDX18, DDX21, and DDX24 are changed in human cells by HIV-1 infection (Krishnan and Zeichner 2004). Second, a recent mass spectrometric proteomic study found that HIV-1 Gag complexes with DHX9, DDX18, DDX21, DDX24, HIV-1 Vpr interact with DDX20, and Env gp120 binds DDX6 (Jager et al. 2012). In a separate study, Rev, in the presence of RNA, was reported to bind DDX1, DDX3, DDX5, DHX9, DDX17, DDX24, DHX36, and DDX47 (Naji et al. 2012); and DDX24 and DHX30 were described to be involved in Rev-influenced packaging of HIV-1 RNA (Ma et al. 2008; Zhou et al. 2008a).

The role of DDX3 in HIV-1 biology was first broached by Yedavalli et al. (2004). They reported that DDX3 is a nucleocytoplasmic shuttling protein that binds CRM1 (see ► [CRM1](#)), a nuclear export factor, and also that DDX3 is involved in the egress from the nucleus of Rev/RRE-

dependent unspliced and partially spliced HIV-1 RNAs (see ► [HIV-1 Rev Expression and Functions](#)) (Yedavalli et al. 2004). Another RNA helicase, DDX1, was found to also provide a similar nuclear-to-cytoplasmic transport of HIV-1 RNAs (Fang et al. 2004). However, among all the RNA helicases postulated to be important for HIV-1, in three recent siRNA-based genome-wide screens for HIV-1 dependency factors, DDX3 was the only RNA helicase found in all three studies to be required for HIV-1 propagation in human cells (Brass et al. 2008; Konig et al. 2008; Zhou et al. 2008b). In addition to DDX3's role in HIV-1 RNA transport, there is emerging evidence that DDX3 can also contribute to the translation of viral RNAs (Liu et al. 2011; Lee et al. 2008). More investigation is needed to parse the mechanistic distinctions between DDX3 activity needed for RNA transport versus RNA translation.

Conclusion

Extant data suggest that the DDX3 RNA helicase plays roles in cell proliferation, tumor progression, and virus infection of human cells. Above, the contributions of DDX3 to several pathological processes are outlined, arguing that this helicase could be an important drug target. It is thus encouraging that progress has been made in the development of small molecule compounds that target DDX3 (see ► [Cellular Cofactors of HIV as Drug Targets](#)) (Yedavalli et al. 2008; Maga et al. 2011; Radi et al. 2012). Going forward, these candidates should help investigators to further dissect the mechanism(s) and function(s) of DDX3.

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Delayed Sexual Debut

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Definition

Early sexual debut, often defined as first sexual intercourse at age 13 or younger, is associated with multiple negative health outcomes, including subsequent sexual risk taking, unintentional pregnancy, and sexually transmitted infections (STI). Evidence-based behavioral interventions (EBI)

aimed at delaying first sex can be delivered to youth as well as parents and are part of a comprehensive HIV prevention strategy, especially when implemented by communities where the prevalence of HIV is high and many young teens are sexually active.

Why Delay Early Sexual Debut?

According to data from the 2011 Youth Risk Behavior Survey, 6% of teens in the USA report they first had sexual intercourse before they were 13 years of age; by 9th grade, 38% of males and 28% of females say they have had sex (Centers for Disease Control 2012). Compared to peers who delay initiation until they are older, young adolescent females are less likely to use condoms or other contraception not only the first time they have sex but throughout their teens (Centers for Disease Control 2006). Among males, even a relatively small difference in age of initiation (12–13 years vs. 14 years) may influence subsequent condom use (Moore et al. 2001). A longitudinal study that followed youth from middle school through young adulthood found that early initiating males and females had a greater number of lifetime sex partners; they also reported higher levels of intimate partner violence, teen pregnancy, and lifetime STI (O'Donnell et al. 2001). Moreover, those who engage in early sexual activity are more likely to be involved in other problem behaviors linked to HIV risk, including alcohol use and bingeing, experimenting with marijuana and other drugs, and substance use before sex.

Ethnic and cultural differences in early sexual debut and sexual activity contribute to disparities in STI and HIV transmission throughout the teen and young adult years. Over three times as many black high school students report having sex before the age of 13 as white teens (14% vs. 4%), and about twice as many have had four or more lifetime sex partners (25% compared to 13%). Differences between whites and Latinos are not as striking: 7% of Latinos report intercourse before they were 13 and 15% have had four or more sex partners. However, over the last decade,

the proportions of black teens and white teens that are sexually active have decreased, while this has not been the case among Latinos. Further, Latinos are less likely to use condoms or other contraceptives at first or subsequent sex: 41% of Latina teens reported using no method of contraception during their last sexual experience, compared with 25% of black females and 10% of white females. While many behavioral interventions – and especially those delivered in schools – are designed to address the needs and risks of both genders, a consistent finding across population groups is the higher level of early initiation reported by males. Overall, males are more than three times as likely to report early initiation as females; 21% of black males and 11% of Latinos report having sex before they are 13 (Centers for Disease Control 2012).

Although the need to intervene early to delay sexual debut is evident, many behavioral interventions are geared toward older teens and may not address the developmental stage, circumstances, or risk contexts of younger adolescents. As Kirby and others point out, interventions to delay sexual debut have often delivered too late to be effective, especially in communities where early initiation, teen pregnancies, and STI and HIV infection are prevalent (Kirby 2007). With studies indicating the onset of puberty among both males and females is now happening at younger ages (Herman-Giddens et al. 2012), the need to provide developmentally appropriate interventions to pre- and early adolescents is likely to become even more pressing. In the USA, this is especially the case for minority and economically disadvantaged youth, for whom disproportionately high levels of early sexual initiation are reflected in ongoing disparities in rates of unintended pregnancy, gonorrhea, chlamydia, HIV, and AIDS. In the USA and globally, early sexual initiation and patterns of subsequent sexual risks also have long-term implications for social as well as physical health, by impeding youths' school completion and economic viability. Indeed, efforts to increase age at first sex have been singled out as a contributing factor in declines in HIV prevalence among youth in sub-Saharan countries with generalized HIV epidemics (Kirby et al. 2005). Thus,

decreasing age at sexual debut is a health priority for multiple reasons, not least of which is the potential impact on reducing HIV transmission and other health disparities.

Behavioral Intervention Approaches: School, Parent, and Community Approaches

Most behavioral interventions that aim to delay sexual initiation target youth directly, primarily through school health and sexuality education programs. In addition, parent education, community-based youth development programs and workshops, and media campaigns have shown promise for reducing sexual risk taking during early and mid-adolescence, especially when targeted to populations and communities where the need is greatest. Across these different types of interventions, however, there is strikingly limited information on what strategies are specifically effective in helping youth postpone sexual initiation and stay abstinent. For example, as part of federal efforts to address unintended teen pregnancies and STI, Mathematica (2012) has identified 31 interventions with moderate to high ratings of effectiveness based on a standardized set of evaluation criteria. However, only five have demonstrated impact on delaying sexual debut or promoting abstinence, even though 17 have been used with adolescents 13 years of age and younger. This underscores a twofold need, first for intervention approaches that target youth before they become sexually active and second for more rigorous evaluations of what programs work to delay initiation until later in adolescence when teens are better prepared to deal with physical, emotional, and social aspects of sexual relationships.

School-Based Approaches to HIV Prevention and Sexuality Education

In 2006, 61% of states required schools to teach students about HIV prevention beginning in elementary school, and 74% required instruction in

middle school; these figures were slightly lower for sex education overall, 49% and 59%, respectively. School district policies were often more restrictive, with only 48% requiring instruction on sexual health and 39% on HIV prevention prior to middle school (Centers for Disease Control 2007). While school-based sex education has the potential to reach youth before they are sexually initiated, the timing and content of lessons have been controversial. Abstinence-only federal and state funding as well as legislation and school policies restricted discussion of condoms and contraception, as well as alternatives to abstinence until marriage (Santelli et al. 2006). Given controversies about the appropriateness of content for older teens, choices for younger teens may be more constrained by disagreements over comprehensive versus abstinence-only or abstinence until marriage sex education.

Multiple literature reviews and several meta-analyses support the benefits of sex education delivered during pre- and early adolescence on sexual risk behaviors. Importantly, studies have consistently shown that sex education neither encourages teens to begin having sex nor increases their sexual activity. The Institute of Medicine has compiled evidence showing that comprehensive sex education programs, which combine abstinence messages with safer sex education and condom availability, are most effective in reducing risks during adolescence (IOM 2000). These programs typically provide youth with information about the benefits of delaying sexual initiation as well as interactive opportunities for developing skills, such as practicing refusal skills, setting personal goals, and considering how peer norms, gender roles, and attitudes influence their behaviors. They are distinguished from abstinence-only or abstinence until marriage curricula because they not only include information about the benefits of abstinence but also address strategies for reducing sexual risks and the use of condoms and other contraception for disease and pregnancy prevention.

As part of the Mathematica review (2012), several sexuality education curricula for use in middle schools have been identified as having moderate to high effectiveness. In general, these

integrate sexual health topics with broader life skills and youth development goals. Examples include the Aban Aya Youth Project for African American youth, Draw the Line/Respect, It's Your Game, and one abstinence-based program, Heritage Keepers. These have been delivered either by teachers or by specially trained health educators and require a minimum of 10–20 classroom sessions. In addition to variation in the amount of information provided on sexual risk reduction, condoms, and other contraception, they differ in whether they are targeted to specific at-risk population groups as well in the extent to which they address gender differences in risks, such as girls who become involved with older boyfriends, or address topics such as dating violence and sexual coercion.

A growing number of public health and national organizations have advocated for the inclusion of comprehensive sex education in elementary as well as middle and high schools (Eisenberg et al. 2008). This support is mirrored by results of multiple surveys that indicate parents, too, overwhelmingly support sex education in school, including provision of age-appropriate medically accurate information on abstinence, contraception, and disease prevention (Kaiser 2004). Yet, in addition to ongoing disagreements about the content of lessons, other barriers to the diffusion of evidence-based school interventions have been identified. For example, time-to-learning requirements and “high-stakes” standardized academic testing have limited classroom opportunities not only for sexuality and HIV education but for health education more broadly. These barriers underscore the importance of more rigorous assessment of the duration and amount of classroom time needed for school-based programs to be effective, either for delaying early initiation or for addressing the needs of youth who are sexually experienced. Other challenging issues include strategies for addressing the needs of sexual minority youth as well as how to address potentially sensitive topics such as dating violence and sexual coercion in general classroom settings.

In addition to providing youth with health and sexuality education, several other school-based

intervention approaches have shown promise, including two programs aimed at younger students, the Good Behavior Game and the Raising Healthy Children Program. The Good Behavior Game is a classroom-management intervention that rewards first and second grade children for displaying on-task behaviors. Delivered by trained classroom teachers, a longitudinal study has demonstrated long-term impact on reducing high risk sexual behaviors as well as drug use into young adulthood (Kellam et al. 2014). This is one of the few programs that have shown such long-term impact. The Raising Healthy Children program for elementary schools as well the middle-school program, Adult Identity Mentoring, are examples of evidence-based youth development programs delivered in schools. These emphasize the connections and social ties that reinforce the importance of avoiding sexual as well as other risks, overlapping content with comprehensive health education programs that also emphasize life skills such as goal-setting, decision making, and maintaining healthy relationship as well as reducing sexual risks.

In sum, school-based programs have the potential to reach youth before they become sexually active, if the timing of delivery and content matches the behavioral risks of youth in diverse communities. In addition, the availability of guidance and health services in the school setting provide opportunities for linking youth to counseling and sexual and reproductive health care.

Parent and Parent-Teen Approaches

Parents are a primary and critical source of information and support for youth undergoing the physical, emotional, and social changes that come with puberty. Research shows not only that positive parent–child communication about sexuality helps teens make good choices but also that teens want their parents' guidance and support (Hacker 2000). Parents can supplement and reinforce school-based sex education by providing information and reinforcing family values and reasons for delaying sexual initiation and risk.

However, many parents report feeling unprepared to address issues of sexuality and sexual health. As a result, studies show that their advice, on reasons for abstinence or on the use of condoms or other contraception, often comes after youth are already sexually active (Kaiser Family 2002). One of the strongest deterrents to positive parenting practices during early adolescence is parents' underestimation of the risks to which their young adolescents are exposed, which may lead to inappropriate or ineffective parenting practices. For example, if mothers discussed condom use with teens before they had sex, their teens were more likely to use condoms at first sex, which is related to more consistent condom use over time; however, this conversation was less helpful if youth were already sexually active (Miller et al. 1998). In addition, parents may underestimate the extent to which their children want to learn from them, and not only from teachers, peers, media, and other sex education channels. Even if they want to play a role in sex education and helping their sons and daughters delay sex until they are older, many parents do not feel they have adequate or accurate information about sexual and reproductive health; this discomfort may be compounded in cultural contexts and communities where family talk about sex has traditionally been limited.

To address these gaps, a number of behavioral interventions have been developed to promote positive parent-child communication. These focus on making parents more comfortable and efficacious when discussing sexual and reproductive health topics with their children. Some have been tailored to address culturally influenced attitudes and beliefs about sexuality and family interactions as well as the increased unintended pregnancy, STI, and HIV risks of at-risk populations of African American and Latino youth. As described in compilations provided by Advocates for Youth (2011) and other clearinghouses, these interventions take a variety of approaches. They include multi-session groups for parents, such as Talking Parents, Healthy Teens; parent-teen (mother-daughter; father-son) workshops, such as Parents Matter! and Keepin' It R.E.A.L.: Mother-Adolescent HIV Prevention Program; multimedia parenting education, such

as Saving Sex for Later's audio-based role model stories that supporting positive parenting practices; parent supplements to school curricula, such as homework assignments for Safer Choices and Youth AIDS Prevention Program; and parent-to-parent networking and support strategies, such as Plain Talk. Other programs, such as Safer Choices and Reducing the Risk have been developed and primarily tested with parents of older teens.

In general, evaluations of these interventions show they are effective in preparing parents to support of their sons and daughters as they make the transition from childhood to adolescence. Parents generally welcome these learning opportunities and report they have benefit from their participation. However, difficulties of community recruitment (as opposed, say, to reaching almost all young teens when they are a captive audience in their middle school classrooms) as well as participation demands may limit their reaching broad audiences. Workshops only reach a segment of the target population of parents who may be sufficiently motivated to attend. Homework assignments may be difficult in households where school attachment or literacy levels are low. Despite the limitations, across different strategies, a number of benefits for youth have been identified, including teens' reports of increased communication and perceptions of greater parental support as well as reductions in sexual and related risk behaviors, like alcohol and drug use. As with many school-based programs, however, there is limited information on the extent to which they specifically help young teens delay sexual initiation and little on long-term impact on sexual health over an extended time.

Community Approaches

Community interventions also can play a role in efforts to reduce early sexual debut, especially as a compliment to what is offered by school and provided by parents. These include youth development programs, strategies aimed at primary care and reproductive health providers, and social marketing campaigns. An advantage of out-of

school programs is that content can be tailored to address levels of risk, gender differences, and sensitive issues such as coercion and relationship violence.

Youth development programs aim to help teens develop assets and resiliencies that are associated with positive behaviors and reduced risks. A review of nine studies found that involving at-risk youth in community service can be effective in reducing sexual risks, including age of initiation, number of sex partners, and related behaviors such as violence and substance use (Guide to Community Preventive Services 2007). In addition to targeting specific youth risks, these facilitate community connectedness—a protective factor for multiple problem behaviors.

Primary care providers can play a unique role in providing youth with access to safe and accurate reproductive health resources but often lack the resources and time. Interventions focused on improving provider-patient relationships in adolescent health care settings have the potential to influence adolescent attitudes and behaviors prior to sexual debut (McKee et al. 2004). In particular, vulnerable urban youth can benefit from improved access to personalized, supportive care from providers in settings where they perceive their privacy is protected. However, like teens' communication with parents, adolescent-provider communications about sexuality can be challenging and often happen only after youth have initiated sexual activity. Six programs identified in the Mathematic review were evaluated when delivered in clinic settings; only two – Sisters Saving Sisters and Safer Sex, both targeted to black teens – included youth under 14 in their evaluation studies and only Safer Sex is noted for having a measurable impact on delay of sexual initiation or abstinence (Mathematica 2012).

Media campaigns based on health communication theory have underscored the role parents play in sex and drug education and keeping teens from engaging in risk. Relationships between parent-child communication and adolescent sexual behavior are well documented in the literature. In addition, HIV/AIDS prevention media campaigns targeting youth directly in the USA and

globally have also found increases in condom use initiation, decisions to be abstinent, and a reduction in number of partners (Noar et al. 2009). While studies have found that in communities where accessibility, availability, and acceptability of condoms are greatest, there are lower incidences of new HIV infections, strategies that support condom distribution programs have not focused on delays in sexual initiation and are not typically targeted to youth under the age of 15. Globally, there has been support for structural interventions that address underlying factors that contribute to early sexual initiation, including poverty, lack of educational opportunities, gender inequity, power imbalances, and forced sex. These most often have been implemented in Africa and other regions with the highest prevalence of HIV. Many of these efforts have been directed to girls around the time of puberty; they address social and economic barriers to school attendance and completion and other strategies that promote gender equality and empowerment (Family Health International 2010).

Limitations

Despite the potential of evidence-based behavioral interventions to help youth delay sexual initiation, there are numerous barriers to their effective dissemination and use by communities where they could have the greatest impact. First, there are relatively few programs that have been specifically targeted to support youth in the transition from middle childhood into adolescence, and relatively few evaluation studies have specifically examined program impact on the timing of sexual initiation. Second, to have the biggest impact on HIV prevention, programs must be directed to the most at-risk populations and highest prevalence communities, yet finding the resources to support programs in these resource-limited settings can be challenging. Across settings, schools have encountered problems devoting classroom time for sex education, the major strategy for reaching almost all youth; it has been difficult to engage parents or youth in voluntary,

afterschool programs. While a growing body of evidence demonstrates that comprehensive sex education can have a positive impact on reducing youths' reports of sexual risk taking, many evaluations are limited by weak study designs and small samples, and youth have not been followed over time to measure impact on pregnancy or STI/HIV rates (Mathematica 2012; Kirby et al. 2005). Comparative information on what works best for what populations is not available, despite sizeable cultural and community differences in age at sexual debut. We also do not know which components of interventions most directly address delay of initiation or how school, parent, and community approaches can be integrated to achieve the greatest impact. Political will and public controversies continue to shape how and when some HIV prevention topics and strategies are addressed, such as information about and access to condoms or other contraception or linkages to sexual health services, especially prior to first sex. There is also little information about patterns of early adolescent risk behaviors and effective prevention strategies for non-heterosexual youth and, especially, young males who may be at risk for early initiation and experimentation. This is especially troubling given the elevated HIV risks of young men who have sex with men and the young age at which many become infected. Despite these limitations, however, the fact is that schools and communities nationwide are selecting and implementing behavioral interventions that seek to reduce early and risky sex, and most surveys have shown that youth, parents, and the public are, in general, highly supportive of these efforts.

Conclusion

Preventing early sexual debut is an important target for HIV prevention programs as well as for broader efforts to address disparities in sexual and reproductive health, including STI, unintended pregnancies, and sexual violence. Program planners can choose from a variety of evidence-based interventions that address the

sexual risks and contexts of younger teens, are appropriate for the communities they serve, and matched to their local opportunities and resources.

Cross-References

- ▶ Peer-Based Intervention Approaches
- ▶ Use of Technology and Social Networking in HIV Prevention

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Dendritic Cell Interactions with HIV-1 Envelope Glycoprotein: Implications for Preventing Transmission

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Definition

Dendritic cells (DCs) are critical sentinels of the immune system and potent antigen-presenting cells. They patrol peripheral tissues including the blood, skin, and mucosal surfaces, poised to sense and respond to incoming microbes. Upon encountering a microbe, DCs engulf it and are triggered to migrate to the lymph nodes. Along the way they degrade the microbe and undergo a process of maturation that includes upregulation of molecules associated with antigen presentation. In the lymph node, DCs present their antigen on major histocompatibility complex (MHC) class I and II molecules to T cells and thus activate an adaptive immune response specific to the microbe. DCs can be one of the first cell types to encounter HIV. The virus has evolved to exploit the natural function of DCs, which includes avoiding degradation in order to gain access to its primary target – the CD4⁺ T cells. DCs are therefore a key target to investigate for strategies to prevent HIV transmission. In this entry we will introduce the reported interactions of different subsets of DCs and HIV, particularly the envelope glycoprotein expressed on the virus surface, as well as strategies to interfere with these interactions to inhibit HIV acquisition.

Introduction

Accumulating data from HIV vaccine clinical trials (► [Prevention Clinical Trials Highlights of](#)

Evidence and Research) and preclinical research suggests that the HIV envelope glycoprotein spike (Env) is likely to be an essential component of a prophylactic vaccine. Env is a heterodimer of the highly glycosylated gp120 and gp41 subunits (► **HIV virion structure**) that form trimers on the virion surface. The trimer mediates attachment and fusion of HIV into host cells and is the sole target for neutralizing antibodies (nAbs). Env is therefore an important focus for HIV prevention strategies in the form of vaccines designed to elicit nAbs and microbicides that directly block infection.

Dendritic cells (DCs) are the most potent antigen-presenting cells and are essential for priming naïve T cells and establishing effective adaptive immune responses. DCs in the genital mucosa are among the first cells to encounter HIV (► **HIV-1 Transmission; Cell-Types Associated with**) and other sexually transmitted infections (► **HIV-1 Sexual Transmission**). They can be infected with HIV, and more importantly, by virtue of their close interactions with CD4⁺ T cells in the lymph nodes, DCs can also transfer virus from the mucosa to these primary HIV target cells with high efficiency. Thus, DCs represent an important link in the chain of HIV transmission and immunity. They play a role in HIV dissemination and seeding of a viral reservoir but they are also critical in governing immune responses to vaccines. Targeting DCs with prevention strategies may be an effective approach to inhibit the establishment of HIV infection.

Dendritic Cells

DCs Prime Adaptive Immune Responses

Human DCs have evolved into multiple distinct and functionally specialized subsets, tailored to handle the microbes associated with their location (Teunissen et al. 2012; Smed-Sorensen and Lore 2013). DCs constantly sample their environment for antigen and can sense the invasion of a pathogen through pattern recognition receptors including C-type lectin receptors (CLRs), toll-like receptors (TLRs), and an array of DNA and RNA sensors. Upon detecting and internalizing a

pathogen, DCs become activated, maturing into potent antigen-presenting cells, and migrate from the peripheral tissue to the draining lymph nodes. Here they present their antigen on MHC class I and II molecules and activate naïve CD8⁺ and CD4⁺ T cells to become cytotoxic or helper T (Th) cells, respectively. CD4⁺ Th cells support the development of cytotoxic CD8⁺ T cells and the production of antibodies (Ab) by B cells. DCs also regulate the magnitude and quality of the ensuing adaptive immune response through the release of cytokines.

DC Subsets Are Functionally Specialized

There are two main subtypes of human DCs – myeloid and plasmacytoid DCs (Table 1). CD123⁺ plasmacytoid DCs (PDCs) are localized primarily in the lymph nodes, secondary lymphoid organs (e.g., spleen, tonsils, mucosal-associated lymphoid tissues), and blood, but migrate into tissues upon inflammation or pathogen exposure. PDCs are uniquely potent producers of interferon- α (IFN α), which plays a key role in the antiviral response, but they can also take up and present antigens to T cells. CD11c⁺ myeloid DCs (MDCs) exist as three major subsets in the blood and multiple subsets in the tissues (Fig. 1). CD1c (BDCA-1)⁺ blood MDCs are superior at antigen uptake and stimulating naïve CD4⁺ T cell responses and are the predominant producers of IL-12p70. CD141 (BDCA-3)⁺ blood MDCs are a very rare subset that are competent at cross-presentation and stimulating naïve CD8⁺ T cell responses and produce large amounts of IFN- λ (Nizzoli et al. 2013; Smed-Sorensen and Lore 2013). CD1c⁺ MDCs can also efficiently cross-prime cytotoxic CD8⁺ T cells although the signaling requirements for optimal priming by the two DC subsets differ (Cohn et al. 2013; Nizzoli et al. 2013; Segura et al. 2013) and may reflect their specialization for different pathogens. The third subset, CD16⁺ MDCs, is relatively poor antigen presenters but are potent secretors of pro-inflammatory cytokines (Smed-Sorensen and Lore 2013).

The skin and mucosa (► **Mucosal Immunity**) are composed of two main compartments – the epidermis and dermis in skin, or stratified

Dendritic Cell Interactions with HIV-1 Envelope Glycoprotein: Implications for Preventing Transmission, Table 1 Specialization of DC subsets

Location	DC subset	Key phenotypic markers	Key cytokine produced	Specialty
Blood, LNs	Plasmacytoid DCs (PDCs)	CD123	IFN α	Innate antiviral response
		BDCA-4		
Blood, tissues	Myeloid DCs (MDCs)	CD11c	IL-12p70	Prime naïve CD4 ⁺ Th cells
		CD1c (BDCA-1)		
		CD11c	IFN- λ	Cross-prime CTLs
		CD141 (BDCA-3)		
		CD11c	TNF	Proinflammatory
		CD16		
Skin – epidermis Mucosa – stratified squamous epithelium	Langerhans cells (myeloid)	CD11c	IL-15	Peripheral tolerance
		CD1a		Support development of CTLs
		Langerin		
Skin – dermis, Mucosa – lamina propria	Dermal DCs (myeloid)	CD11c	Low IL-15	Support development of CTLs
		CD1a		
		MR		
		CD11c	IL-10	Support Ab production
		CD14		
		DC-SIGN		
		MR		
		CD11c	Not determined	Not determined
		CD141		

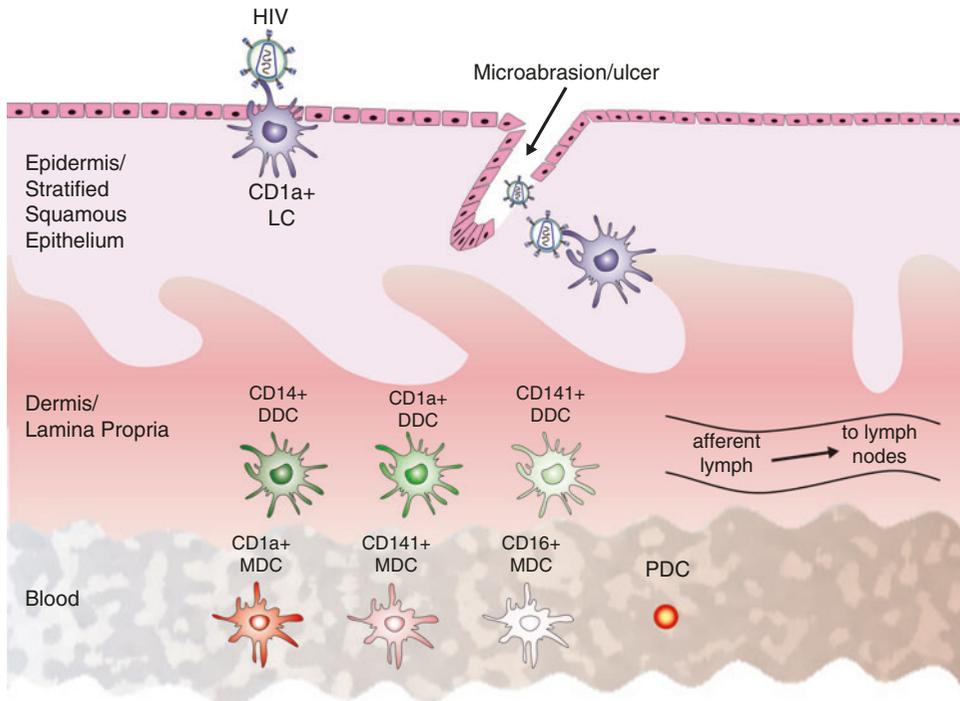
squamous epithelium and lamina propria in mucosa. Langerhans cells (LCs) are the major DC subset found near the surface, in the epidermis/epithelium. Several subsets of dermal or interstitial DC subsets reside deeper in the dermis/lamina propria (Fig. 1), including CD1a⁺CD14⁻, CD1a⁻CD14⁺, and CD141⁺ subsets (Harman et al. 2013b). In the skin, LCs play a dual role, maintaining epidermal tolerance by stimulating skin-resident regulatory T cells but also responding in an immunostimulatory fashion to pathogens and efficiently priming cytotoxic T cells and CD4⁺ Th cells that support their development. In contrast, CD14⁺ DDCs primarily prime CD4⁺ Th cells that support the proliferation and differentiation of B cells into Ab-producing cells (Teunissen et al. 2012). These differential roles are governed by the distinct cytokine profiles of the two subsets with the specialized production of IL-15 by LCs and IL-10 by DDCs (Banchereau et al. 2012). Due to the low frequency of DCs in vivo, DCs are commonly derived from monocytes in vitro as a reliable way of generating high numbers of DCs.

These model monocyte-derived DCs (MDDCs) most closely resemble dermal DCs in terms of gene expression (Harman et al. 2013a).

DC Interactions with HIV Env

DC Binding and Uptake of HIV/Env

In the infection process, HIV first anchors to the host cell via Env binding to the high-affinity receptor CD4. Env binding is followed by interactions with the co-receptor CCR5 or CXCR4 that allows fusion with the plasma membrane and entry of the virus. In DCs (► [HIV-1 Transmission; Cell-Types Associated with](#)) this may result in a low level of productive infection (*cis* infection). As DCs are nonproliferating cells, the replication of HIV is generally low and is also restricted by host factors such as SAMHD1 (Vicenzi and Poli 2013). A more significant and unique feature of DCs is that they can capture and transfer virus to closely associated CD4⁺ T cells without becoming infected themselves, in a process termed *trans*



D

Dendritic Cell Interactions with HIV-1 Envelope Glycoprotein: Implications for Preventing Transmission, Fig. 1 Skin and mucosal DC subsets interact with HIV at the point of entry. Genital skin and mucosal surfaces represent a portal of entry for HIV. Multiple phenotypically and

functionally distinct DC subsets interact with HIV in these tissue as the virus enters through breaches in the epidermis/epithelium or is actively transported across the luminal wall by Langerhans cells. DCs contribute to virus dissemination as well as the initiation of anti-HIV immune responses

infection. DCs efficiently capture and endocytose HIV via pattern recognition receptors, including CLR that have affinity for the high mannose glycans on Env. The normal function of CLR is to facilitate antigen uptake for efficient processing and presentation to T cells, but HIV has evolved to subvert this pathway and can be routed to intracellular compartments where the virus remains intact (see section “[The Role of DCs in HIV Dissemination](#)”).

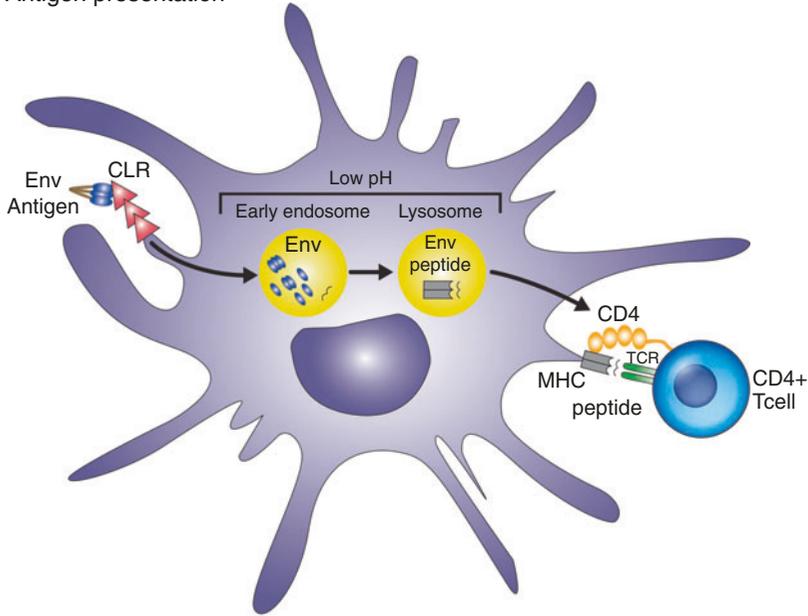
The different DC subsets express unique repertoires of CLR, enabling the DCs to capture a variety of pathogens via their glycosylated surface proteins. In relation to HIV binding, LCs uniquely express high levels of langerin. The various dermal DCs express combinations of dendritic cell-specific ICAM-3 grabbing non-integrin (DC-SIGN), mannose receptor (MR), dendritic cell immunoreceptor (DCIR), and DEC-205 (Harman et al. 2013a; Sandgren et al. 2013). The

expression patterns of CLR on lamina propria DCs are currently less well defined. Blood DCs express a more restricted CLR repertoire including DCIR, DEC-205, and BDCA-2 (the latter on PDCs only) and tend to rely on CD4 for Env binding. In the context of soluble Env protein alone, this is a functional pathway for internalization and antigen presentation (Sandgren et al. 2013). HIV also exploits non-glycan dependent binding to other DC-expressed pattern recognition receptors, including siglec-1 and syndecan-3, for capture and transfer to CD4⁺ T cells (Tsegaye and Pohlmann 2010).

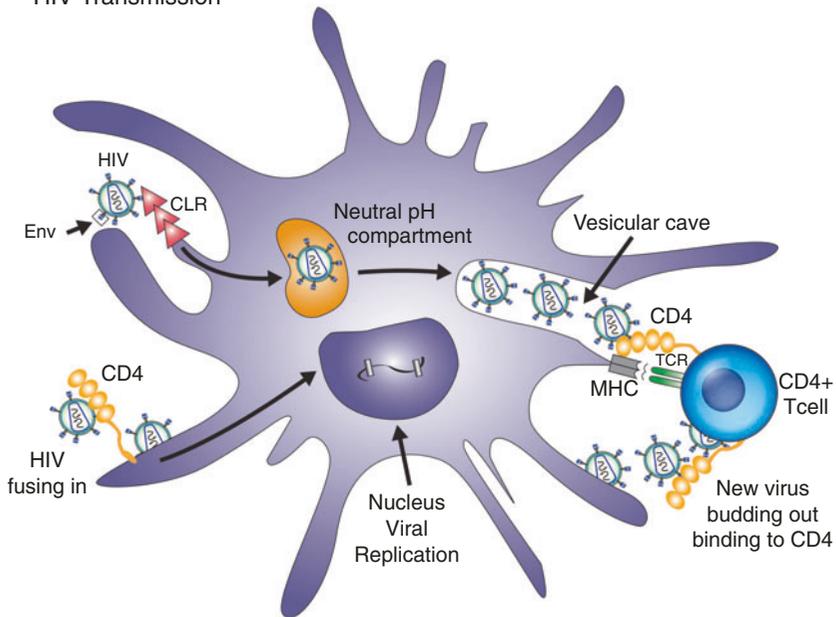
The Role of DCs in HIV Dissemination

As mentioned above, Env binding to certain CLR triggers endocytosis of HIV leading to two outcomes (Fig. 2). The virus can be degraded and subsequently presented to T cells. Alternatively, approximately 5–10% of the virus subverts

a Antigen presentation



b HIV Transmission



Dendritic Cell Interactions with HIV-1 Envelope Glycoprotein: Implications for Preventing Transmission, Fig. 2 HIV subverts antigen presentation pathways in DCs to gain access to its target cell, CD4+ T cells. (a) DCs utilise CLR to bind and take up pathogens, which it processes and then presents to T cells to stimulate

an immune response. (b) HIV can also be taken up via this route without being destroyed and transferred directly to CD4+ T cells interacting with the DC. At later times, newly synthesised virus in an infected DC buds out in close proximity to CD4+ T cells for efficient spread

endolysosomal trafficking and is preserved in pH-neutral compartments or deep plasma membrane invaginations continuous with the cell surface, referred to as vesicular caves (Cunningham et al. 2013). Upon contact with a CD4⁺ T cell, the virus recycles rapidly to the interface between the two cells and is transferred directly to the T cell where it can replicate explosively. The latter is a very efficient route of infection for T cells – much more efficient than direct infection by cell-free virus (► [Inhibition of HIV-1 Spread: Cell-Free Versus Cell-Cell](#)). Furthermore, DCs have been shown to preferentially transfer virus to responding antigen-specific CD4⁺ T cells that cluster with them (Lore et al. 2005), and the simultaneous activation of these T cells by antigen recognition enhances HIV transmission (Rodriguez-Plata et al. 2013).

The result of these interactions between HIV and different DC receptors is that DCs can transfer HIV to CD4⁺ T cells in two waves. The first phase of virus transfer occurs within 24 h and results from transfer of virus to bystander CD4⁺ T cells in the absence of DC infection. The second wave begins 48–72 h after infection and is a result of de novo virus production in infected DCs (Cunningham et al. 2013). DCs thus play a critical role in the capture and transfer of HIV from the portals of entry to the regional lymph nodes, dissemination of the virus to T cells, and the seeding of a viral reservoir.

Mucosal DCs are especially relevant to sexual transmission of HIV and dissemination to the draining lymph nodes. Mucosal LCs have been observed to extend their dendrites between the tight junctions of the epithelial cells, or even to migrate across the epithelium right into the lumen, to sample antigen. In fact, HIV attracts DCs in the gut (Selection of CCR5 Using Viruses; Transmission) and exploits their migratory capacity to hitch a ride across the mucosa to the T cell-rich areas in the gut and in the lymph nodes (Harman et al. 2013b).

Immunosuppressive Effects of Env

DC Dysfunction in HIV Infection

DC-mediated immune responses are subverted by HIV and are insufficient to control dissemination

of the virus (Smed-Sorensen and Lore 2011; Miller and Bhardwaj 2013). For example, not all the virus taken up by DCs is degraded and DCs do not fully mature in response to HIV, which may lead to suboptimal antigen presentation and T cell activation in the lymph nodes. HIV Env is also able to shut down autophagy in DCs, which compounds this dysfunction by decreasing MHC class II antigen presentation and stimulation of HIV-specific CD4⁺ T cells. This concomitantly allows accumulation of virus and enhanced transmission to T cells (Blanchet et al. 2010). DCs, both in vitro and in simian AIDS models, have also been shown to become sensitized by Env to undergo apoptosis upon exposure to activation stimuli (Wijewardana et al. 2010; Chen et al. 2013). The result is significant DC depletion occurring early in acute infection and dysfunction, which may lead to defective or skewed Th cell responses. The DCs that do remain are hyperfunctional in response to TLR7/8 agonists (HIV also triggers TLR7/8) in terms of maturation, and cytokine and chemokine secretion, which may contribute to the systemic immune activation (► [Chronic Immune Activation](#)) observed in chronic HIV (Miller and Bhardwaj 2013).

Env-Mediated Immunosuppression

While the factors governing the fate of HIV inside a DC (transfer vs. degradation) are unclear, the glycan composition of Env (Virus Structure), which varies from strain to strain, may play an important role. It has been shown that virus modified to have an oligomannose-enriched envelope was captured more efficiently by DCs and was routed more efficiently into the endolysosomal pathway and degraded, resulting in reduced viral transfer to T cells. It was also presented to Env-specific CD4⁺ T cells more efficiently (van Montfort et al. 2011). The glycan composition of Env determines the binding affinity of the glycoprotein for different CLRs that can subsequently target it to different compartments in the endolysosomal pathway. These results may have promising implications for Env-based protein subunit vaccines, but Env has also been associated with immunosuppressive effects through its interactions with mannose-binding CLRs.

Echoing what has been observed in HIV-infected patients, multiple *in vitro* studies have shown that Env glycoproteins can impair critical DC functions including maturation, cytokine production, and activation of T and B cells and can induce DC apoptosis. Exposure to certain strains of Env results in defective maturation of DCs in response to classical maturation stimuli (Shan et al. 2007), as well as impaired production of effector cytokines such as TNF, IL-12, and IFN α (Fantuzzi et al. 2004; Martinelli et al. 2007; Chung et al. 2012) and the induction of anti-inflammatory IL-10 (Shan et al. 2007; Sarkar et al. 2013). Furthermore, Env exposure can lead to a decreased capacity to stimulate T cell proliferation (Fantuzzi et al. 2004; Fernando et al. 2007; Shan et al. 2007; Hu et al. 2008) and B cell activation (Chung et al. 2012). As mentioned above, exposure to Env also sensitized DCs to undergo extensive apoptosis upon activation by various stimuli that would normally promote DC function, including CD40 ligand, TLR ligands, and proinflammatory cytokines both *in vitro* and *in vivo* (Chen et al. 2013). These immunosuppressive effects have all been shown to be dependent on Env interactions with mannose-binding CLRs, such as DC-SIGN and MR (Shan et al. 2007; Chung et al. 2012; Chen et al. 2013). In line with these observations, immunization of humanized mice with Env glycoproteins where the mannose moieties were occluded or removed, thus preventing binding to CLRs and presumably circumventing this route of DC dysfunction, resulted in enhanced Env-specific Ab titers and T cell responses (Banerjee et al. 2009, 2012). As mentioned above, the position and type of N-linked glycans on Env may again play a critical role in these negative effects on DC function (Shan et al. 2007). Using different strains of Env or HIV, other studies have not observed any impairment of maturation in MDDCs or primary MDCs and PDCs in response to various TLR ligands or inflammatory stimuli (Lore et al. 2005; Harman et al. 2009; Vani et al. 2012a, b). Env exposure in these instances also did not inhibit DC antigen presentation or stimulation of T cell proliferative responses (Lore et al. 2005; Vani et al. 2012a). Importantly,

MDCs and PDCs do not express the main culprit receptors DC-SIGN and MR, but these are highly expressed on MDDCs. The presence or absence of distinctive glycosylation sites on Env is known to facilitate mother-to-child transmission of HIV and be associated with disease progression, confirming that this phenomenon likely plays a role *in vivo* (Baan et al. 2012, 2013).

Preventing HIV Transmission

Microbicide Strategies

HIV is most commonly transmitted via heterosexual intercourse (► [HIV-1 Sexual Transmission](#)); thus, preventative strategies need to operate at the genital mucosal surfaces. Two such strategies include microbicides (► [HIV-1 Transmission Blocking Microbicides](#)) (topical applications that can be formulated to block HIV infection) and mucosal Abs (that can be elicited by vaccination (► [HIV-1 Transmission Blocking Vaccines; How Feasible Are They?](#))). Given the critical role that Env plays in attracting and infecting DCs and subsequent HIV transmission to T cells, it is a primary target for blocking strategies to interrupt these events and prevent the establishment of HIV infection. A number of compounds have been identified that can block Env interactions with host cells, namely, via DC-SIGN and CD4 binding, that have the potential to be developed into microbicides (Table 2). These include sugars found in human breast milk and other subsequently developed multimeric glycomimetics (structural mimics of mannose and fucose polysaccharides) (Anderluh et al. 2012), as well as purpose-designed CD4-binding proteins, e.g., designed ankyrin repeat proteins (DARPs) (Schweizer et al. 2008).

Compounds that target the actual virus may be preferable over agents that bind directly to cell-surface receptors such as DC-SIGN and CD4 as they are less likely to influence the natural functions of these receptors in immunity. It has not yet been fully validated whether DC-SIGN (which acts as an adhesion molecule and pathogen capture receptor) or CD4 (which binds to MHC II to stabilize the immune synapse and amplify the T cell receptor signal) can be targeted by inhibitors

Dendritic Cell Interactions with HIV-1 Envelope Glycoprotein: Implications for Preventing Transmission,**Table 2** Compounds that can inhibit Env/DC interactions

Agent	Function
Lewis-X motif containing sugars	Compete with HIV for binding to CLRs, e.g., DC-SIGN
Glycomimetics	Compete with HIV for binding to CLRs, e.g., DC-SIGN
DARPinS	Compete with HIV for binding to CD4
Soluble CD4/DC-SIGN/langerin	Bind to HIV Env and thus block HIV binding to cellular receptor
Small-molecule CD4 mimics	Bind to HIV Env and thus block HIV binding to CD4
Algal lectins	Bind to HIV Env and thus block HIV binding to DC-SIGN and DC-mediated transfer to T cells
Dendrimers	Bind to HIV Env and thus block HIV binding to cellular receptor
Env-specific nAbs	Bind to HIV Env and thus block HIV binding to CD4 and DC-mediated transfer to T cells. Opsonize virus for degradation

in vivo without unwanted effects. To this end, various soluble HIV receptors have been tested including soluble CD4 and small-molecule CD4 mimics (Teixeira et al. 2011), DC-SIGN, and langerin. However, such soluble HIV receptors have so far demonstrated limited success. For instance, although soluble CD4 was found to be effective at blocking binding of cell-free virus, it actually enhanced infection with cell-associated virus (Haim et al. 2009), and during natural transmission via semen, vaginal secretions, breast milk, or blood, a significant proportion of virus is expected to be cell associated (► [Inhibition of HIV-1 Spread: Cell-Free Versus Cell-Cell](#)). A number of lectins have been isolated from algae and cyanobacteria that bind to Env and block HIV binding to DC-SIGN in vitro. Compounds including griffithsin, cyanovirin-N, and scytovirin were even more potent at inhibiting DC-SIGN-mediated HIV transfer to T cells and are being investigated for their microbicide potential (Huskens and Schols 2012). Dendrimers are synthetic, highly branched nanoparticles that, by virtue of their highly charged surface, can exhibit antiviral activity by binding to and blocking the

entry of a virus. SPL7013 (Starpharma) is a dendrimer that has been developed as the active ingredient in VivaGel, which is now in clinical trials as a HIV and herpes simplex virus microbicide (Parboosing et al. 2012).

A novel therapeutic strategy may be to develop a mechanism for inhibiting Env-induced DC migration through tissues, to inhibit dissemination of the virus from the mucosa. A signaling pathway via CCR5 was recently identified, whereby Env enhances filopodium formation and trans-endothelial migration of DCs. As described above, these processes are central for initial DC homing to and uptake of HIV as well as migration to the lymph nodes and virus transfer to CD4⁺ T cells in vivo. An active fragment of the protein Slit2 was used to inhibit filopodium formation and DC migration, which could contribute to a potential protective strategy (Prasad et al. 2012).

Env-Based Protein Subunit Vaccines

As previously mentioned, Env represents the sole target on HIV for nAbs and as such is considered to be a critical component of a prophylactic HIV vaccine (► [HIV-1 Transmission Blocking Vaccines; How Feasible Are They?](#)). The early Env-based protein subunit vaccine (AIDSVax by VaxGen), based on Env monomers, was unsuccessful at eliciting broadly nAbs against primary HIV strains. However, a more recent trial (RV144) combining a modified canarypox vector (ALVAC) with AIDSVax in a prime-boost strategy resulted in a reduction in susceptibility to HIV infection and the detection of HIV-specific Ab responses, albeit non-neutralizing, in some vaccinees. This trial demonstrated that vaccination can be effective at conferring a degree of protection against HIV acquisition (► [Preventing HIV-1 Transmission Through Vaccine-Induced Immune Responses](#)) and provides the benchmark for the development of more potent vaccine regimens. Areas for optimization include primarily the design of stable trimeric Env immunogens (► [HIV-1 Transmission Blocking Vaccines; New Designed Immunogens](#)) to elicit broadly nAbs but also the mode of antigen delivery, such as the site of injection and cell types targeted, and the activation of cells receiving the vaccine, usually

achieved through coadministration of an adjuvant. Finally, it may not be enough to elicit Env-specific broadly nAbs systemically. It is likely that they will need to be present at the mucosal surfaces in order to effectively block HIV transmission.

Broadly nAbs (► [Role of Antibodies in HIV Transmission](#)) directed toward the gp120 surface unit of Env, as well as the gp41 transmembrane unit of Env, have been detected in sera from HIV-infected individuals. Such nAbs have also been proven to block transmission of HIV in macaques. nAbs directed toward the membrane proximal region of Env may be particularly successful at blocking transfer of HIV from DCs to T cells (Sagar et al. 2012). However, trials testing Env immunogens have to date been unsuccessful at eliciting such nAbs. Consequently, efforts are currently underway to map the broadly nAb response to understand both the physical characteristics of these Abs and their cognate epitopes on Env, as well as how they evolved, in terms of their germline sequence and degree of somatic hypermutation. The hope is that this information will inform the rational design of new Env immunogens (Burton et al. 2012).

Targeting DCs to Enhance Vaccination

One of the critical factors in the establishment of an effective humoral response is the provision of appropriate CD4⁺ T cell-mediated help. T follicular helper (Tfh) cells that are specialized for B cell help and are essential for the formation of germinal centers may be of particular importance. As DCs are critical for priming and polarizing CD4⁺ Th cells, the potency with which they do this following vaccination is likely to influence the quality of a vaccine-induced B cell response. Specifically, the efficiency with which DCs take up vaccine antigens, their degree of maturation, and their mode of antigen processing and presentation to CD4⁺ T cells, especially Tfh, are likely to be central in this process.

Extensive efforts have been directed toward targeting vaccine Ags to DCs via specific CLRs, including DC-SIGN, DEC-205, and DCIR, to improve Ag uptake and presentation. This strategy can augment T and B cell responses and reduce the dose of Ag required in a vaccine

setting; however, none of these targeting antibodies have yet been tested with HIV Env. Given the functional specialization of the DC subsets, the choice of targeting receptor and the expression pattern of that receptor on the DC subsets will likely influence the immunological outcome. Uptake of antigen via different CLRs leads to different efficiencies and modes of antigen presentation (Delamarre and Mellman 2011). For example, targeting DEC-205 enhances cross-presentation of exogenous antigen on MHC I to CD8⁺ T cells, whereas DC-SIGN targeting results in efficient MHC II presentation to CD4⁺ T cells. In the case of Env, its high glycosylation naturally targets it to DCs via certain CLRs, including DC-SIGN. Additionally, in blood DCs, including PDCs, that display restricted CLR repertoires, the high-affinity interaction between Env and CD4 also enables efficient uptake and presentation of Env to CD4⁺ T cells (Sandgren et al. 2013).

Another receptor that appears attractive for antigen targeting is CD40. Although it is not a CLR, it is expressed on all DC subsets and also on other APCs including B cells and monocytes. Stabilized Env trimers fused to CD40 ligand (normally expressed on CD4⁺ Th cells) induced activation of DCs via CD40 signaling and licensed them for naive CD4⁺ T cell priming (Melchers et al. 2011). As CD40 ligand also promotes proliferation of B cells and Ab production, this targeting approach may enhance anti-Env responses from two angles.

In terms of targeting DCs, another critical aspect may be the site of vaccine delivery. Most vaccines to date are administered intramuscularly; however, the muscle is poorly populated with immune cells. Generally DCs and other innate immune cells are recruited into the muscle to take up the vaccine and transfer it to the lymph nodes where an adaptive immune response is triggered. The skin, in contrast, has a well-developed immune network with multiple DC subsets as described above and may thus represent a more potent site for vaccine delivery. In fact, intradermal vaccination that delivers antigens to the direct vicinity of the DC network has allowed for large dose sparing without compromising adaptive immune responses (Fehres et al. 2013).

For a successful prophylactic HIV vaccine, it may be critical to combine targeting of antigen to specific DC subsets along with potent, targeted activation of these same cells (Smed-Sorensen and Lore 2013). Protein subunit vaccines are usually poorly immunogenic and require coadministration with an adjuvant to activate immune cells. Adjuvants are used to enhance the immune response and can be used to tailor the type of immune response generated. A major mechanism of adjuvant action is thought to be the recruitment and activation of APCs, such as DCs, to the site of vaccination. The activation, or maturation, of DCs involves upregulation of key molecules associated with antigen presentation such as MHC class II and co-stimulatory molecules CD40, CD80, and CD86, as well as chemokine receptors such as CCR7 that direct the DC to migrate from the periphery to the lymph nodes, all of which endow the DC with a potent capacity to prime T cells.

The most commonly used adjuvant in humans is alum, which consists of aluminum phosphate or hydroxide salts, to which vaccine antigens are adsorbed. Despite being in use for over 80 years, the mechanism of action of alum has only recently been elucidated and includes stimulation of the innate immune system, which is in part dependent on activation of the NALP3 inflammasome. Alum also renders antigens particulate and enhances Ag uptake by DCs (Garçon et al. 2011).

A distinctly different class of adjuvants is emulsion adjuvants, including MF59 (Novartis) and AS03 (GlaxoSmithKline) which are licensed for use in influenza vaccines. It is proposed that these adjuvants act by creating an immunocompetent environment at the site of injection, recruiting critical innate immune cells including neutrophils, monocytes, and DCs, although their effects on DC maturation and capacity to prime T cells remain to be defined (Garçon et al. 2011).

A more targeted approach can be achieved using defined TLR ligands as adjuvants (Duthie et al. 2011). TLR ligands efficiently induce cytokine production and maturation of DCs which collectively augment their ability to present antigens on MHC I and MHC II to stimulate antigen-specific T cell responses. TLR signaling also

enhances migration of DCs to the lymph nodes. The various DC subsets express unique repertoires of TLRs and this offers opportunities to target specific subsets and fine-tune the DC response (Smed-Sorensen and Lore 2013). For example, it was recently shown that DC subsets require specific combinatorial TLR stimulation to enable optimum priming of T cell responses (Matthews et al. 2012; Nizzoli et al. 2013).

Conclusion

The interactions between HIV Env and DCs are complex and depend on the glycosylation of Env and the type of DC, among other things. This impacts on the initial transmission of the virus at mucosal surfaces, the dissemination of the virus from the site of exposure throughout the body, the widespread infection of CD4⁺ T cells, and finally the generation of anti-HIV immune responses. Env can, in some contexts, have a negative effect on DC function, yet it is also considered to be an essential component in a prophylactic HIV vaccine. In the future, the cumulative knowledge of the interactions between Env and DCs can hopefully be used in the development of potent microbicides and/or optimized vaccine formulations to stimulate appropriate DC subsets while overcoming Env-induced immune subversion and thus allow the generation of effective protection against HIV acquisition.

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Differential Diagnosis of HIV-Associated Neurocognitive Disorders

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Definition

Cognitive dysfunction in human immunodeficiency virus (HIV)-infected patients is common and can be a difficult diagnosis for the treating physician in the face of multiple potential causes.

Cognitive dysfunction persists in up to 50% of HIV-infected patients in the post-highly active antiretroviral therapy (post-HAART) era, with milder forms predominating (Cysique et al. 2004; Cohen and Gongvatana 2010). The differential diagnoses are divided into those relevant to virally suppressed patients and those that are not, with HIV-associated neurocognitive disorder (HAND) common to both groups.

In patients who are virally suppressed, the diagnoses more commonly encountered, apart from HAND, are substance abuse, psychiatric illness, and hepatitis C, both through its direct effects on cognition and through hepatic encephalopathy. The clinical significance of ageing and neurodegenerative diseases such as Alzheimer's and vascular cognitive impairment is not presently clear though the risk factors for degenerative diseases are common in HIV disease raising the possibility of increased significance in the future. In patients who are not virally suppressed, cognitive dysfunction is more commonly related to HAND, but central nervous system (CNS) opportunistic infection and malignancy such as primary CNS lymphoma are also important considerations. Cognitive dysfunction after the recent commencement of HAART suggests an immune reconstitution inflammatory syndrome (IRIS) related to a previously subclinical opportunistic infection, most commonly progressive multifocal leukoencephalopathy, but HAND as an IRIS phenomenon directly related to HIV is also a possibility.

Virally Suppressed or Early HIV Disease (CD4+ Count >500/ μ L)

Substance Abuse

Drug use is a major problem for a subset of the HIV population, as a contributor to cognitive dysfunction as well as being a vector of transmission through high-risk behaviors, such as sharing of unclean needles and high-risk sexual activity (Foley et al. 2008). It also contributes to increased morbidity and mortality through higher risk of complications such as infective endocarditis and coinfection with hepatitis C, which in itself is a

risk factor for cognitive dysfunction directly and through hepatic encephalopathy, often even in mild forms.

Cognitive impairment from long-term substance abuse is well studied. Long-term stimulant abuse for instance leads to lasting generalized cognitive impairment, rather than in specific cognitive domains (Spronk et al. 2013). In studies focused on the HIV population, substance abuse was additive to the effects of HIV on cognition, leading to greater declines in attention, psychomotor function, and executive abilities (Foley et al. 2008). Substance abuse is also a major factor that negatively affects engagement and response to medical services, such that these patients are at increased for HIV disease progression and mortality (Margolin et al. 2002).

The effects of acute drug intoxication and withdrawal can have significant overlap with the clinical manifestations of HIV. Drug intoxication in common with HAND may present with apathy, disorientation, and poor concentration. Similarly drug withdrawal features such as agitation, altered consciousness, and seizures may also be present in CNS infections.

Treatment of substance abuse disorders in conjunction with HIV can improve outcomes for both conditions. The treating physician should be aware of potential interactions between HAART and treatments for substance abuse, as well as engaging a multidisciplinary team to address all issues (Gourevitch and Friedland 2000).

Psychiatric Illness

Psychiatric illness especially major depression is more common in the HIV population due to direct damage to subcortical brain areas but also due to psychiatric factors such as stress related to chronic illness and social aspects, for example, isolation and stigma. Depression is underdiagnosed in the HIV population, and its prevalence increases as HIV disease progresses (Asch et al. 2003). Patients with depression in turn are at increased risk of progression of HIV disease and mortality.

Depression may also be a side effect of medications used to treat HIV and its complications. For example, efavirenz is a non-nucleoside reverse transcriptase inhibitor with prominent

neuropsychiatric side effects that can exacerbate or cause depressive symptoms. These usually respond to the removal of the drug.

Several clinical symptoms and signs are common to HAND and depression. Mood symptoms in HAND are common including apathy, irritability, insomnia, and anxiety, while cognitive deficits may also occur in major depression especially impaired concentration and memory. HAND is associated with apathy rather than sadness and anhedonia, critical features that distinguish between major depression and HAND. Major depression may also present with nonspecific somatic symptoms such as headache, lethargy, and gastrointestinal symptoms, all of which have a wide differential in the HIV-infected patient. Psychiatric illness needs to be firmly at the forefront of the physician in order to make accurate diagnoses.

Successful management of depression improves the adherence to HIV and psychiatric treatment. Pharmacotherapy must be cautiously chosen as there can be considerable risk of interaction with HAART, and side effect profiles should be individualized to the patient.

Hepatitis C Virus

Hepatitis C virus (HCV) infection is known to be a cause of cognitive impairment as a direct manifestation and also indirectly through hepatic encephalopathy. Up to 30% of HIV-infected patients are coinfecting with HCV (Letendre et al. 2005) though there is a wide variability, and up to two thirds of coinfecting patients have cognitive impairment to some degree (Hinkin et al. 2008). It is curious that it seems to be independent of HCV viral load in the plasma (Garvey et al. 2010). Apart from hepatic encephalopathy which in its mild form can be difficult to diagnose, HCV can cause cognitive impairment probably through direct brain infection. Indeed, there are data supporting HCV infection of macrophages, microglia, and astrocytes (Wilkinson et al. 2009), the same brain cell types implicated in HAND pathogenesis. Treatment of HCV can lead to improvement in cognition (Thein et al. 2007), though the frequency and degree of improvement require further definitive data.

Moreover, the efficacy of the new non-interferon-based therapies in relation to cognition also needs further study.

Ageing

The life expectancy of HIV-infected patients has steadily improved over the last decade and is now on par for many subsets of the HIV population with their non-HIV-infected counterparts (Hogg et al. 2013). This means that more and more patients are living to the age in which they will suffer from the effects of normal ageing. Given that the prevalence of HAND has not decreased with effective virological control, there is a concern that the longer duration of HIV infection together with age may have a compounding detrimental effect on cognitive function (Brew et al. 2009).

HAND has some interesting similarities with the cognitive dysfunction seen in ageing. The neuropsychological parameters of processing speed, working memory, and effortful memory retrieval are deficient in both ageing and HAND, while semantic knowledge, language, and visuospatial abilities are relatively preserved (Brew et al. 2009). Additionally, there is intersection between the neuropathology found in HIV and ageing particularly in relation to the frontal lobes and hippocampus, as well as impairment of the blood-brain barrier and disturbances in the cellular disposal of toxins (Brew et al. 2009). Functional brain imaging studies have shown that HIV infection is equivalent to a 21-year increase in brain age (Ances et al. 2010).

As can be appreciated, ageing and HAND may act in concert to increase the cognitive deficits that are common to both, with potential acceleration and worsened severity of the ultimate cognitive deficit. Definitive prospective data, however, are needed.

Neurodegenerative Diseases and Mechanisms in HIV

There are several areas of similarity in the mechanisms of HAND and neurodegenerative disease. Inflammation, highlighted by elevated levels of tumor necrosis factor α (TNF α), interleukin 6 (IL-6), monocyte chemotactic protein

1 (MCP-1), and quinolinic acid in the cerebrospinal fluid (CSF) and serum, is seen in HAND as well as neurodegenerative diseases (Brew et al. 2009). Chronic inflammation, through interferon γ (IFN γ) and TNF α , leads to changes in the ubiquitin-proteasome system to the immune-proteasome system, switching its function from degrading large proteins to cleaving antigenic proteins for MHC-1 presentation. This exerts a potentially harmful slowing of protein turnover leading to accumulation of misfolded ubiquitinated proteins of pathological ageing, which is a hallmark neuropathological change in neurodegenerative diseases (Nguyen et al. 2010). HIV provides a chronic long-term substrate of inflammation due to its persistent long-term carriage in the CNS.

Normal neuronal autophagy has a protective function in the CNS to maintain homeostasis; however, neuronal autophagy is both inhibited by HIV and is disrupted in neurodegenerative diseases. Furthermore, P-glycoprotein expression is deliberately inhibited in some HIV therapies through the use of ritonavir in order to enhance antiretroviral activity of protease inhibitors. However, the P-glycoprotein system is thought to play a role in the normal clearance of amyloid from the brain and has been implicated in neurodegenerative disease as well as HIV-infected patients on HAART (Brew et al. 2009).

These pathogenetic mechanisms, that are common between HIV and neurodegenerative diseases, can be seen to provide evidence that some patients may develop cognitive impairment as a consequence of premature ageing and accelerated expression of the diseases of ageing, the neurodegenerative diseases (Brew and Cysique 2010).

Alzheimer's Disease

There exists significant overlap in HAND and Alzheimer's disease (AD) in terms of similar pathogenic mechanisms as shown above, common risk factors, neuropathology, measurable biomarkers, and potential response to pharmacological agents. In AD, the brain tissue is notable for the presence of extracellular deposits of beta amyloid, especially 1–42 (A β 42), in the form of plaques and aggregations of microtubule-

associated, tau-forming, neurofibrillary tangles. The CSF contains low levels of A β 42 and elevated total tau (t-tau) as well as phosphorylated tau (p-tau) (Brew and Cysique 2010). In HIV, there is evidence for increased amyloid deposition in the brains of patients at autopsy even in those treated with HAART, and there is evidence for reduced levels of A β 42 in the CSF of patients with HAND. As described above in mechanisms related to P-glycoprotein inhibition, HAART especially ritonavir may be associated itself with abnormal A β 42 deposition in the brain (Becker et al. 2009).

Interestingly, risk factors that are known to be associated with AD, such as insulin resistance, midlife raised lipids, lowered leptin levels, APOE4 status, and elevated CD69 monocytes have also been shown to be associated with the development of HAND (Brew and Cysique 2010).

HIV and AD also share a common anatomical substrate of the brain, namely, the hippocampus (Brew et al. 2009), while there is preliminary evidence that AD treatment in the form of cholinesterase inhibitors is beneficial in HAND (Simioni et al. 2013).

Clinically it can on occasion be difficult to distinguish HAND from AD especially in mild cases. Careful neuropsychological testing can be helpful in identifying the “subcortical” cognitive domains of processing speed, working memory, attention, and executive functioning (Schouten et al. 2011) characteristic of HAND as opposed to the “cortical” deficits of AD such as episodic memory, language disturbances, and loss of visuospatial skills. Motor symptoms also appear earlier in the course of HAND but are unlikely to be the presenting complaint. Motor disturbances such as unsteady gait and tremor are relatively early manifestations, while impaired saccadic eye movements, bradykinesia, hyperreflexia especially of the legs, and frontal signs occur later (Brew 2001). This is in direct contrast to AD which is unlikely to cause significant motor disturbances until a late stage.

While both diseases can be progressive, HAND can have a fluctuating course over time not usually seen with other neurodegenerative diseases.

In addition to these clinical differences, neuroimaging can also be a useful diagnostic tool. While focal or diffuse cerebral atrophy and white matter changes shown on MRI are nonspecific, more advanced techniques of MR spectroscopy, functional MRI, single-photon emission computed tomography (SPECT), and positron emission tomography (PET) with amyloid PET tracers are promising to be potential discriminators between HAND and other neurodegenerative diseases (Ances et al. 2012).

Vascular Cognitive Impairment

HIV infection and the use of HAART, especially protease inhibitors, are well known to cause changes to fat distribution as well cause metabolic abnormalities including dyslipidemia, diabetes mellitus, insulin resistance, and hepatic steatosis. Hypertension has also been linked to HIV infection and in particular HAART (Becker et al. 2009). This induction of the metabolic syndrome leads to an increased incidence in cardiovascular disease such as stroke and myocardial infarction in the HIV population (Currier et al. 2008). The metabolic syndrome is also a risk factor for dementia and vascular cognitive impairment. Even though the traditional cardiovascular risk factors have an increased prevalence in the HIV population, studies correcting for these traditional risk factors still show higher rates of cardiovascular disease. The pathogenesis of vascular disease in HIV is most likely related to inflammation and immune dysregulation affecting endothelial function and coagulation (Fedele et al. 2011). Clinically, vascular cognitive impairment has a very similar presentation to HAND, characterized by cognitive slowing and impaired executive function. It may not be clearly related to stroke events, and imaging may only show white matter disease indicative of microangiopathic changes of small cerebral vessels. On the other hand, the clinical presentation of vascular dementia (VaD) is broad, reflecting the variety of vascular territories that can be involved. Features that would be suggestive of vascular dementia in an HIV-infected patient would be localizing cortical syndromes, a stepwise progression reflecting

cumulative vascular incidents, history of stroke, and neuroimaging consistent with previous large or small vessel ischemia. VaD can be diagnosed in isolation but also commonly in conjunction with neurodegenerative diseases such as Alzheimer's disease.

Studies on the relative risk of traditional cardiovascular risk factors and HIV serostatus, CD4+ counts, and viral replication on cognitive function in older HIV-infected patients show a much higher association with traditional risk factors than HIV factors. This suggests that close attention to traditional cardiovascular and metabolic risk profiles is mandatory to limit cognitive dysfunction in the HIV population (Becker et al. 2009).

Not Virally Suppressed with Advanced HIV Disease (CD4+ Count <200 μ L)

Central Nervous System Opportunistic Infections

CNS infections have the potential to cause cognitive dysfunction; however, most are easily distinguished from HAND by the systemic features, acuity of the illness, headache, and the progression of cognitive dysfunction to confusion to stupor with advancing severity. In general the opportunistic infections that infect the CNS occur when CD4+ counts are low and profound immunosuppression is present. Deficits occur over days to weeks with the slowest of progressing infections and over months with HAND. Systemic features such as fever are not commonly present, even in diseases such as cryptococcal meningitis, tuberculosis, or toxoplasmosis. Headache, a common symptom of CNS infection, can be a feature of HAND, or indeed a prodrome to it, but is usually less severe than in CNS infection. The focal features of presentation with tuberculosis and toxoplasmosis are also out of keeping with HAND, which is a nonfocal disorder (Brew 2001). However, there are two infectious diseases in the HIV context that warrant special mention, as they have the potential, at least early on, to mimic a presentation of HAND.

Differential Diagnosis of HIV-Associated Neurocognitive Disorders, Table 1 Differential diagnosis for cognitive impairment in HIV infected patients

	Cognitive impairment	Associated signs	Patient profile	Neuropathology	Neuroimaging	
HIV-associated neurocognitive disorder (HAND)	Attention		Prior AIDS defining illness	Impaired BBB	Frontal, hippocampal, and basal ganglia atrophy	
	Working memory		Longer duration of HIV infection	Disturbed toxin removal	Symmetric white matter lesions	
	Executive function		Low CD4+ count	Impaired autophagy	Distinct patterns on MRS, SPECT, and PET scans	
	Processing speed			Disturbed ubiquitin-proteasome complex		
Asymptomatic neurocognitive impairment (ANI)	Asymptomatic or symptomatic impairment defined by $\geq -1 \sigma$ from the mean in two cognitive areas as above	Depressive symptoms				
		Insomnia				
Mild neurocognitive disorder (MND)		Lethargy				
		Apathy				
HIV-associated dementia (HAD)	Severely affected impairment defined by $\geq -2 \sigma$ from the mean in two cognitive areas as above	Unsteady gait				
		Tremor				
Ageing	Processing speed	Impaired saccadic eye movements				
		Bradykinesia				
	Working memory	Hyperreflexia				
		Frontal signs				
	Memory retrieval	Psychosis or mania		Age > 70	Decreased myelination	Frontal and hippocampal atrophy
					Impaired BBB	
					Disturbed toxin removal	
Alzheimer's disease	Declarative memory		Age > 70	Impaired autophagy	Frontal and hippocampal atrophy (beyond that seen in ageing)	
				Amyloid (A β 42) plaques		White matter lesions
	Language		APOE4 status	Neurofibrillary tangles	Distinct patterns on fMRI, SPECT, FDG-PET	
	Visuospatial skills		Family history			Amyloid PET tracer uptake increased

Vascular cognitive impairment and dementia	Executive function	Stepwise progression	Hypertension Diabetes Metabolic syndrome Smoking IVDU Coinfection HCV End-organ damage of long-term substance abuse	Infarcted brain tissue	Large vessel cortical and subcortical infarction
	Psychomotor retardation	Cortical Syndromes Gait disturbance (falls) Urinary symptoms			Small vessel ischemia and lacunar infarction White matter lesions
	Generalized impairment Working memory (esp. alcohol) Attention				Ventricular and sulcal enlargement (esp. alcohol)
Substance abuse	Concentration	Depressive symptoms			Normal
	Memory	Sadness			
	Executive Function	Anxiety Insomnia Mania Psychosis Self harm			
CNS opportunistic infections	Generalized impairment	Progression to confusion and stupor	Low CD4+ count (<200/μL)	Meningitis or encephalitis	<i>Numerous depending on specific infection</i>
		Fever	No prophylactic antimicrobials	Demyelination (PML)	
		Meningism	IRIS	Abscess	
		Focal neurological deficits		SOL	
		Seizures			

AIDS acquired immunodeficiency syndrome, *HIV* human immunodeficiency virus, *BBB* blood-brain barrier, *MRS* magnetic resonance spectroscopy, *SPECT* single-photon emission computed tomography, *PET* positron emission tomography, *FDG-PET* fludeoxyglucose-PET, σ standard deviation, *fMRI* functional magnetic resonance imaging, *IVDU* intravenous drug user, *HCV* hepatitis C virus, *CNS* central nervous system, *IRIS* immune reconstitution inflammatory syndrome, *PML* progressive multifocal leukoencephalopathy, *SOL* space-occupying lesion

Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is a subacute demyelinating condition resulting from the infection of oligodendrocytes and astrocytes by the polyomavirus JC. It was relatively common before the advent of HAART in patients with depressed CD4+ counts. PML onset is gradually progressive over several weeks and develops in the absence of fever. Classic PML presents with cortical signs, hemiparesis, speech disturbances, headache, ataxia, and cognitive dysfunction; however, atypical forms are increasingly being recognized (Koralnik 2006). CSF examination for JC virus DNA by polymerase chain reaction (PCR) is the usual method of diagnosis, but its sensitivity is significantly diminished in HAART-treated patients. MRI features of PML occasionally can be similar to those seen in HAND and include bilateral non-enhancing subcortically localized white matter changes. More typically, PML lesions tend to be more asymmetric and more demarcated than those seen in HAND (Brew 2001). PML may also occur in the context of rapid immune reconstitution after the commencement of HAART. In this immune reconstitution inflammatory syndrome (IRIS), enhancement of demyelinating plaques can be seen on neuroimaging (Gheuens et al. 2013).

Cytomegalovirus Encephalitis

Cytomegalovirus (CMV) is a herpesvirus that can cause an encephalitis most commonly in immunosuppression related to HIV infection. The mean CD4+ count of patients with CMV encephalitis is 13 cells/ μ L. The clinical features include fluctuating confusion and apathy, with evidence of poor memory, headache, and ataxia, a not dissimilar picture to the more severe forms of HAND. Symptoms develop over an average of 3.5 weeks but with a range up to 3 months (Brew 2001). Some patients however have evidence on examination of brainstem involvement with nystagmus, ophthalmoplegia, and cranial neuropathies that do not occur in HAND. It is common also for patients with CMV encephalitis to have or have had CMV infection elsewhere,

usually in the retina (Brew 2001). MRI can show ventriculoencephalitis with periventricular enhancement and increased signal on T-2-weighted images or diffuse multifocal scattered micronodules. CSF PCR for CMV DNA is the usual method of diagnosis, keeping in mind that it may be positive in the absence of symptoms.

Conclusion

Cognitive dysfunction in HIV-infected patients is common and has a variety of causes. HIV not only directly infects brain tissue, it also increasingly appears able to overlap with and possibly mimic some neurodegenerative processes. It contributes to vascular risk factors as well as causing the immunosuppression that leads to opportunistic infections. As outlined above, the cognitive impairment of HAND differs from other causes of cognitive impairment, but at times the clinical distinction can be challenging. The physician confronted with such patients needs to work methodically through all potential causes, utilizing discriminating tests where possible while understanding at all times the likelihood and potential additive effects of two or more pathologies occurring concurrently.

Cross-References

- ▶ [Comorbidity: Progressive Multifocal Leukoencephalopathy](#)
- ▶ [HAND Adjunctive Therapies: Reversing Neuronal Injury](#)
- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [HIV-2 Neurological Manifestations](#)
- ▶ [Medication Adherence and HIV-Associated Neurocognitive Disorders \(HAND\)](#)
- ▶ [Neurocognitive Functioning in HIV-infected Substance Users](#)
- ▶ [Neuroinflammation and HAND: Therapeutic Targeting](#)
- ▶ [Overview of HIV CNS Infection](#)

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Diffuse Large B-Cell Lymphoma

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Definition

Diffuse large B-cell lymphoma (DLBCL) is an AIDS-defining, aggressive lymphoid neoplasm in which normal lymph node architecture is replaced by sheets of large, atypical lymphoid cells. It is thought to arise from mature B cells and is comprised of large, noncleaved centroblasts and immunoblasts with abundant cytoplasm and prominent nucleoli. As the most common form of non-Hodgkin lymphoma (NHL) in both HIV-positive and HIV-negative individuals, there is an increasing appreciation of its morphologic, genetic, and biologic heterogeneity.

Epidemiology and Risk Factors

DLBCL accounts for roughly 30% of all NHLs in the HIV-negative population. By comparison, up to 75% of systemic NHL subtypes in HIV-seropositive individuals are comprised of DLBCL or variants thereof (Cote et al. 1997). As a whole, standardized incident ratios for NHL in HIV-positive individuals have decreased over the last two decades from over 1,000 in the early 1990s to below 200 in the mid-2000s due to the advent and widespread use of combination antiretroviral therapy or cART (Patel et al. 2008). Primary central nervous system (PCNS) lymphomas account for approximately 15% of AIDS-related lymphomas (ARL) and are morphologically classified as DLBCL; however, given recent insights into the tumor micro-environment and unique biology of this disease, PCNS lymphoma likely represents a distinct ARL entity and will not be further addressed in this section.

Risk factors for DLBCL and ARLs in general are linked to the degree of host immune-suppression and viral activity. In the pre-cART era, a CD4 count less than 50 and plasma HIV RNA levels greater than 100,000 were strongly linked to the development of ARL; however, in the era of cART, most patients with newly diagnosed ARLs will have CD4 counts greater than 200 (Kaplan et al. 2012).

Pathogenesis

The mechanisms by which HIV exerts its oncogenic effects in human lymphoid tissue are complex and not yet fully elucidated. While B cells are not considered a direct target of HIV, it has been proposed that immune dysregulation as a consequence of HIV viremia results in aberrant somatic hypermutation and loss of T-cell immunity against pathogenic viruses such as Epstein-Barr virus (EBV) or human herpesvirus 8 (HHV8, also known as Kaposi's sarcoma-associated herpesvirus or KSHV) (Kaplan et al. 2012). The loss of regulation leads to hypergammaglobulinemia and polyclonal B-cell hyperplasia in milieu of constant antigenic stimulation. In addition, myeloid dendritic cells (mDC) play a pivotal role in propagating HIV-related B-cell disease states via increased expression of B lymphocyte stimulator (BLyS) surface expression, which is in turn correlated with disease progression (Fontaine et al. 2011).

Presentation, Diagnosis, and Staging

Patients with ARLs typically present with advanced-stage disease, constitutional symptoms, marrow involvement, and extranodal distribution in unusual sites such as the gingiva, rectum, and biliary tree. DLBCL in particular involves the gastrointestinal tract in many cases, with patients reporting abdominal pain, bleeding, diarrhea, or severe nausea.

Definitive diagnosis requires tissue biopsy with sufficient material to correlate morphologic, immunohistochemical, and immunophenotypic characteristics. Excisional biopsy is the preferred

method for obtaining tissue for subtype classification. Core needle biopsy may be utilized for sampling otherwise inaccessible or high-risk areas, although fine-needle aspiration should be avoided due to the potential for sampling error and lack of cytological architecture. Biopsy material should be processed for standard B-cell immunohistochemical and flow cytometric markers in addition to a comprehensive morphologic assessment by an experienced hematopathologist. Evaluation of the proliferative index using Ki-67 or MIB-1 staining helps to distinguish aggressive from very aggressive subtypes, which in turn may influence the choice of chemotherapeutic regimens. In addition to adequate peripheral tissue sampling, unilateral bone marrow aspirate and core biopsy should be obtained to evaluate for lymphomatous involvement. Central nervous system (CNS) evaluation is not routinely indicated unless there are greater than two areas of extranodal involvement in the context of elevated LDH, marrow involvement, epidural disease, paranasal sinus disease, or testicular involvement.

Along with pretreatment assessment of renal and hepatic function, serum lactate dehydrogenase (LDH), phosphorus, and calcium should be obtained to evaluate potential tumor lysis, especially in the presence of bulky or rapidly progressive disease. If not already known, hepatitis B serologies should be obtained, and in patients with a positive core antibody, positive surface antigen, or circulating levels of hepatitis B DNA, appropriate antiviral therapy should be initiated due to the risk of reactivation or exacerbation with rituximab-containing regimens.

Contrast computed tomography (CT) of the chest, abdomen, and pelvis is recommended as a baseline assessment of disease distribution as per the Cotswold-modified Ann Arbor staging system. In contrast to the HIV-negative population, interpretation of positron emission tomography with [18F] fluorodeoxyglucose (FDG-PET or PET hereafter) in HIV-positive individuals can be confounded by persistent generalized lymphadenopathy related to viral load and opportunistic infections secondary to immune suppression. As such, its role in staging, interim scanning, and

risk-adapted therapy for HIV-related DLBCL remains an area of active investigation. However, end-of-treatment response assessment carries a similarly high negative predictive value (NPV) as seen in the HIV-negative population, suggesting a possible role for response assessment in individuals with limited disease without suspected coinfection (Hentrich et al. 2011).

Prognosis

The previously poor prognosis of patients with HIV-associated DLBCL in the pre-cART era stemmed from a variety of factors including the presence of concomitant opportunistic infections, aggressive and advanced-stage disease at presentation, and toxicities associated with the use of multiagent chemotherapy in a severely immunocompromised population. By contrast, prognosis in the era of cART has improved to the point that outcomes now approach those of the HIV-negative population. In one study relatively early in the cART era, improvement was limited to individuals demonstrating a virological response to cART (Antinori et al. 2001), highlighting the importance of a functional or recovering immune system through the course of therapy. This improved immunocompetency of HIV-positive patients has resulted in lymphoma-specific factors playing a more important role in predicting clinical outcome than was previously the case. The International Prognostic Index (IPI) is a well-validated model incorporating disease stage, patient age, presence of extranodal involvement in two or greater areas, performance status, and serum LDH into a prognostic index with high discriminatory power in HIV-negative individuals. Several studies have examined its utility in ARLs and HIV-associated DLBCL specifically, demonstrating a similar ability to risk-stratify patients at diagnosis. The lowest-risk individuals with either none or one of the aforementioned risk factors appear to maintain an overall survival approaching 70% at 5 years, nearly identical to the comparable HIV-negative population (Lim et al. 2005; Bower et al. 2005). In addition,

multivariate analyses have shown a CD4-positive lymphocyte count less than 100 as being associated with significantly worse outcomes, though it is unclear if this has retained prognostic significance in the era of cART.

The use of gene expression profiling (GEP) has identified distinct cell of origin (COO) signatures in DLBCL. Lymphomas with a post-germinal center (non-GC), also called activated B cell (ABC), subtype are associated with a markedly worse prognosis compared to lymphomas with a germinal center (GC) signature in HIV-negative lymphomas. The data in HIV-associated DLBCL are less clear. Retrospective data from two trials of the US AIDS Malignancy Consortium (AMC) suggest no difference in lymphoma-specific nor overall survival. Conversely, a planned subset analysis of patients with HIV-associated DLBCL receiving dose-dense rituximab and the infusional regimen EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) at the National Cancer Institute (NCI) reported a substantial difference in PFS between GC and non-GC subtypes (95% vs. 44%).

The true prognostic relevance of COO in HIV-related DLBCL notwithstanding routine incorporation of GEP into clinical decision making remains a work in progress due to the expense and limited availability of testing outside academic centers. Attempts at correlating GEP with immunohistochemistry have been complicated by a lack of reproducibility or standardization of diagnostic algorithms. Despite these challenges, however, the unique biology and disparate prognosis of non-GC subtypes in the HIV-negative population have spurred investigation into targeted agents and novel multiagent regimens. For example, constitutive activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) in these tumors is a potential target for proteasome inhibition, and this is supported by preliminary studies indicating increased activity of the drug bortezomib in relapsed non-GC DLBCL compared with GC DLBCL in HIV-negative patients. Recently, inhibitors of Bruton's tyrosine kinase (BTK) have been shown to induce responses in relapsed HIV-negative non-GC DLBCL and are currently undergoing further testing in clinical trials.

Clinical Management and Initial Treatment

Early attempts at treatment of ARL in general were limited by the baseline immunodeficiency and cytopenias commonly found in HIV-positive individuals at presentation. Administration of cytotoxic therapy compounded these deficiencies, thereby increasing the risk of opportunistic infections that further compromised delivery of adequate treatment. Through the bolstering immunologic effects of cART and improved supportive care, clinicians are now able to administer even high-dose therapy with a manageable side effect profile.

The standard-of-care treatment for DLBCL in HIV-negative individuals combines the anti-CD20 monoclonal antibody rituximab (R) with a multiagent chemotherapy regimen including cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) administered every 21 days for six to eight cycles. This regimen has produced five-year OS rates of approximately 60% in randomized phase III trials, averaged over all risk groups. Historically, CHOP and CHOP-like regimens had not achieved anywhere near this degree of success in HIV-positive individuals treated in the pre-cART era, with two-year OS no better than 20% (Kaplan et al. 2012). Given these dismal outcomes and concern for hypogammaglobulinemia in already immunocompromised hosts, prospective trials sought to evaluate the role of rituximab in combination with chemotherapy in the HIV-positive population.

A prospective multicenter German trial evaluated 72 patients, risk-stratified by World Health Organization (WHO) performance status >3 , CD4 count less than 50, and prior opportunistic infection. The 48 patients with zero or one risk factor were considered "standard risk" and achieved a CR rate of 79% with median survival not reached at 47 months' follow-up. This was in contrast to the 29% CR rate obtained in the 24 "high-risk" patients with 2 or more risk factors. A phase II trial of 61 patients with one or no risk factors including CD4 < 100 , prior AIDS, or Eastern Cooperative Oncology Group (ECOG) performance score >2 produced two-year OS rates of 75%.

The AMC conducted the only randomized, phase III prospective trial comparing standard dose CHOP with R-CHOP in patients with HIV-related DLBCL. One hundred fifty patients were administered CHOP or R-CHOP in a 2:1 fashion while receiving cART, growth factor support, and prophylaxis against *Pneumocystis jiroveci*. Patients randomized to rituximab had a statistically nonsignificant improvement in CR rate from 47% to 58% and a median OS of 2.6 years compared to 2.1 years in the CHOP cohort. However, 14% of the patients in the R-CHOP group suffered infection-related deaths compared to two percent in the CHOP group. While majority of these deaths occurred in patients with a CD4 count less than 50/ μ l, and neutropenic antibiotic prophylaxis was not administered in the study, these findings initially raised concerns over the use of rituximab in HIV-positive individuals with severe immunodeficiency.

Subsequent studies evaluating the combination of rituximab and infusional chemotherapy firmly established rituximab as not only safe given the proper supportive care but also highly effective in the treatment of HIV-related DLBCL. The infusional regimen EPOCH was developed intramurally at the National Cancer Institute on the basis of in vitro studies suggesting greater tumor kill as a result of continuous – rather than bolus or episodic – exposure to multiagent cytotoxic therapy. Importantly, this regimen adapted a pharmacodynamic approach to dosing, with subsequent dose increases or reductions based on nadir CD4 counts in preceding cycles. A modified, dose-adjusted (da) EPOCH regimen was employed in another multicenter AMC phase II trial whereby patients were randomized to receive rituximab either concurrently with chemotherapy or sequentially after each cycle. Although the study was not designed to evaluate overall survival, 73% of patients receiving rituximab concurrently achieved a CR, compared with 55% in the sequential arm. Deaths related to sepsis were uncommon due to the routine use of neutropenic antibiotic prophylaxis with fluoroquinolones.

A phase II intramural NCI trial evaluating daEPOCH with dose-dense rituximab delivered one cycle of chemotherapy beyond

documentation of complete response for a minimum of three cycles. The five-year PFS and OS were 84% and 68%, respectively, and there were no treatment-related deaths. As stated above, along with CD4 count, cell of origin was an independent predictor of outcome.

In summary, several trials have demonstrated both efficacy and safety of this infusional approach. An ongoing phase III, multicenter randomized controlled trial comparing R-CHOP with daEPOCH-R for the treatment of DLBCL in HIV-negative patients may further inform the choice of treatment for DLBCL in HIV-positive individuals.

Treatment of Relapsed/Refractory Disease

As is the case for HIV-negative individuals, the goal of treatment for relapsed/refractory HIV-associated DLBCL is to achieve a response sufficient enough to proceed with high-dose chemotherapy followed by autologous stem cell rescue (ASCT). The PARMA study was the first multicenter randomized trial to establish the superior event-free and overall survival benefit of this approach for chemosensitive relapsed/refractory DLBCL in HIV-negative patients. Though such an approach would have been impractical in the pre-cART era, it is now considered the standard of care for fit HIV-positive patients with chemosensitive disease at relapse.

The largest prospective trial evaluating the efficacy of ASCT in HIV-positive lymphomas looked at a total of 50 patients, 22 of whom had DLBCL. Thirteen of the 22 patients received transplant, with 11 of those (42%) in continuous CR at the data cutoff time point. For the DLBCL subset, the overall and progression-free survival post-transplant was 81.5% and 83%, respectively, at 44 months (Re et al. 2009). Importantly, there were no treatment-related deaths, and median time to neutrophil and platelet engraftment was 10 and 12 days, respectively, similar to that observed in the HIV-negative population. Although somewhat smaller in sample size, other prospective and retrospective studies have

confirmed the efficacy and safety of ASCT in HIV-associated, relapsed/refractory DLBCL with chemosensitive disease. Predictably, long-term survival seen in these studies was largely confined to patients with well-controlled viral loads. As this is a population with significant pretransplant treatment exposure, CD4 counts are generally low prior to ASCT and therefore not a reliable index for prediction of outcomes or toxicities.

The role of allogeneic stem cell transplantation remains an area of active investigation. An ongoing multicenter trial conducted by the US Bone Marrow Transplant Clinical Trials Network and the AMC is intended to provide the first prospective data on efficacy and transplant-related toxicities; however, until completion of this study with sufficient follow-up, it is recommended that allogeneic transplantation for HIV-positive patients occur within the context of a clinical trial at an academic institution.

Management of cART and Supportive Care

The optimal point at which cART should be incorporated during treatment has been evaluated in several studies but remains an unresolved issue. Given the previously discussed importance of sustained virological suppression on outcomes, the prospect of uncontrolled HIV replication during cytotoxic and lymphocyte-depleting therapy argues for the continuation of cART during treatment. Conversely, potential pharmacokinetic and pharmacodynamic interactions may lead to lower effective chemotherapeutic drug exposure or, importantly, increased toxicities. In particular, the CYP 3A inhibition associated with protease inhibitors (PIs) has been associated with increased myelosuppression without an increased rate of infectious complications. As shown by the NCI EPOCH study, high response rates can be seen without the concomitant use of cART. At the same time, concerns over cART noncompliance – and therefore suboptimal viral control and subsequent emergence of viral resistance – due to nausea or vomiting are increasingly unfounded given the highly effective antiemetics and supportive care

currently available. If there are concerns over excess toxicity or specific drug interactions, consideration may be given to raltegravir-based regimens, which are less frequently associated with these potential interactions. Ultimately, in the absence of prospective, randomized controlled data, the decision to incorporate cART during chemotherapy must be made on a case-by-case basis, taking into account the above considerations and individual risk/benefit ratio. However, in general, most physicians with expertise in this field now feel that it is preferable to continue antiretroviral therapy through the course of chemotherapy when possible. Should toxicities necessitate discontinuation of cART, it is best to continue to hold these agents until after the completion of all chemotherapy.

Use of pegylated granulocyte colony-stimulating factor (G-CSF) ameliorates chemotherapy-induced neutropenia, and infectious complications are minimized by the use of prophylactic fluoroquinolone antibiotics and azoles during periods of protracted neutropenia. All patients should receive *Pneumocystis jiroveci* prophylaxis (e.g., dapsone, inhaled pentamidine, atovaquone) regardless of initial CD4⁺ cell count. Caution should be exercised when using trimethoprim-sulfamethoxazole given its potential to exacerbate myelosuppression with concurrent chemotherapy.

Conclusion

The prognosis and treatment of DLBCL in HIV-positive individuals has improved dramatically in the era of cART. As a result of improved immunocompetency secondary to antiviral therapy, supportive care, and evolving treatment strategies, remission rates for individuals with at least certain types of DLBCL rival those of the HIV-negative population, and patients with good-risk features can reasonably expect to be cured of their disease. Results of ongoing trials examining the role of gene expression profiling, allogeneic stem cell transplantation, and novel targeted therapies will clarify the optimal strategy for treatment of relapsed or otherwise poor-prognosis disease.

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E

Eastern Europe and Central Asia, Specific Characteristics of HIV/AIDS Epidemic

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Introduction

While there is increasing evidence that HIV incidence is in decline among general populations worldwide, Eastern Europe and Central Asia are notable exceptions. There, HIV infections have increased 13% since 2006 (World AIDS Day Report 2012).

Several factors contribute to these rising rates. First, the political transition in the early 1990s led to dramatic economic dislocations as well as an expansion of criminal economies. Second, a highly structured public health system rooted in the Soviet tradition has been unable to effectively transition to meet post-Soviet challenges. These changes resulted in a dramatic increase in

injection drug use (IDU), associated with an increase in opiate smuggling from the Central Asian state of Afghanistan.

Initially the HIV epidemic in the region was driven by people who inject drugs (PWIDs). However, while risk factor profiles vary substantially between countries of the region (Gouws and Cuchi 2012), surveillance data suggest heterosexual transmission has now become the leading mode of HIV transmission in this region (World AIDS Day Report 2012).

Nonetheless, substance use remains an important driver of new infections. Implementation of known interventions to reduce transmission in IDUs has been impeded by challenges in scaling up opioid substitution therapy (OST) as well as other harm reduction efforts such as needle exchange. The use of OST is illegal in Russia and access to these services is limited in other countries. Antiretroviral therapy in the region became widely (but not universally) available after 2002. However, as noted below, access to therapy may be reduced in the setting of substance use. Complicating control of HIV has been the emergence of different non-injection drugs such as stimulants. These can lead to an elevated rate of HIV transmission due to their association with high-risk sexual behaviors (Platt et al. 2013).

The epidemic varies between Eastern Europe and Central Asia. For the purposes of this chapter, Eastern Europe is defined as the countries of

Belarus, Estonia, Latvia, Lithuania, Moldova, Russia, and Ukraine, and Central Asia represents the countries of Kazakhstan, Kyrgyzstan, Uzbekistan, Tajikistan, and Turkmenistan.

Eastern Europe

Epidemiology

The rate of diagnosed cases of HIV infection per 100,000 population in Eastern Europe has increased from 11.7 in 2004 to 22.3 in 2014 (ECDC and WHO Regional Office for Europe 2014). The highest rates of new HIV infections are in Russia, Ukraine, and Estonia. Notably, the epidemic continues to rise in the most populous countries of the region. For example, in 2012, Russia reported a 12% increase in the number of new HIV cases. In 2014, AIDS diagnoses per 100 000 population: 23 in Ukraine, 8.5 Latvia, 6.6 Georgia, 5.7 Moldova and Armenia, 5.0 Belarus, and 1.4 Estonia (ECDC and WHO Regional Office for Europe 2014- ref: Table 15).

HIV Prevention and Care

Needle and syringe programs (NSPs) are present in nearly all countries in Eastern Europe. However, coverage of NSPs is variable across and generally low, largely because of low levels of needle-syringe provision by NSPs in Russia, although in Russia and in other countries PWIDs can purchase needles in pharmacies. High NSP coverage is present in Estonia and Lithuania (Uusküla et al. 2011).

Most countries have implemented OST programs (with Russia a notable exception); however, overall, the scale of programs was very limited with one person receiving OST for every 100 PWIDs (Mathers et al. 2010). One of the success stories has been the launching opioid substitution therapy in Ukraine in 2004 (initially limited to the buprenorphine use and methadone since 2007). The number of people in Ukraine receiving OST has increased threefold from 2009 to mid-2012. However, even given its success there is less than 3% OST coverage among PWID in Ukraine (Bojko et al. 2015).

HIV Care and Treatment (ART)

Limited data is available on the characteristics of people accessing HIV care in Eastern European countries. The most common HIV-associated opportunistic infections diagnosed in 2011 were pulmonary tuberculosis (36%), wasting syndrome (25%), and esophageal candidiasis (23%). The status of TB in the setting of HIV is remarkable for the region. In 2011, tuberculosis notification rates per 100,000 ranged from 25.4 in Estonia to 150.7 in Moldova, and the estimated proportion of new cases of TB (including relapses) who were HIV positive varied from 0.9% in Lithuania to 20% in Ukraine (ECDC and WHO Regional Office for Europe 2013).

Additionally, access to care for patients with TB/HIV patients is worse compared to Western Europe. Initial TB therapy in Eastern Europe is suboptimal, with less than two-thirds of patients receiving at least three active drugs (Efsen et al. 2014). Highest rates of resistance to at least one anti-TB drug was reported in Estonia, Latvia, and Lithuania (34–41%). Multidrug resistant (MDR) TB was also most frequently observed in these same countries, occurring in 11–23% of all new cases and 29–58% among previously treated cases (ECDC and WHO Regional Office for Europe 2013). Mortality is high among HIV-infected patients with MDR, and they were more likely to die than the other group of those with HIV disease.

A high proportion of patients present late for care. For example, it has been documented that 57.9% of those in HIV care in Eastern European sites were late presenters (defined as HIV diagnosis with a CD4 count $<350/\text{mm}^3$ or an AIDS diagnosis within 6 months of HIV diagnosis) (Mocroft et al. 2013; ECDC and WHO Regional Office for Europe 2014- ref: Table 14). Officially, HIV care and ART is universally available for those in need in the region. However, studies have revealed multiple barriers to obtaining HAART. These barriers include a labyrinthine bureaucracy controlling access to ART, limitations created by an expectation that access to ART was conditional on treated drug use in a setting of limited drug treatment opportunity, and the lack of integration across HIV,

tuberculosis, and drug treatment programs (Pecoraro et al. 2014).

An additional significant coinfection given the burden of IDU is hepatitis C (HCV). This region has high rates of HIV/HCV coinfection – currently up to 80% among people living with HIV and seeking treatment in Estonia and Ukraine and over 90% in Russia (Central and Eastern European Harm Reduction Network 2007). Access to pegylated interferon-based therapies remains a challenge given the high cost of a 48-week treatment course which exceeds 14,000 USD with even lower access to new, oral direct-acting agents.

In addition to the structural level barriers, stigma toward HIV-infected people operating at multiple levels has been documented. In Russia, many people living with HIV (PLH) had been refused general health care, and negative attitude toward treating HIV-positive patients has been noted among medical students. These barriers may to some degree explain the high proportion of late presentations for HIV care and the low acceptance of ARV. ART has relatively low penetration in Eastern Europe. The percentage of adults (aged 15+) living with HIV accessing antiretroviral therapy in Eastern Europe and central Asia is only approximately 20% [19–22%] (UNAIDS 2016).

Special Populations

There is little data defining HIV risk among female sex workers in the region. The highest rates of HIV prevalence among female CSWs is in Moldova. Not surprisingly, IDU has been associated with HIV seropositivity in many CSW studies.

High rates of HIV disease have also been documented in prisons. Specifically, studies have found rates higher than 10% in Estonia and Ukraine (Dolan et al. 2007). A recent review suggested that regionally, the prevalence of HIV in prisons was approximately 4% (95% CI 1.4–8.0). Little information is available on the implementation and efficacy of HIV prevention programs within the correctional institutions in the region.

Eastern Europe has been experiencing increasing rates of both internal and external migration. Typically, this is due to economic, political, or

social factors. Low socioeconomic status, lack of access to services, separation from family, and limited risk awareness all contribute to migrants' HIV vulnerability.

Central Asia

Epidemiology of HIV

As with Eastern Europe, HIV rates are rising in Central Asia (CA). As with Eastern Europe, new HIV infections in Central Asia (CA) have risen primarily among people who inject drugs (PWIDs), female sex partners of PWID, men who have sex with men (MSM), female sex workers (FSWs), and migrant workers. While it is estimated that 1% of adults inject drugs in CA, the number exceeds 10% in areas along major drug trafficking routes, representing one of the highest rates of injection drug use in the world. As a result, high rates of HIV infection have been noted in various seroprevalence studies. Regional studies have shown that HIV prevalence among PWID varies from 0% to over 13% in different regions in Kazakhstan. Similar variation has been shown in Kyrgyzstan, Tajikistan, and Uzbekistan. Supporting this, additional data showed that 33% of Uzbekistan's cumulative registered cases and 72% of those in Kazakhstan were transmitted through the use of shared injection equipment (Thorne et al. 2010).

In the past 3 years, there has also been a steady rise in the incidence of sexual transmission of HIV as PWID in CA have infected their sexual partners (Boltaev et al. 2013). For example, data from Kazakhstan in 2011 showed heterosexual activity as the primary mode of transmission, which represented 50.7% of new cases surpassing IDU. The rise in sexual HIV transmission, particularly to the sexual partners of PWID, has been reported in each of the Central Asian countries except Turkmenistan. Another key population affected by HIV in CA is MSM. National reports estimate HIV prevalence among MSM to range from 1% to 2% in Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan in 2011 and as high as 6.8% in Tashkent, Uzbekistan.

HCV

While robust data on the magnitude of HIV/HCV coinfection are lacking, HCV prevalence is high in PWID, as noted above. Given the known potential for HIV transmission through sharing contaminated injection equipment, studies suggest that the majority of PWID infected with HIV will also be coinfecting with HCV. In a recent study with 364 couples (728 individuals) from Almaty, Kazakhstan, where at least one member of the dyad reported recent injection drug use, 90.2% of PWID were HCV positive (El-Bassel et al. 2013).

Prisoners have high rates of injection drug use and blood-borne infections. In Kyrgyzstan, 38% of inmates in one study test are positive for HCV, while in Kazakhstan, 91% of HIV-infected prisoners are HCV positive (Larney et al. 2013).

TB

Tuberculosis incidence rates in Kazakhstan, Uzbekistan, Tajikistan, and Kyrgyzstan are extremely high, and the percentage of both new cases and previously treated cases of TB that demonstrate multidrug resistance in Kazakhstan, Uzbekistan, Tajikistan, and Kyrgyzstan are among the highest in the world (Schluger et al. 2013). According to WHO, the percentage of TB cases with HIV coinfection is 2% in Kazakhstan and Tajikistan and 3% in Uzbekistan (WHO 2012). This situation requires substantial effort and improvement in diagnosis and treatment to control CA's staggering TB epidemic, especially that of MDR-TB. Furthermore, there is limited attention to adherence to TB treatment protocols for people who use drugs. Effective programs have been created in a number of regions around the world, and these models should be adopted in CA.

HIV Testing and Counseling

The WHO European Action Plan for 2012–2015 released in 2011 calls for reducing the number of undiagnosed people by increasing early uptake of HIV testing and counseling services, especially those most at risk (WHO 2011). Despite notable progress in scaling up HIV testing in CA, the number of key populations (such as PWID, MSM, and FSWs who inject drugs) who have been tested remains well below WHO's recommended

coverage of more than 90%. Coverage of PWID with recent testing has increased in Kazakhstan and in Tajikistan from 2009 through 2011; however, these numbers have declined in Kyrgyzstan and Uzbekistan. Highlighting some of the challenges, a study in Kazakhstan with 580 PWID found that 25% of the study sample had never been tested for HIV (El-Bassel et al. 2013). Nonetheless, coverage of PWID with a recent HIV test is higher than coverage among MSM with an HIV test in most countries of the region. There is unquestionably an urgent need to increase HIV testing among these key populations in CA.

HIV Treatment and Care

Currently, the Ministries of Health in CA countries recommend ART for HIV-infected individuals with CD4+ count <350 or WHO clinical stage III/IV which is analogous with the 2010 WHO guidelines. As of January 2013, the proportion of estimated ART-eligible PWID on treatment among those who were enrolled in HIV care in Kazakhstan, Kyrgyzstan, and Tajikistan ranged between 51% and 88% (Kazakhstan RAC 2012; Kyrgyzstan RNC 2013; Tajikistan RAC 2011). However, these statistics must be considered with caution because a large number of PWID are not enrolled in care, and ART coverage may actually be much lower. Solid data on ART among other key populations is limited, and access to ART treatment is limited by multiple barriers.

Conclusion

The region has a growing HIV epidemic in the world occurring in a setting of multiple gaps of translation of evidence into policy and programs. To meet the needs of key populations especially PWID, their sexual partners, MSMs, sex workers, and their social networks, it is critical to establish comprehensive programs and measure their impact. Additionally, it is critical to identify and address structural barriers in order to enhance access to needed services. This process must involve overcoming all the barriers noted above, as well as addressing discriminatory practices and laws and

transforming the health system through increased awareness that health is indeed a human right.

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Elderly: Epidemiology of HIV/AIDS

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Definition

The introduction of highly active antiretroviral therapy in the mid-1990s, along with new infections in adults over the age of 50, has resulted in increasing numbers of adults over 50 living with

HIV and surviving into the later stages of life. These adults face a new set of challenges and threats to their health from cardiovascular disease and cancer as well as neurocognitive disease, bone disease, and frailty. In this chapter we describe the epidemiology of common chronic illnesses in the aging HIV population, as well as their relation to the HIV virus and traditional risk factors. Means to prevent, predict, and treat these conditions in the HIV-positive population are an area of ongoing study by HIV clinicians and researchers.

Introduction

Biologically, aging is a consequence of the rate at which an organism accumulates random damage and its ability to subsequently perform repairs in responding to these insults (Brothers and Rockwood 2014). The aging process is more pronounced – occurring more rapidly and at younger ages – in individuals infected with human immunodeficiency virus (HIV). Accelerated aging in HIV is caused by a combination of accumulated random damage and a reduced ability to mitigate these insults. Longstanding HIV infection produces vulnerability to various biological insults, while the virus causes inflammation and immune suppression that reduces physiological response to damage.

Aging among HIV-infected individuals is also associated with growth in the availability of life-saving medications. Highly active antiretroviral therapies (HAART) that reduce viral loads and immune suppression have led to dramatic improvements in life expectancy of HIV-infected individuals, while fundamentally altering the landscape of the disease. The incidence of once-feared complications of HIV infection and AIDS, including opportunistic infections and AIDS-related cancers, is dramatically reduced in the post-HAART era. However, noncommunicable, age-associated illnesses have increased in parallel, in part a result of increased longevity and, in part, a consequence of viral and behavioral factors. As such, HIV-infected adults experience at least some

shorter lifespan, high levels of comorbid disease, and earlier age-associated functional decline than their uninfected counterparts. Despite improvements in immune function in the setting of HAART, HIV-infected individuals still have elevated inflammatory biomarkers (Interleukin-6 or IL-6), altered coagulation (D-dimer), monocyte activation (soluble CD14 or sCD14), and at least somewhat suppressed immune systems that all contribute to age-associated complications (Brothers and Rockwood 2014). These virus-related factors interact with behavioral and social factors like smoking rates more than double that of the uninfected population and high rates of diabetes, cardiovascular, and renal disease in HIV-infected persons. Long-term HAART exposure has itself been linked to metabolic, cardiovascular, and bone diseases and acts to potentiate the effects of aging (Deeks 2009).

This chapter covers the epidemiology of aging in the HIV population and explores common causes of illness and death in this group. Therapy for HIV has curbed rates of death from infection and cancers related to abnormal immune function so that diseases most affecting the elderly HIV population today mirror those affecting the HIV-negative population, for whom heart disease and cancer remain the leading causes of death. Such is the case for cardiovascular disease, bone disease, non-AIDS-related cancers, cognitive dysfunction, and frailty – each profiled here. Each, however, also presents with unique characteristics in the HIV population as a result of interactions with virologic factors, medications, and comorbid medical illnesses that often result in accelerated or altered progression of disease and higher levels of disability and death. These discrepancies pose new challenges in the care of the aging and elderly HIV patient while models for predicting disease occurrence and progression continue to be studied.

Epidemiology

Median life expectancy among HIV-infected persons in high-income countries has risen steadily

since HAART was introduced (mid-1990s) from 55 years of age in 1996 to 70 years in 2005, while the rate of new infections has remained constant in this time (Pathai et al. 2014; Rubinstein et al. 2014). Previous projections estimated that by 2015, 50% of the HIV-positive population in the United States would be over the age of 50 (Effros et al. 2008). Even in lower-income settings such as KwaZulu-Natal, South Africa – the country with the highest prevalence of HIV and highest incidence of new infections (AIDSInfo) – the overall life expectancy increased from 49.2 years in 2003 to 60.5 years in 2011 (Sabin 2013). Between 2005 and 2013, moreover, while the number of adults and children living with HIV infection worldwide grew from 32.1 million to 35 million, the number of deaths due to AIDS fell by nearly 1 million, from 2.4 million to 1.5 million (AIDSInfo 2015).

Two distinct epidemiologic phenomena are responsible for the establishment and growth of the elderly HIV-positive population. First, immune reconstitution using HAART has led to decreased rates of AIDS-related morbidity and mortality, resulting in increased survival into older age of individuals infected at a young age. Effective therapy with HAART has meant a narrowing of the gap in life expectancy between HIV-positive and HIV-negative populations and a changing epidemiology of disease and illness in the aging HIV-infected population globally. However, such gains in life expectancy have been unevenly distributed and are mediated by socio-demographic, lifestyle, and disease-related factors, including markers of severity of disease prior to initiation of HAART (Sabin 2013). In some populations, life expectancies can range from 10 to 30 years less in infected individuals than uninfected counterparts and come closest to approaching normal life expectancy among individuals who have remained on therapy for several years and successfully achieved near normal CD4+ counts (defined as $CD4 > 500$) (Deeks 2009). There is evidence of higher rates of non-AIDS conditions linked with aging and chronic inflammation, such as cardiovascular disease, cancers, bone disease and fractures, kidney and liver

failure, and cognitive dysfunction in HIV-positive individuals (Deeks 2009). The cumulative effect of this multi-organ decline is a syndrome of increased frailty, polypharmacy, and falls – all occurring at earlier ages than observed in the HIV-negative population (Deeks 2009; Pathai et al. 2014).

Second, new infections in individuals above the age of 50 continue to occur as a result of low levels of knowledge regarding HIV infection and its transmission, increased rates of sexual activity among middle- and older-age individuals, low utilization of condoms, and physiologic changes postmenopause that cumulatively increase vulnerability to infection. In the United States, 10.8% of all new HIV infections annually are in adults aged 50 and older (Althoff et al. 2014). Risk factors for infection and modes of transmission mirror those in younger age groups, with most cases transmitted by male-to-male sexual contact in men and heterosexual contact for women ([HIV among older Americans](#)). Newly infected individuals over the age of 50 are often uniquely disadvantaged, however, as they are more likely than younger individuals to be diagnosed late in the course of infection and are twice as likely to progress from HIV infection to AIDS within the first year after diagnosis. This trend is evident in data from the Centers for Disease Control and Prevention (CDC); in 2011, elderly HIV-positive Americans represented nearly a quarter of all new AIDS diagnoses, and in 2010 more than half of all deaths in HIV-infected adults ([HIV among older Americans](#)). Late diagnosis is itself a consequence of a failure to recognize and seek care for symptoms of HIV and of decreased routine HIV testing and other prevention efforts by healthcare providers.

Frailty

The changing demographic of the HIV-infected population, from young to older ages has created demand for a better understanding of aging and frailty in this group. Frailty, as a medical condition, refers to the relative difference in

vulnerability among individuals of the same age to physiological and pathological stressors (Brothers and Rockwood 2014). Some decline in function is expected with aging. However, being frail entails an increased likelihood of poor outcomes and comorbid conditions as compared to others of the same age. To be considered frail, it has been suggested that at least three of five clinical findings must be met: the person must exhibit weakness, slowness, unintentional weight loss, low energy, and low physical activity (Brothers and Rockwood 2014). Together, frailty is associated with greater risk of disability, hospitalization, and death than persons of the same age that are not frail.

Frailty is common among older HIV-infected persons. Estimates of its prevalence range from as low as 5% in women enrolled in the Women's Interagency HIV Study to as high as 19% of HIV-infected participants in South Africa (Althoff et al. 2014). A US-based cohort of men who have sex with men in the Multicenter AIDS Cohort Study (MACS) estimated the prevalence at 12% among HIV-positive men compared to 9% in their uninfected comparison group (Althoff et al. 2014). In both infected and uninfected populations, the condition is associated with lower levels of education and income, unemployment, and comorbid medical and psychiatric disease. However, additional risk factors related to HIV disease and higher levels of comorbid conditions, including diabetes, cardiovascular disease, and dyslipidemia, contribute to elevated levels and accelerated progression of frailty. HIV-positive individuals with poorly controlled diseases – defined as having low CD4+ cell counts, detectable viral loads, past AIDS diagnoses, and high-risk factors like injection drug use – appear to experience increased frailty compared to HIV-negative populations, whereas their low-risk, well-controlled HIV-positive counterparts do not (Womack et al. 2013).

Current knowledge on means to effectively prevent frailty and the decline it heralds is theoretical only, suggesting the need for further research. Clinical approaches are geared toward earlier initiation of HAART along with aggressive management of known comorbidities like the use

of statins and aspirin for secondary prevention of cardiovascular disease.

Bone Disease

Osteopenia (defined by low bone mineral density) and osteoporosis (low bone mineral density in conjunction with diminished bone strength and abnormal bone architecture) are the most prevalent metabolic complications of HIV infection, affecting as many as 50–60% and 15% – respectively – of HIV-positive adults in high-income settings (Ali et al. 2014; Bhavan et al. 2008). The increased risk of fragility fractures corresponds to significant morbidity and mortality in this population. In the United States, HIV-positive adults experienced 60% more fractures of the spine, hip, and wrist than their HIV-negative counterparts in one study period (Walker Harris and Brown 2012). Similar trends have been observed in low- and middle-income settings as well, where the development of fractures has been demonstrated at higher rates and younger ages than in comparable uninfected populations (Ali et al. 2014).

The mechanisms leading to osteopenia, osteoporosis, and fractures are multifactorial and linked to chronic immune activation by HIV infection and HAART exposure. Other common factors and mechanisms include comorbid renal disease, vitamin D deficiency, and related bone resorption, as well as typical behavioral risk factors, including high rates of smoking, alcohol consumption, and physical inactivity. In studies, osteoporosis in HIV-infected adults was most closely associated with older age, lower body mass index (BMI), longer duration since HIV diagnosis and more advanced disease (Bhavan et al. 2008). Exposure to HAART is associated with bone resorption of approximately 2–6% loss of bone mineral density (BMD) after therapy for 48–96 weeks – an effect well beyond that expected with normal aging (Walker Harris and Brown 2012).

Osteoporosis is diagnosed using a combination of dual-energy, x-ray absorptiometry (DXA)-measured BMD and clinical evidence of fragility fractures (Walker Harris and Brown 2012).

Workup in the HIV-positive patient involves searching for and treatment of secondary causes of low BMD common to this group, including vitamin D deficiency, secondary hyperparathyroidism, and BMI less than 20 kg/m² (Bhavan et al. 2008). Traditional therapy for osteoporosis includes bisphosphonates. However, the use of these agents can be limited by side effects and can be associated with increased risk of atypical femoral fractures after use for more than 5 years (Ali et al. 2014). Just as in the non-HIV population, suggestions for HIV-infected individuals are to take vitamin D and calcium supplementation and adjust the HAART regimens to reduce bone mineral effects (Ali et al. 2014; Walker Harris and Brown 2012). Lifestyle modifications recommended for the non-HIV population, including increased physical activity with weight-bearing and muscle-strengthening exercise, smoking and alcohol cessation, and measures to reduce fall risk, are recommended for HIV-infected persons as well (Walker Harris and Brown 2012).

Cardiovascular Disease

In the post-HAART era, cardiovascular diseases (CVD) have emerged as a leading cause of morbidity and mortality in HIV-infected adults, with effects most pronounced among elderly HIV-positive individuals (Bhavan et al. 2008). CVD in this population includes coronary artery disease (CAD) – the most common cause of cardiovascular death in both infected and uninfected adults – stroke, heart failure, peripheral vascular disease, and cardiomyopathy, among other less common diagnoses. Estimates of the prevalence of all CVD in this population approach 10%, with rates increasing with age (Esser et al. 2013). The full extent of the virus's role in the development of CVD is incompletely understood, but CVD in HIV is likely mediated by a combination of three pathways: (1) HIV-infected individuals are at high risk for CVD through other mechanisms related to a high propensity toward certain behaviors including tobacco and alcohol abuse; (2) HIV or HAART may increase the development of

traditional cardiovascular risk factors, including diabetes, hyperlipidemia, and obesity and lipodystrophy; and (3) HIV or HAART – via inflammation, endothelial dysfunction, or other yet undiscovered means – acts to increase cardiovascular risk independent of traditional risk factors for disease (Currier et al. 2008). Emphasis will be placed here on the development of atherosclerotic cardiovascular disease (ASCVD), as it represents the most common cardiovascular risk in elderly HIV-positive adults.

Direct comparisons of groups of HIV-positive and HIV-negative adults have revealed significant differences in the rate of development of ASCVD and resulting myocardial infarction (MI) and stroke – in this case a result of virus-mediated vascular damage or elevated rates of traditional risk factors. HIV-infected adults are 1.5–2 times as likely to suffer from an MI than HIV-negative adults of similar age and risk profile (Currier et al. 2008). Other studies have shown up to a sixfold increased rate of ischemic cardiomyopathy and CAD-associated hospitalization in the HIV population. CAD is the primary risk factor for MI, ischemic stroke, and cardiovascular death and occurs at increasing rates as we age. In HIV, the pattern of normal risk progression with age is accelerated.

In an effort to disentangle the contributions of various risk factors in the development of ASCVD and predict the risk of MI, researchers have developed risk models incorporating both traditional risk factors (diabetes, hypertension, age) and HIV-specific risk factors (protease inhibitor exposure, viral load, total years infected, and markers of inflammation) that mirror models used in the uninfected population (e.g., the Framingham Risk Equation). One of the largest of these is derived from the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study, a longitudinal cohort composed of more than 30,000 HIV-positive adults in Europe, Australia, and the United States (Friis-Moller et al. 2010). When tested against the Framingham Risk Score – the gold standard in predicting MI in the uninfected population – the D:A:D model was better able to predict the development of MI events.

In addition to risk models to predict disease, tests aimed at accurately measuring surrogates of

atherosclerotic disease as it develops (carotid intima-media thickness) and more aggressive strategies for preventing and treating ASCVD have been designed and implemented. Studies using carotid intima-media thickness (CIMT) have helped to elucidate additional mechanisms of increased stroke and MI risk in this population, including findings of relatively higher rates of unstable coronary artery plaque in HIV-positive persons compared to matched HIV-negative controls (Hulten et al. 2009). Increasingly, aggressive therapy and prevention strategies for HIV populations have been advocated including empiric therapy with statins, even in persons without elevated lipid levels. An ongoing investigation, the Randomized Study to Prevent Vascular Events in HIV (REPRIEVE) trial, will help clinicians understand the utility of such a strategy. REPRIEVE randomizes HIV-positive persons without an ASCVD history to receive either a low-dose statin or placebo (Release MGHN 2014). As the relationship between HIV and ASCVD is better characterized through studies such as REPRIEVE, clinicians' abilities to prevent and treat ASCVD in the aging HIV-positive population will be augmented.

Non-AIDS Cancer

Following the introduction of HAART, rates of AIDS-defining cancers (ADC) – including Kaposi sarcoma, primary CNS lymphoma (PCNSL), and non-Hodgkin lymphoma (NHL) – have decreased by more than 70%, while non-AIDS-defining cancers (NADC) such as lung, anal, and oropharyngeal malignancies common to the uninfected population increased in prevalence more than threefold (Rubinstein et al. 2014). In the post-HAART era, oncogenic viruses and chronic malignancies related to behavioral risk factors like smoking account for the majority of malignancies occurring in the HIV-positive population (Rubinstein et al. 2014). Smoking rates in the HIV-positive population have been estimated to be as high as 50% in the United States, compared to just 18% in the general adult population, and these individuals are subsequently at an elevated

risk of developing and dying from lung and oropharyngeal cancers, for example. High levels of alcohol consumption – as much as double the rate seen in the HIV-negative population – can also be tied to an elevated risk of NADC.

Still, the increase in NADC exceeds that expected based on age alone and remains elevated despite controlling for smoking (Rubinstein et al. 2014). Together, ADC and NADC annually account for 26–30% of deaths among HIV-infected individuals in the United States and more than 4000 new cases of cancer each year. Today, rates of ADC remain highest in resource-poor settings with low access to HAART, and NADC rates have been observed in low- and high-income settings alike.

Oncogenic viruses have long been known to contribute to ADC. This relationship is exhibited most notoriously in the link between human herpes virus 8 (HHV-8) and Kaposi sarcoma, which at its peak in the early 1990s affected nearly one in every two HIV-positive Americans (Society AC). Epstein-Barr virus was similarly implicated in HIV-associated lymphomas, including Burkitt's lymphoma, immunoblastic B-cell lymphoma, and primary central nervous system (CNS) lymphoma (Rubinstein et al. 2014). NADC too exhibit links with oncogenic viruses: human papillomavirus (HPV) has been linked to nearly all cases of anal and cervical carcinoma and many cases of head and neck carcinoma, while hepatitis B and C viruses that occur commonly in the HIV-infected population greatly increase the risk of development of hepatocellular carcinoma. Each of these viruses is believed to promote cancerous replication by disrupting normal cell cycle regulation and by inhibiting the expression of naturally present tumor suppressor genes. These changes are thought to interact with pro-oncogenic properties of HIV in creating increased susceptibility to cancerous states, though the exact mechanism of this transformation is poorly understood.

CD4+ cell counts in isolation are often ineffective indicators of malignant potential as ADCs and NADCs have been detected in patients with depleted and elevated CD4 counts alike. Patients on HAART are often candidates for the same chemotherapy offered to uninfected patients as

treatment for NADC, though attention must be paid to interactions between chemotherapeutic agents and HAART drugs.

Neurocognitive Dysfunction

Aging and HIV infection have each been shown to contribute to progressive cognitive changes manifested in dysfunctions in learning, mood, attention, memory, perception, and problem solving. Whether this decline is a result of the HIV virus's effect on the CNS, normal aging, or an interaction of the two remains unclear, however. Early epidemiologic studies prior to HAART demonstrated higher prevalence of HIV dementia in the elderly compared to HIV-positive young adults suggesting some interaction between the virus and age-related changes in the brain (Bhavan et al. 2008). These findings were supported by a decrease in rates of the most severe form of cognitive dysfunction, HIV-associated dementia (from 16% to 5%), and CNS infections after the rollout of HAART (Heaton et al. 2011). Still, estimates of the prevalence of all HIV-associated neurocognitive disease (HAND) remain as high as 50%, and neurocognitive deficits continue to be more common in HIV-positive than HIV-negative individuals often regardless of treatment status, severity of disease, or age (Gannon et al. 2011).

HAND today represents a group of syndromes differentiated by the degree of impairment and disability each produces. From least to most impairment, the syndrome includes asymptomatic neuropsychological impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) (Gannon et al. 2011). Increasingly, cases of HAND represent ANI and MND, characterized by psychomotor retardation and tremor, as opposed to the more severe HAD, which presents with a combination of neuropsychiatric abnormalities including trouble with attention, concentration, learning, memory, psychomotor skills, and processing severe enough to affect the performance of activities of daily living.

Multiple theories have emerged to try to explain neurocognitive deficits detected among

HIV-positive persons. First, HIV increases rates of cardiovascular disease via three separate mechanisms, detailed above, leading to a form of vascular dementia which may manifest as neurocognitive dysfunction. Second, chronic viral infection – even at low levels of replication – leads to smoldering inflammation, which has been demonstrated to cause glial damage in the frontal white matter of the brain leading to cognitive changes (Gannon et al. 2011). This theory was supported by a study after the advent of HAART that demonstrated twice the rate of cognitive impairment in elderly HIV-positive persons with detectable viral loads in cerebrospinal fluid (CSF) compared to those without detectable CSF viral loads (Cherner et al. 2004). Other groups have shown that insulin resistance associated with HAART may itself induce cognitive dysfunction (Bhavan et al. 2008). And while HAART has decreased rates of virus-related CNS damage, it too may have neurotoxic effects independent of the HIV virus (Gannon et al. 2011). Finally, comorbid social factors more prevalent in the HIV-positive population such as drug and alcohol abuse, low socioeconomic status, and unemployment may influence cognitive function.

The tenants of HAND treatment remain effective treatment of HIV with HAART, with emphasis on use of agents that can penetrate and lower viral loads in the CNS, along with investigation and treatment of curable causes of neurocognitive dysfunction including infections (syphilis), metabolic derangements (vitamin B12 deficiency, hypothyroidism), and comorbid psychiatric disease, common in elderly adults (Bhavan et al. 2008). Investigations of other drugs to target processes of neuronal damage and CNS inflammation, as well as strategies for the prevention of HAND, remain under study.

Health Maintenance and General Considerations for the Care of the Elderly HIV Patient

Given the changing demographics of the HIV-infected population, clinicians will begin to encounter a changing face to the virus. Improved

access to HAART and continuing levels of new infections among adults over the age of 50 contribute to an elderly HIV-positive population with unique comorbidities and treatment challenges requiring specialized care. HIV infection increases the risk for and interacts with comorbid aging-related diseases in order to cause accelerated disease progression and an increased likelihood of disability and death in the elderly HIV-positive person. HIV clinicians, while specialists, are frequently the primary care provider for the HIV-infected person and thus should be familiar with the health maintenance recommendations for this group (Aberg et al. 2014). Particular attention should be given to the use of screening tools for cardiovascular, neurocognitive, bone, and malignant disease and emphasis on risk factor modification to decrease rates of tobacco and alcohol abuse and improve early management of hyperlipidemia, diabetes, and hypertension to prevent disease progression.

Due to the high rates of comorbid medical illness in the elderly HIV patient, issues of polypharmacy, medication side effects, and interactions can pose an additional challenge to care. Physiologic differences in immune function between the elderly and young adults contribute to differing responses to HAART as well. Elderly HIV patients demonstrate more successful viral suppression than younger patients, likely a result of better medication compliance, improved access to HIV medications and fewer high-risk behaviors within the older population (Bhavan et al. 2008). Despite improved viral suppression, however, markers of immune reconstitution suggest the elderly lack robust immune activity even in the presence of an undetectable HIV viral load. Increased rates of renal and liver disease in elderly HIV-infected adults contribute to problems metabolizing drugs and exacerbate interactions between medications. Further research is needed to better understand the mechanisms behind viral suppression, age, and immune function, as well as the long-term safety and efficacy of HAART for the elderly.

Geriatric HIV care, like geriatric care for the uninfected patient, requires a nuanced approach to a clinic visit. Clinicians must be able to effectively communicate with patients, and integrating social

support systems into the broader care plan may prove beneficial (Bhavan et al. 2008). In addition to HIV-specific primary care recommendations (referenced above), the HIV clinician should incorporate standard geriatric screening for other problems such as elder abuse, financial difficulties, safe driving practices, and end-of-life preferences into the care plan of this unique population. A systematic approach to the care of HIV-positive elderly adult can be integrated into a multi-disciplinary team's daily practices to improve the care of elderly patients and their quality of life.

Conclusions

Elderly HIV-infected adults, like the uninfected elderly, face a number of health challenges as a consequence of increasing age and comorbidity. These problems are often potentiated among HIV-infected individuals by viral effects on immune suppression, chronic inflammation, and medication side effects and interactions. Cardiovascular disease and non-AIDS cancers are today among the largest contributors to disease and death in HIV-infected elderly, along with neurocognitive disease, bone disease, and frailty. Other common comorbidities, including chronic kidney disease and liver disease, are not described here but contribute to deaths from cardiovascular and other causes and further exacerbate issues of frailty. Ongoing study in the field aims to elucidate the mechanisms of increased risk of these diseases in HIV-infected adults compared to uninfected adults as well as means to predict, prevent, and treat disease. Care for the HIV elderly, meanwhile, follows the same principles as care for the uninfected geriatric adult and should aim to eliminate polypharmacy, reduce risk of falls, and intervene on preventable and reversible causes of injury and death.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Cardiovascular Complications](#)

- ▶ [Differential Diagnosis of HIV-Associated Neurocognitive Disorders](#)
- ▶ [Epidemiology of AIDS-Defining Malignancies](#)
- ▶ [Epidemiology of Non-AIDS-Defining Malignancies](#)
- ▶ [Global NeuroAIDS](#)
- ▶ [HIV-Associated Cancers](#)

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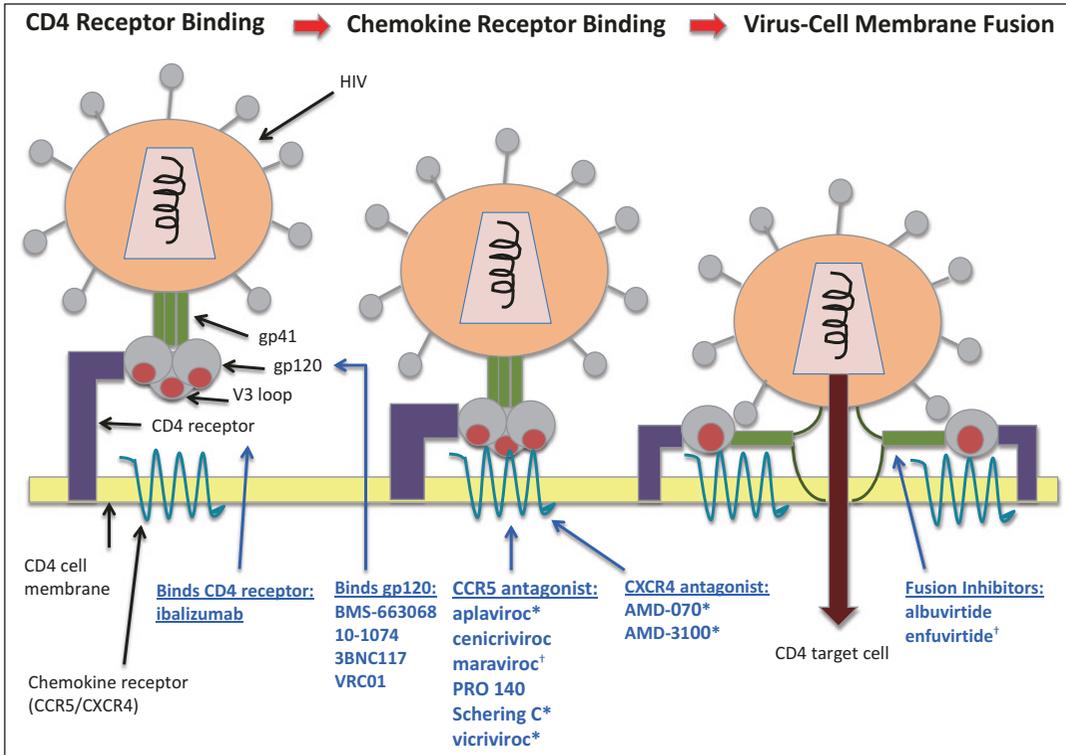
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Entry Inhibitors

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Definition

Entry inhibitors are one of the six mechanistic classes of antiretroviral drugs used for the treatment of HIV infection. HIV entry inhibitors



Entry Inhibitors, Fig. 1 Steps in HIV entry and inhibition. †US FDA approved for HIV treatment. *Development for HIV treatment discontinued

interfere with the entry of HIV into its target cell, the human CD4⁺ T-lymphocyte. By inhibiting this step in the HIV life cycle, entry inhibitors and ultimately slow the progression of HIV infection. In targeting the first step in the HIV life cycle before the virus enters the cell, entry inhibitors are distinct from other classes of antiretroviral agents for HIV infection that target HIV after it has entered and infected a CD4⁺ cell. Some HIV entry inhibitors are also distinct in targeting a host-cell receptor, rather than a viral protein.

Introduction

There are three major steps involved in HIV entry into a human CD4⁺ cell: (1) attachment to the CD4 receptor, (2) attachment to the chemokine receptor, and (3) fusion of the viral membrane

with the host-cell membrane. These steps are shown in Fig. 1. Several viral proteins are necessary for this process. The HIV surface protein gp120 binds to the CD4 receptor on the surface of a CD4⁺ cell. This leads to a conformational change in gp120, which increases its affinity for a coreceptor and exposes the HIV envelope protein gp41. HIV gp120 subsequently binds to either the CCR5 or CXCR4 coreceptors on the CD4⁺ cell. gp41 then penetrates the CD4⁺ T cell membrane, which facilitates fusion of the virus and CD4⁺ cell membranes, allowing entry of the virus into the cell. HIV entry inhibitors work by interfering with one aspect of this process. This chapter discusses HIV entry inhibitors that have been approved by the US Food and Drug Administration (FDA) as well as investigational agents that reached clinical trials. A summary of these agents is provided in Table 1.

Entry Inhibitors, Table 1 HIV entry inhibitors

Full name	Abbreviation or alternate name	Mechanism of action	Status	Adult dose for HIV treatment
albuvirtide	ABT, FB006M	Fusion inhibitor	Investigational, Phase 3	320 mg IV weekly
AMD-070	N/A	CXCR4 chemokine receptor antagonist	Development discontinued due to hepatotoxicity	N/A
AMD-3100	N/A	CXCR4 chemokine receptor antagonist	US FDA approved (2008) for stem cell harvesting; development for HIV treatment discontinued due to suboptimal virologic response	Not approved for HIV treatment
aplaviroc	APL, GW 873140	CCR5 chemokine receptor antagonist	Development discontinued due to hepatotoxicity	N/A
BMS-663068	BMS-663068	CD4 attachment inhibitor, binds HIV gp120	Investigational, Phase 3	600 mg PO BID
cenicriviroc	CVC, TAK-652	CCR5 chemokine receptor antagonist	Investigational, Phase 2	100 mg or 200 mg PO daily (dosage adjusted based on coadministered ARVs)
enfuvirtide	ENF, Fuzeon, T-20	Fusion inhibitor	US FDA approved for HIV treatment (2003)	90 mg SQ BID
ibalizumab	N/A	CD4 attachment inhibitor, binds CD4 receptor	Investigational, Phase 3	2,000 mg IV loading dose followed by 800 mg IV every 2 weeks
maraviroc	MVC, Selzentry, Celsentri, UK-427,527	CCR5 chemokine receptor antagonist	US FDA approved for HIV treatment (2007)	150 mg PO BID 300 mg PO BID 600 mg PO BID (dosage adjusted based on coadministered ARVs)
PRO 140	N/A	CCR5 chemokine receptor antagonist	Investigational, Phase 2/3	350 mg SQ weekly
Schering C	SCH-C	CCR5 chemokine receptor antagonist	Development discontinued due to QT prolongation	N/A
10-1074	N/A	CD4 attachment inhibitor, binds HIV gp120	Investigational, Phase 1	30 mg/kg IV (frequency TBD)
3BNC117	N/A	CD4 attachment inhibitor, binds HIV gp120	Investigational, Phase 2	30 mg/kg IV (frequency TBD)
vicriviroc	VCV, SCH 417690, Schering D, SCH-D, MK-4176	CCR5 chemokine receptor antagonist	Development for HIV treatment discontinued due to suboptimal virologic effect; phase 1 for HIV prevention	N/A
VRC01	N/A	CD4 attachment inhibitor, binds HIV gp120	Investigational, Phase 2	40 mg/kg IV (frequency TBD)

Abbreviations: ARVs antiretrovirals, BID twice a day, IV intravenous, PO by mouth, SQ subcutaneous, TBD to be determined

CD4 Attachment Inhibitors

Small-Molecule CD4 Attachment Inhibitors

Fostemsavir as of 10/2016

BMS-663068 is an oral investigational small-molecule HIV CD4 attachment inhibitor. The compound is a methyl phosphate prodrug that is rapidly converted by alkaline phosphatase in the gastrointestinal lumen to the active compound, BMS-626529, that subsequently is rapidly absorbed. The active compound specifically binds to HIV gp120 and inhibits its binding to the CD4 receptor on the cell surface. In vitro, BMS-656529 demonstrated a broad range of virologic activity against a group of clinical HIV isolates.

In phase 1 testing, BMS-663068 demonstrated potent virologic activity over 8 days (Nettles et al. 2012); however, 14% of individuals had decreased baseline susceptibility to the compound due to polymorphisms in the HIV envelope. In a phase 2b study, 251 treatment-experienced individuals who had a prestudy viral isolate with a half maximal inhibitory concentration (IC₅₀) to the prodrug BMS-626529 of less than 100 nM received tenofovir and raltegravir together with BMS-663068 400 mg or 800 mg twice daily, BMS-663068 600 mg or 1,200 mg once daily, or ritonavir-boosted atazanavir (control regimen). At week 24, virologic suppression to <50 copies/ml was seen in the BMS-663068 groups at rates of 80% (400 mg twice daily), 69% (800 mg twice daily), 76% (600 mg once daily), and 72% (1,200 mg once daily) versus 75% in the atazanavir/ritonavir group. Serious adverse events were described in 7% of individuals on BMS-663068 versus 10% on atazanavir/ritonavir, and 2% versus 4%, respectively, discontinued because of adverse events. Individuals continued to be followed and based on 48-week responses, the 1,200 mg daily dose was selected for all BMS-663068 groups and 67% of individuals continued study regimens through 96 weeks (DeJesus et al. 2016). At week 96, HIV RNA was suppressed to <50 copies/ml in 61% (BMS-663068) versus 53% (atazanavir/ritonavir) in a modified intent-to-treat analysis and grade

2–4 related clinical adverse events occurred in 8.5% (BMS-663068) versus 31% (atazanavir/ritonavir), mostly due to atazanavir-associated hyperbilirubinemia.

Ultimately, pharmacokinetic/pharmacodynamic modeling using data from phase 2 studies in HIV-1-infected individuals ($N = 244$, clinical trials.gov identifiers: NCT01009814 and NCT01384734) suggested that BMS-663068 dosed at 600 mg BID would have the optimal risk-benefit profile, and this dose was selected for further investigation. BMS-663068 is now in phase 3 evaluation in heavily treatment-experienced individuals with multidrug resistant HIV (NCT02362503). The US Food and Drug Administration designated BMS-663068 as “breakthrough therapy” in July 2015, a designation for compounds used to treat a serious or life-threatening disease with preliminary clinical data that demonstrate the potential for substantial improvement over existing therapies.

Monoclonal Antibodies

Two to three years after HIV infection, approximately 10–20% of individuals develop serum antibodies that can neutralize a broad spectrum of HIV viral strains. Recent advances have led to the discovery of multiple potent human monoclonal antibodies that are broadly neutralizing across many HIV-1 subtypes. These antibodies target different epitopes on the HIV envelope as shown in Fig. 2. Therapy with broadly neutralizing antibodies (bnAB's) differs from other therapies for HIV infection in that not only is there associated virologic activity but the potential to enhance host immunity to HIV infection. Such compounds are currently being explored in clinical trials for HIV treatment and prevention, both individually and as antibody combinations.

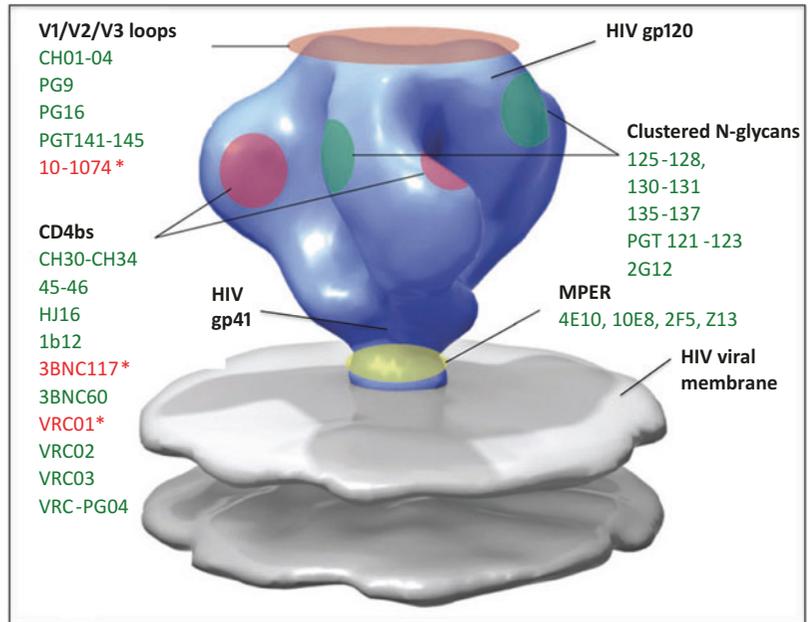
Monoclonal Antibodies that Bind to gp120

10-1074 10-1074 is an investigational broadly neutralizing monoclonal antibody that specifically binds to the base of the V3 loop within HIV-1 envelope gp120. This antibody was originally cloned from a single B cell from an individual infected with clade A HIV-1 with virologic

Entry Inhibitors,

Fig. 2 HIV binding sites for broadly neutralizing monoclonal antibodies.

*Antibodies that are farthest along in clinical development and discussed in the text. *Abbreviations:* *CD4bs* CD4 binding site, *MPER* membrane-proximal external region, *V* variable region



E

control not on antiretroviral therapy. In preclinical trials, while 10-1074 monotherapy did not control HIV-1 infection in an ART-untreated humanized mouse model, administration of 10-1074 alone to mice with virological suppression receiving standard combination antiretroviral therapy was able to sustain HIV suppression. Administration of 10-1074 in combination with other potent human antibodies was able to control established HIV infection in humanized mice as long as antibody remained at target levels (Horwitz et al. 2013).

A phase 1 open-label, dose-escalation study evaluating the safety, tolerability, and pharmacokinetics of 10-1074 at doses of 3 mg/kg, 10 mg/kg, and 30 mg/kg in HIV-infected and uninfected individuals is underway (NCT02511990). Clinical trials are also studying the safety, pharmacokinetics, and antiretroviral activity of the combination of 3BNC117 and 10-1074 in HIV-infected and uninfected individuals as well as the role of 10-1074 in HIV prevention.

3BNC117 3BNC117 is an investigational broadly neutralizing antibody that targets the HIV-1 envelope gp120-CD4 binding site. The antibody was initially cloned from a single

memory B cell isolated from an individual infected with clade B HIV-1 with virologic control not on antiretroviral therapy. 3BNC117 is unique in its potency and breadth and is currently being investigated in clinical trials.

In the first-in-man phase 1 clinical trial (Caskey et al. 2015), individuals were administered a single intravenous infusion of 3BNC117 at increasing doses (1 mg/kg, 3 mg/kg, 10 mg/kg, or 30 mg/kg) and were followed for 24 weeks after the last infusion. Pharmacokinetic data from 22 individuals (12 HIV-uninfected, 17 HIV-infected individuals) showed a compound half-life of 17.6 days in HIV-uninfected and 9.6 days in viremic HIV-infected individuals. When administered at 30 mg/kg, 3BNC117 induced rapid decreases in plasma HIV-1 RNA levels, with mean HIV RNA dose-dependent declines of approximately 0.8–2.5 log copies/mL. Peripheral blood mononuclear cells (PBMCs) obtained before and 28 days after antibody infusion in 5 individuals dosed at 30 mg/kg demonstrated emergence of 3BNC117-resistant viral strains in some individuals. At 28 days, high-level resistance (defined as a greater than fivefold reduction in susceptibility to 3BNC117) was seen in 2 out of 5 individuals dosed at 30 mg/kg.

Overall, 3BNC117 was generally safe and well tolerated. Five individuals reported ophthalmic complaints (2 increased lacrimation, 2 conjunctival erythema, and 1 blurry vision); however, a causal relationship with 3BNC117 was not established. No serious adverse events or grade 3 or 4 adverse events deemed to be related to 3BNC117 occurred.

3BNC117 was recently administered to 13 HIV-infected individuals who were virologically suppressed on antiretroviral treatment to determine the delay in the time to viral rebound following a period of treatment interruption. Two or four 30 mg/kg infusions of 3BNC117 given to a total of 13 individuals were generally well tolerated. The infusions were associated with a delay in viral rebound for 5–9 weeks after 2 infusions, and up to 19 weeks after 4 infusions, or an average of 6.7 and 9.7 weeks respectively, compared with 2.6 weeks for historical controls. Rebound viruses arose predominantly from a single genotype. Viruses emerging in the presence of high serum antibody concentrations showed decreased 3BNC117 susceptibility (Scheid et al. 2016). Phase 2 clinical trials are ongoing to determine the safety, pharmacokinetics, and antiretroviral activity of administering multiple doses of this antibody (NCT02588586). In addition, administration of this antibody is being considered for trials of HIV prevention.

VRC01 VRC01 is an investigational broadly neutralizing monoclonal antibody which binds to the CD4 binding site of gp120 on the HIV envelope. In preclinical trials, this antibody neutralized over 90% of 190 diverse strains of HIV. In phase 1 dose-escalation studies of HIV-infected individuals (study VRC 601), a maximal dose of 40 mg/kg IV was administered. Intravenous VRC01 had a half-life of 11 days in HIV-infected individuals (Lynch et al. 2015). A single administration of the antibody lead to a 1.1–1.8 log copies/ml decline in HIV RNA in 6 out of 8 individuals. The 2 individuals with minimal decline in plasma viremia were found to have predominantly VRC01-resistant virus prior to treatment. Adverse events assessed as possibly related to study product administration, including transient localized pruritus at the

subcutaneous injection site and flushing, were mild in severity and resolved with no residual effects. Two pilot studies of VRC01 explored its effect in HIV-infected individuals with virologic suppression on antiretroviral therapy on virologic rebound following a treatment interruption but showed only a modest delay; some individuals developed VRC01-resistant viral strains.

There are several additional ongoing clinical trials involving VRC01. AIDS Clinical Trials Group (ACTG) A5342 is a phase 1 study that administers either intravenous VRC01 or placebo in a crossover design to HIV individuals with suppressed HIV RNA on ART and examines the impact on the HIV latent cell reservoir (NCT02411539). In addition, phase 2 studies are evaluating the safety and efficacy of VRC01 for HIV prevention in reducing HIV acquisition in HIV-uninfected women in Sub-Saharan Africa (study HPTN 081; NCT02568215) and in HIV-uninfected men and transgender persons who have sex with men in North and South America (HPTN 085; NCT02716675). Also, VRC01 has been modified to increase its binding affinity and increase its duration of activity; the safety and pharmacokinetics of this long-acting formulation (VRC01LS) is currently being evaluated in both intravenous and subcutaneous formulations in HIV-uninfected adults (NCT02599896).

Monoclonal Antibodies that Bind to the CD4 Receptor

Ibalizumab (TMB-355, Previously TNX-355)

Ibalizumab is an investigational monoclonal antibody that binds to the CD4 receptor on host CD4+ cells. This antibody provides potent inhibition against a broad range of HIV-1 strains *in vitro*; ibalizumab neutralized 92% of a panel of over 100 HIV-1 pseudoviruses representing multiple clades.

A multicenter phase 2a study was conducted in 82 treatment-experienced HIV-1 infected individuals with multidrug-resistant HIV-1. All individuals received an optimized background antiretroviral regimen based on treatment history and resistance testing and then added either ibalizumab dosed intravenously at 15 mg/kg

every 2 weeks (Arm A) or 10 mg/kg weekly for 9 doses followed by the same dose every 2 weeks (Arm B) or placebo. Reductions in plasma HIV-1 RNA levels at 24 and 48 weeks were 0.95 and 0.71 log copies/ml in Arm A and 1.16 and 0.96 log copies/ml in Arm B, respectively, and were statistically significant greater than those seen in the placebo arm.

A 24-week Phase 2b randomized, double-blinded study was conducted in 113 treatment-experienced HIV-1-infected individuals. Individuals received intravenous ibalizumab dosed at either 800 mg every 2 weeks (Arm A) or 2,000 mg every 4 weeks (Arm B) in combination with an optimized background regimen based on treatment history and resistance testing. A plasma HIV-1 RNA level below detection was achieved after 24 weeks of therapy in 44% of individuals on Arm A and 8% of individuals on Arm B (Bruno and Jacobson 2010).

NCT02475629 is an ongoing phase 3 study of ibalizumab plus an optimized antiretroviral background regimen in treatment-experienced individuals with multidrug resistant HIV-1. In this study, individuals are administered a loading dose of 2,000 mg intravenous ibalizumab on day 7, followed by ibalizumab 800 mg intravenous once every 2 weeks, plus an optimized background regimen beginning on day 14. The primary outcome measures are day 14 HIV RNA level reduction and the proportion of individuals achieving a ≥ 0.5 log copies/ml decrease from day 7/baseline HIV RNA. In February 2015, ibalizumab was designated “breakthrough therapy” from the US Food and Drug Administration, a designation for investigational compounds used to treat serious or life-threatening diseases with preliminary clinical data suggesting the potential for improvement over existing therapies.

Chemokine Receptor Antagonists

CCR5 Antagonists

Aplaviroc (APL, GW 873140)

Aplaviroc was an investigational CCR5 antagonist that demonstrated potent *in vitro* activity

against CCR5-tropic (R5) HIV-1. Aplaviroc was tested in clinical studies in HIV-uninfected and treatment-naive HIV-1-infected individuals, but clinical development was discontinued due to drug-associated idiosyncratic hepatotoxicity (Currier et al. 2008). Study individuals receiving aplaviroc demonstrated increased rates of elevations of hepatic transaminases and bilirubin compared to control arm individuals, and one individual experienced aplaviroc-related severe hepatic cytolysis. The mechanism of drug toxicity is not known but thought to be compound-specific, rather than class-specific.

Cenicriviroc (CVC, TAK-652)

Cenicriviroc is an investigational oral small-molecule CCR5/CCR2 chemokine receptor antagonist. The compound was first developed clinically as an antiretroviral agent for HIV treatment as a CCR5 antagonist and more recently is being explored as an anti-inflammatory drug based on its activity as a CCR2b receptor antagonist. Cenicriviroc does not antagonize binding to other chemokine receptors. Cenicriviroc demonstrates potent activity *in vitro* against CCR5-tropic HIV-1 (R5) and HIV-2 (R5) isolates but has no activity against CXCR4-tropic (X4) HIV-1. Cenicriviroc is active against HIV subtypes A-G. Cenicriviroc is orally bioavailable and demonstrates a long plasma half-life of 3–40 h, allowing once-daily dosing. The compound is metabolized by the liver, is a substrate of CYP3A4 and CYP2C8, and is $>98\%$ bound to plasma proteins.

A phase 1 study demonstrated potent virologic activity over 10 days. A phase 2 study enrolled 143 individuals with CCR5-tropic HIV-1 infection with HIV RNA levels at least 1,000 copies/ml and CD4 cell counts of at least 200 cells/uL (Thompson et al. 2016). Individuals received coformulated tenofovir disoproxil fumarate/emtricitabine and were randomized 2:2:1 to add cenicriviroc 100 or 200 mg or efavirenz 600 mg. The study regimen was a 6-pill twice-daily regimen with cenicriviroc (or placebo) dosed with breakfast and efavirenz (or placebo) dosed fasting at bedtime. HIV RNA was suppressed to <50 copies/ml at week 24, the primary endpoint of the study, in 76% (CVC 100 mg), 73% (CVC 200 mg), and 71%

(efavirenz) and at week 48 in 68%, 64%, and 50%, respectively. Virologic failure was observed in 11% on CVC versus 7% on efavirenz; drug-resistance mutations emerged in 5 CVC individuals versus none on efavirenz, and R5/X4 dual/mixed virus emerged in 1 CVC individual. Treatment-emergent adverse events of at least moderate severity were seen in 9% in each of the CVC groups versus 36% in the efavirenz group ($P = 0.001$). CVC was also associated with an increase in monocyte chemoattractant protein (MCP)-1 levels and a transient decrease in soluble CD14 levels, markers of inflammation. Based on these results, CVC 200 mg daily was selected as the dose for further development and phase 3 studies in treatment-naïve HIV-infected individuals were originally planned but ultimately not begun.

Lenacapavir currently is being studied as a CCR2b antagonist for its anti-inflammatory effects in HIV-infected individuals for neurocognitive impairment (NCT02128828) and arterial inflammation (ACTG 5363) and in HIV-uninfected individuals for nonalcoholic steatohepatitis (NASH), liver fibrosis (NCT02217475), and primary sclerosing cholangitis (NCT02653625).

Maraviroc (MVC, Selzentry, Celsentri, UK-427,527) Maraviroc is the first US Food and Drug Administration-approved chemokine receptor inhibitor and the second approved HIV entry inhibitor (2007). The drug is unique among approved antiretroviral drugs in targeting not HIV itself but the human CCR5 cell-surface receptor. Maraviroc blocks the binding of CCR5-tropic HIV-1 and HIV-2 (also called R5 HIV) to the receptor but has no activity against CXCR4-tropic (X4) HIV. Maraviroc is administered orally at a standard dose of 300 mg twice daily. Potent CYP3A4 inducers decrease maraviroc concentrations, and potent CYP3A4 inhibitors increase maraviroc concentrations, necessitating dose adjustments.

The phase 3 MOTIVATE (*Maraviroc versus Optimized Therapy in Viremic Antiretroviral Treatment-Experienced Patients*) studies were two large, parallel, randomized, double-blind, placebo-controlled multicenter trials in

treatment-experienced HIV-infected individuals with R5 virus that demonstrated the safety and efficacy of maraviroc and supported its approval (Gulick et al. 2008). The studies enrolled 1,049 HIV-infected individuals from Canada and the United States (MOTIVATE 1) and Australia, Europe, and the United States (MOTIVATE 2) with treatment experience or virologic failure with 3 prior classes of HIV drugs (nucleoside analogues, nonnucleoside analogues, and protease inhibitors), and an HIV RNA level of at least 5,000 copies/ml. Individuals underwent genotypic and phenotypic resistance testing that, along with the treatment history, was used to design an optimized background regimen of antiretroviral drugs. Individuals were then randomized 2:2:1 to add maraviroc once daily, maraviroc twice daily, or placebo and were followed for virologic response and safety. At 48 weeks, the primary study endpoint, maraviroc was associated with a significantly greater decrease in HIV RNA level of about 1.0 log copies/ml compared to placebo in both studies. Maraviroc did not demonstrate virologic activity in a study of treatment-experienced individuals with non-R5-HIV (X4 or mixed infection) and demonstrated suboptimal virologic responses in treatment-naïve individuals with R5 virus.

Oral maraviroc was explored for HIV prevention in a phase 2 study of HIV-uninfected at-risk men-who-have-sex-with-men and women as PrEP (*Pre-Exposure Prophylaxis*) either as monotherapy or combined with emtricitabine (FTC) or tenofovir disoproxil fumarate (TDF) versus the control regimen of TDF/FTC and demonstrated comparable safety and tolerability (NCT0105114). Maraviroc also was explored for HIV prevention delivered through a vaginal ring but failed to achieve target drug levels due to decreased elution from the ring.

Side effects with maraviroc are uncommon. The prescribing information contains a boxed warning for hepatotoxicity based on a single case report, but a retrospective review of maraviroc clinical trials did not confirm a risk for hepatotoxicity. Postural hypotension can occur at higher maraviroc doses (typically 600 mg twice daily and higher). Drug resistance

to maraviroc most commonly develops in the setting of ongoing viral replication with the outgrowth of preexisting X4 HIV. With its unique mechanism of action, maraviroc is not cross-resistant to other HIV antiretrovirals.

No dose adjustment is required for individuals with mild-moderate hepatic or renal impairment, but maraviroc is contraindicated in individuals with severe renal impairment (CrCl <30 mL/min) and should not be used in severe hepatic impairment. Maraviroc is pregnancy category B, although there are insufficient data to support its use with only a few case reports in pregnant women.

Maraviroc is not used commonly today for HIV treatment because of its requirement for viral tropism testing (to determine the presence of R5 and/or X4 HIV) and is not recommended in treatment guidelines as initial therapy because of suboptimal virologic responses in studies of treatment-naïve individuals. With its novel mechanism of action, maraviroc is active against multidrug-resistant HIV and can be used as a part of combination regimens in treatment-experienced participants with R5 virus, although typically fewer than 50% of such individuals have R5 virus only.

PRO 140

PRO 140 is an investigational humanized monoclonal antibody that targets the host-cell CCR5 chemokine receptor that has been designated by the Food and Drug Administration for fast-track approval, a designation for a compound to treat a serious or life-threatening condition that fills an unmet medical need. PRO 140 is a non-competitive, allosteric inhibitor and is active against viruses resistant to small-molecule antagonists of CCR5 such as maraviroc.

PRO 140 demonstrated virologic activity both in vitro against R5 viruses and in vivo in HIV-1-infected individuals with CCR5-tropic virus. A phase 2a study was conducted to evaluate the antiviral activity, tolerability, and pharmacokinetics of weekly or biweekly subcutaneous doses of PRO 140 in HIV-1 infected individuals with R5-tropic HIV-1 (Jacobson et al. 2010). Individuals were dosed with placebo, 162 mg, or 324 mg

of PRO 140 at days 1, 8, and 15. A fourth study arm dosed 324 mg of PRO 140 on days 1 and 15 and placebo on day 8. Pharmacokinetic studies revealed terminal half-lives of 3.4 and 3.7 days with 162 and 324 mg dosed weekly, respectively. Subcutaneous PRO 140 demonstrated mean log reductions in HIV-1 RNA level of 0.15, 0.75, 1.20, and 1.51 for the placebo, 162 mg weekly, 324 mg biweekly, and 324 mg weekly dose groups, respectively, by day 22. HIV RNA levels remained suppressed between successive doses. Treatment was generally well tolerated with no drug-related serious adverse events. Administration-site reactions, including induration (20%), pain (9%), and irritation (7%), were infrequent, mild, and transient. Low titers of anti-PRO 140 antibodies were detected in 7 (5%) individuals; however, there was no clear effect on pharmacokinetic profiles or virologic activity. All viruses were susceptible to PRO 140 before and after therapy, and the maximum percentage inhibition of R5 viruses was 98–100%.

PRO 140 is undergoing phase 2 trials evaluating weekly subcutaneous injections for maintenance of virologic suppression in individuals suppressed on ART (NCT02175680) as well as those with poor ART adherence (NCT01272258) and a phase 3 trial evaluating the safety and tolerability of weekly injections in individuals with limited treatment options (NCT02483078).

Vicriviroc (VCV, SCH 417690, Schering D, SCH-D, MK-4176) and Schering C

Vicriviroc is an investigational small-molecule CCR5 antagonist that was first developed clinically as an antiretroviral agent for HIV treatment and received US FDA fast-track status for clinical development in 2005. Vicriviroc demonstrates potent activity in vitro against CCR5-tropic HIV-1 (R5) isolates but has no activity against CXCR4-tropic (X4) HIV-1. Vicriviroc is metabolized by the CYP3A4 hepatic enzyme system and plasma levels are enhanced by coadministration with ritonavir, the potent CYP3A4 inhibitor.

A phase 2 study in treatment-naïve individuals enrolled 92 individuals with R5 HIV and randomized them to receive one of 3 doses of vicriviroc (25, 50, 75 mg once daily) for 14 days and then

added zidovudine/lamivudine or to the standard-of-care 3-drug regimen zidovudine/lamivudine and efavirenz. This study was stopped early because of increased virologic failure rates in the 25 and 50 mg vicriviroc arms.

Phase 2 studies in treatment-experienced individuals demonstrated significant virologic activity of vicriviroc. In parallel identically designed phase 3 studies, VICTOR-E3 and VICTOR-E4, 857 treatment-experienced HIV-infected individuals with documented resistance to at least two prior antiretroviral drug classes had their antiretroviral regimen optimized on the basis of treatment history and drug-resistance testing to include at least two fully active antiretroviral drugs, and then were randomized 2:1 to receive vicriviroc or matching placebo (Casiero et al. 2012). The primary study endpoint, the proportion of individuals with HIV RNA suppressed to <50 copies/ml, was 64% in the vicriviroc arm versus 62% in the placebo arm ($p = 0.6$).

Based on the increased virologic failure rates in the treatment-naïve study and the lack of a significant virologic benefit in the large treatment-experienced studies, further clinical development of vicriviroc for HIV treatment was discontinued in 2010. Vicriviroc currently is being explored for HIV prevention in phase 1 studies using the compound in an intravaginal ring, either alone or in combination with a second agent, MK-2048, an investigational HIV integrase inhibitor (Microbicide Trials Network [MTN] study 027 [NCT02356302], NCT02419456.)

A related compound, SCH-351125 (SCH-C, Schering C), a CCR5 antagonist that is structurally distinct, was discontinued from clinical development due to QT interval prolongation.

CXCR4 Antagonists

AMD-3100 (JM-3100, Plerixafor, Mozobil) and AMD-070 (AMD-11070)

AMD-3100 is an investigational bicyclam CXCR4 antagonist that interferes with HIV binding to the host-cell CXCR4 chemokine receptor. AMD-3100 demonstrates potent activity in vitro against CXCR4-tropic HIV-1 and HIV-2 (X4) isolates but has no activity against CCR5-

tropic (R5) HIV-1. A phase 1 study of AMD-3100 administered at escalating doses by continuous intravenous for 10 days to 40 HIV-infected individuals with HIV RNA at least 5,000 copies/ml demonstrated dose-proportional concentrations, up to 3.4-fold increases in white blood cell counts, but no significant change in HIV RNA – although the one enrolled individual with X4-virus only demonstrated a decrease in HIV RNA of 0.9 log copies/ml (Hendrix et al. 2004). Based on these results, further development of AMD-3100 for HIV infection was discontinued.

The notable leukocytosis was found to be due to AMD-3100 interfering with the binding of SDF-1, the natural ligand of the CXCR4 receptor, that functions to retain hematopoietic stem cells in the bone marrow and clinical studies ensued. AMD-3100, renamed plerixafor, was ultimately approved by the US Food and Drug Administration in 2008 for use in stem cell mobilization for bone marrow transplantation in individuals with hematologic malignancies (non-Hodgkin's lymphoma and multiple myeloma).

A follow-on compound, AMD-070, an orally bioavailable investigational CXCR4 antagonist, was tested in pilot studies of HIV-uninfected and HIV-infected individuals, but development was ultimately abandoned due to hepatotoxicity in laboratory assays and animal studies.

Fusion Inhibitors

Albuvirtide (ABT, FB006M)

Albuvirtide is an investigational HIV fusion inhibitor that is a peptide analogue of enfuvirtide, modified with 3-maleimimidopropionic acid (MPA). Albuvirtide demonstrates potent activity in vitro against HIV-1 of different subtypes, including variants resistant to enfuvirtide. The compound is administered parenterally and can irreversibly conjugate to serum albumin, conferring a long plasma half-life that could allow weekly (or less frequent) dosing.

The first clinical study of albuvirtide was a phase 1 trial that enrolled 55 HIV-infected individuals who received single intravenous doses escalating from 20 to 640 mg (or placebo).

Albuvirtide was generally safe and well tolerated and demonstrated a half-life of 11 days with virologic activity demonstrated over 6–10 days following dosing. A phase 2a study enrolled 12 HIV-infected individuals who received albuvirtide 160 or 320 mg given intravenously once daily for 3 days, followed by 2 weekly administrations. The compound was generally safe and well tolerated and associated with dose-dependent HIV RNA reductions of 0.7 (160 mg) and 1.0 (320 mg) log copies/ml.

A follow-up study enrolled 20 HIV-infected individuals who were randomized to receive albuvirtide 160 or 320 mg given intravenously weekly with standard-dose lopinavir/ritonavir twice daily for 7 weeks (Zhang et al. 2016). At week 7, HIV RNA decreased 1.9 (160 mg) and 2.2 (320 mg) log copies/ml, HIV RNA was suppressed to <50 copies/ml in 11% (160 mg) and 56% (320 mg), and there were no serious adverse events.

Albuvirtide currently is under study in a phase 3 trial of a target 420 HIV-infected individuals experiencing virologic failure of their first antiretroviral regimen in which all individuals receive standard-dose lopinavir/ritonavir and are randomized to add either albuvirtide 320 mg given intravenously once weekly or standard daily doses of tenofovir disoproxil fumarate and lamivudine (NCT02369965).

Enfuvirtide (ENF, Fuzeon, T-20)

Enfuvirtide was the first US Food and Drug Administration-approved HIV entry inhibitor (2003). The drug is a linear synthetic 36-amino acid peptide that has a structure analogous to the heptad repeat region (HR2) of HIV-1 membrane glycoprotein 41 (gp41). As part of viral-CD4 cell membrane fusion, the heptad repeat (HR) regions of HIV gp41 (HR1 and HR2) fold in on and bind to one another. Enfuvirtide, as an analogue of HR2, inhibits this binding and acts as a viral-cell membrane fusion inhibitor. As a peptide, enfuvirtide requires parenteral administration and with its short half-life of about 4 h, enfuvirtide requires dosing every 12 h. The US FDA-approved dose of enfuvirtide is 90 mg every 12 h by subcutaneous injection.

The TORO (*T-20 vs. Optimized Regimen Only*) studies were two large, parallel, randomized, controlled, open-label, multicenter phase 3 trials in treatment-experienced HIV-infected individuals that demonstrated the safety and efficacy of enfuvirtide and supported its approval. The studies enrolled 1,013 HIV-infected individuals from the Americas (TORO 1, Lalezari et al. 2003) and from Europe and Australia (TORO 2, Lazzarin et al. 2003) with treatment experience or virologic failure with 3 prior classes of HIV drugs (nucleoside analogues, non-nucleoside analogues, and protease inhibitors) and an HIV RNA level of at least 5,000 copies/ml. Individuals underwent genotypic and phenotypic resistance testing that, along with the treatment history, was used to design an optimized background regimen of 3–5 antiretroviral drugs. Individuals were then randomized 2:1 to add enfuvirtide (or not) and were followed for virologic response and safety. At 24 weeks, the primary study endpoint, enfuvirtide was associated with a significantly greater decrease in HIV RNA level of about 0.8–0.9 log copies/ml in both studies.

The main side effect of enfuvirtide is injection site reactions characterized by pain, erythema, induration, and subcutaneous nodules, and these are seen in nearly all individuals. Drug resistance to enfuvirtide is conferred by substitutions in the HR1 region of gp41 at positions 36–40, 42, and 43 that decrease binding of the drug; these substitutions develop commonly in the presence of ongoing viremia; enfuvirtide is considered to have a low barrier to resistance. With its unique mechanism of action, enfuvirtide is not cross-resistant to other HIV antiretrovirals. As a peptide, enfuvirtide does not have significant interactions with other drugs. No dose adjustment is required for renal or hepatic impairment.

There are limited efficacy data in pediatric individuals aged 6–16. Enfuvirtide is pregnancy category B, although there are insufficient data to support its use in pregnant women. Enfuvirtide is not used commonly today because of its requirement for twice-daily subcutaneous dosing. With its novel mechanism of action, enfuvirtide is active against multidrug-resistant HIV and may

be used in heavily treatment-experienced individuals with few or no remaining HIV treatment options.

Conclusions

In summary, entry inhibitors are antiretroviral drugs that prevent HIV from entering target cells. Entry inhibitors interfere with the one of three major steps involved in the HIV life cycle: (1) CD4 receptor attachment, (2) chemokine receptor attachment, or (3) viral-cell membrane fusion. There currently are only two entry inhibitors that are US FDA approved for HIV treatment (enfuvirtide, maraviroc), but a number of agents under investigation hold promise for both HIV treatment and prevention.

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Epidemic Kaposi Sarcoma, Pathogenesis and Presentation

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Definition

Kaposi sarcoma (KS) is a vascular neoplasm caused by infection with human herpesvirus 8 (HHV-8, also known as Kaposi sarcoma-associated herpesvirus) which may involve the

skin, mucous membranes or viscera. Epidemic KS is the form of the disease found among persons with HIV infection; it is the most common malignancy in persons with HIV worldwide.

Introduction

Kaposi sarcoma (KS) remains among the most common cancers seen in persons with human immunodeficiency virus (HIV) infection more than three decades after it was initially described as a key-presenting manifestation of the acquired immunodeficiency syndrome (AIDS) in 1981. While new cases of KS have decreased dramatically in the United States with the widespread availability of combination antiretroviral therapy (cART), the incidence remains threefold higher than prior to the HIV pandemic, and the cancer (► [Cancers Related to HIV](#)) is the most common in the entire male population of several countries in sub-Saharan Africa. Much has been learned about the pathogenesis of KS and its clinical manifestations in HIV-infected persons, and many of these discoveries have led to effective therapies for the disease. However, KS remains a cancer that infrequently responds completely to therapy, and continued research into the biology of the malignancy will likely lead to more effective, safer, and less expensive therapies that may be used in the coming years.

Pathogenesis

The dramatically increased incidence of KS among persons with severe CD4 T-cell deficiencies (► [Central Memory CD4+ T cells](#)) led investigators to seek an infectious etiology of the tumor. In 1994, a novel human herpesvirus (human herpesvirus 8, HHV-8, or Kaposi sarcoma-associated herpesvirus) was shown to be the cause of KS (Chang et al. 1994). Subsequently, it has been observed that infection with HHV-8 is necessary, but not sufficient, for the development of KS. In the pre-cART era, only half of severely immunosuppressed individuals with HHV-8 infection developed KS (Martin

et al. 1998), and in some regions of sub-Saharan Africa, the majority of the general population is infected with HHV-8 but few go on to develop KS (Dollard et al. 2010). Although many aspects of the pathway to tumorigenesis have been worked out for KS, a number of gaps in the understanding of the pathogenesis remain.

Several epidemiologic patterns of KS have been recognized; these include classic KS (arising in elderly men in regions surrounding the Mediterranean Sea), endemic KS (arising in individuals in Africa before the AIDS epidemic or who are not infected with HIV), epidemic KS (associated with HIV infection), and transplantation-associated KS. It is now recognized that these are all forms of the same disease.

Acquisition and Dissemination of HHV-8

Behavioral, seroepidemiologic, and virologic studies have consistently underscored the importance of salivary transmission and acquisition of HHV-8 (Pauk et al. 2000). In areas where KS is not endemic, HHV-8 is most often acquired as a sexually transmitted infection after the time of sexual debut. In regions of the world where KS is endemic, HHV-8 is most often acquired in childhood with persistent acquisition into adulthood. The timing of primary acquisition of HHV-8 may play an important role in determining whether the infection will remain asymptomatic or progress to KS. Precedent with other viral infections, such as hepatitis B virus, shows that infection earlier in life may be associated with immune tolerance that is permissive of viral replication. HHV-8 replication is associated with a higher risk of developing KS (Engels et al. 2003) and therefore may explain why one of the two peaks in KS incidence in endemic regions occurs shortly after the time that HHV-8 is acquired.

Primary infection with HHV-8 likely occurs in oral epithelial cells (Pauk et al. 2000), and dissemination to other anatomic sites is thought to be an important next step in the progression to KS. Infection of circulating B lymphocytes may then lead to wider anatomic dissemination, which may in turn allow for infection of circulating endothelial cells. Endothelial cells are almost certainly the primary origin of the pathognomonic

cell of KS tumor, the “spindle cell,” though the absence of an animal model which completely recapitulates all of the features of the progression from primary viral infection to tumorigenesis makes definitive conclusions about the process challenging.

KS is an unusual tumor, in that it does not share some of the key characteristics of almost all other malignant tumors. First, while the spindle cell is central to the pathogenesis of the malignancy, it comprises the minority of many KS lesions, and the neoplasm is always a heterogeneous mix of cell types. Inflammatory cells (B and T lymphocytes as well as monocytes) and vascular proliferations predominate. Spindle cells tend not to be clonal and cannot proliferate in culture in the absence of growth factors or in nude mice, and KS tumors are dependent on persistent HHV-8 replication for survival.

The development and maintenance of KS tumors is clearly therefore reliant on host, viral, and environmental factors, as summarized below.

Viral Factors

Like all herpesviruses, HHV-8 (► [Kaposi's Sarcoma Associated Herpesvirus](#)) has two genetic programs: the lytic stage, in which viral replication occurs, and the latent stage, in which the virus persists with expression of an extremely restricted number of gene programs. Latency is maintained through a tethering of the HHV-8 episome to the host chromosomes by the latency-associated nuclear antigen (LANA or ORF73). In addition to maintaining the viral genome through cell division, LANA and other viral latency genes play a clear role in carcinogenesis. LANA also inhibits traditional tumor suppressors (p53 and Rb) and upregulates the expression of proto-oncogenes. The viral FLICE-like inhibitory protein (v-FLIP) is antiapoptotic, and v-cyclin and the kaposins may contribute to neovascularization, disruption of the cell cycle, and inflammation. During lytic infection a larger number of viral genes are expressed. Key among the lytic viral genes for the initiation and propagation of the KS tumor includes the viral interleukin 6 analogue (v-IL6) and the viral G protein-coupled receptor (v-GPCR) which each increases angiogenesis in

a number of different ways and the viral ORFK15 that incites a proinflammatory cytokine and chemokine response permissive to KS tumors.

Genomic Heterogeneity

Human herpesviruses exact efficient and accurate replication of their DNA genome due in part to the presence of a proofreading DNA polymerase. However, the large size of the genome and the number of viruses produced in some portions of the lytic life cycle can lead to genomic heterogeneity (Zong et al. 2002). Distinct “strains” of HHV-8 can be detected between individuals that tend to cluster by geographic region, though within an individual are stable over time. These strains are determined based on sequencing small regions of one or a limited number of genes that are thought to be hypervariable. It is unclear as to how these genetic variations affect the clinical manifestations of disease, and this will be an interesting area for future research.

Coinfection

In the field of cancer biology, increasing attention is being paid to the microbial community in a given anatomic compartment and how that may alter the susceptibility to cancer within an individual. Both in vitro models and data from clinical cohorts support a role for coinfections to potentiate HHV-8 replication, which may in turn increase the odds of developing KS from asymptomatic infection. The HIV *Tat* protein (► [Tat Expression and Function](#)) increases the production of HHV-8 in culture (Aoki and Tosato 2004), and relationships have been established between the detection of HIV in the plasma and both the frequency of detection of HHV-8 at mucosal sites (Johnston et al. 2009) and the risk for development of KS over time. Taken together with epidemiologic evidence that HIV infection increases the risk of developing KS over that seen in immunocompromised transplant recipients (Grulich et al. 2007), the data are compelling that HIV coinfection strongly potentiates the development of KS. Similarly, cytomegalovirus coinfection (► [Immunopathogenesis of HIV Co-infections](#)) increases HHV-8 replication (Vieira et al. 2001), though the clinical relevance of this interaction

has not been established. Observations that HHV-8 lytic replication may be triggered by the engagement of toll-like receptors 7 and 8 (TLR 7/TLR8) (Gregory et al. 2009), whose ligands are typically single- or double-stranded RNA, raise the possibility that RNA viruses inhabiting the human virome could also play a role in KS tumorigenesis. No data to date has evaluated the role of the gut microbiome and predisposition to KS, but with the understood importance of the oral cavity in HHV-8 acquisition and dissemination, the preliminary evidence that oral inflammation is associated with HHV-8 oral replication, and increasing appreciation of the role of the oral microbiome in oral inflammation, further research is necessary to determine if there is an association between the microbiome and the susceptibility to KS.

Host Factors

Cellular Immunity

The inverse association between the incidence of KS and the quantity of CD4 T lymphocytes in HIV-infected individuals highlights the importance of T cells in the biology of KS, but to date, the exact mechanisms by which T-cell deficiencies predispose to KS are not clear. Early studies were mixed but overall found either a weak or no association between the absolute number of T lymphocytes and the risk of classic or endemic KS. In the setting of HIV infection, weak CD4 T-cell responses to latent HHV-8 antigens can be detected, but have not been correlated with risk of developing KS (Sabbah et al. 2012). CD8 T-cell responses have been identified to both latent and lytic (Ribechini et al. 2006) HHV-8 antigens, with a suggestion that the LANA protein is immunodominant. Observations from small numbers of patients with KS show that cytotoxic T-cell responses in patients with KS are most robust after immune reconstitution with cART (► [Immunological Responses to Antiretroviral Therapy](#)) and that an inverse relationship exists between robustness of cytotoxic T-cell response and the presence of HHV-8 in the peripheral blood (Bihl et al. 2009). Taken together, it is clear that while T-cell immunity is important in the pathogenesis of KS, other immunologic factors must also contribute.

Fewer studies have examined the role of B lymphocytes or NK cells in the development of KS. Reduced numbers of NK cells and impaired function have been observed with classic KS, and deficits in NK cell function are restored after cART.

Humoral Immunity

Given the importance of HHV-8 replication in the development of KS, it is possible that control of viral replication with neutralizing antibodies could reduce the progression to KS. Reduced levels of neutralizing antibodies to HHV-8 are found in the peripheral blood of persons with KS compared with asymptotically infected controls. Neutralizing epitopes have not been identified.

Inflammation

The development of KS at sites of surgical incisions or trauma (the “Koebner phenomenon”) has been held out as an example of the importance of inflammation in the development of KS. Worsening or newly emergent KS lesions have been described as a complication cART initiation, often attributed to “immune reconstitution inflammatory syndrome” (► [Immunological Response to ART](#)) (Achenbach et al. 2012). The detection of higher quantities of leukocyte esterase in the oral mucosa (traditionally a marker for the presence of inflammatory white blood cells from clinical specimens) was associated with high quantities of HHV-8 detected in that compartment (Casper et al. 2004).

While no studies to date have definitively characterized the inflammatory cytokines or “milieu” that may predispose to KS in vivo, careful study of HHV-8 has allowed a greater understanding of how inflammation may lead to KS. While the virus acts aggressively to downregulate certain specific immune responses that could result in its elimination (i.e., Th1 responses) (Th1 & Th2 Cells), several viral proteins act to increase the Th2 response (Th1 & Th2 Cells) (chemokine ligands), recruit T lymphocytes to areas of HHV-8 replication, and subsequently produce inflammatory cytokines such as TNF-alpha, IL-6, etc. which may allow for the rapid development of the KS tumor. Additional research is

needed to understand how differences in the inflammatory response to HHV-8 may lead to differential rates of progression to KS.

Environmental Factors

Given the widespread geographic and demographic variations in KS incidence, it is tempting to speculate that environmental factors may also modify the risk of developing KS from HHV-8 infection. However, epidemiologic studies to investigate these associations are challenging due to the relative rarity of the disease, the difficulty in finding comparable controls for cases in case-control studies, and even the absence of biologically plausible environmental factors to examine in such studies. There are few similarities in the environments of the diverse populations where KS is endemic, such as East Africa, Southern Italy, and Western China. However, exposure to iron-rich soil, plants which produce phorbol esters, or arthropods have all been proposed as environmental risk factors for KS in at least one study. Perhaps more consistent is the strong association between the use of amyl nitrates (“poppers”) and the risk of KS among the community of men who have sex with men in resource-rich regions. Again the biologic plausibility of this association has not been established, and it remains possible that the use of amyl nitrates is a surrogate for another exposure that is the true cause of increased risk for KS.

Exploiting Knowledge of KS Pathogenesis for Clinical Therapeutics

The mainstay of treatment for KS (► [Management of AIDS-related Kaposi's Sarcoma](#)) currently is cytoreductive chemotherapy. While this treatment in many is highly effective, even in resource-rich settings, the combination of chemotherapy and cART may leave residual disease in more than half of patients treated (Nguyen et al. 2008). Thus, the knowledge gained from understanding the pathogenesis of KS can inform more effective and less toxic therapies for the disease. Strategies aimed at inhibiting HHV-8 replication have been ineffective in treating KS, likely because the current antiviral drugs are only moderately effective in reducing HHV-8 replication, the possibility that

these DNA synthesis inhibitors may still allow production of early viral gene products, and the fact that only the minority of cells in KS tumors contain lytically replicating virus. Inhibition of angiogenesis was thought to be a promising target for these extremely vascular tumors, but results to date have been disappointing. Modulation of the inflammatory response has shown some evidence of success; interferon administration results in modest tumor response, interleukin-12 has shown activity in pilot studies, and more promise has been seen with the immunomodulatory drugs thalidomide and lenalidomide. Finally, drugs that inhibit specific pathways important for tumorigenesis such as matrix metalloproteinases and tyrosine kinases have limited activity in small clinical trials. It is clear that due to the complexity of the disease process, the optimal therapy for KS will likely entail drugs that inhibit multiple targets, and future studies will need to define optimal treatment strategies.

Presentation

KS is a disease with diverse clinical manifestations. An HHV-8 “primary infection syndrome” has been described in African children, transplant recipients, and HIV-negative men who have sex with men, typically consisting of fever, cytopenias, and cutaneous manifestations (rash or even KS), but it is thought that the majority of cases of incident KS occur sometime after primary infection. In resource-rich regions where the HIV epidemic has matured, KS often presents in patients who have either been marginalized from HIV care or who are not able to adhere to HIV therapy, though occasionally is identified as the AIDS-defining illness. In resource-poor areas, KS often is the diagnosis which prompts HIV testing, but may also be identified among patients participating in HIV care programs. In resource-rich regions, KS most commonly presents at low CD4 T-cell counts (Biggar et al. 2007), though the disease is increasingly being identified in persons with higher CD4 counts and suppressed HIV replication. In places where the tumor was endemic prior to the HIV pandemic, the range of CD4 counts for persons

presenting for care appears to be wider than what has been reported in the United States and Europe. In the United States, Europe, and Australia, epidemic KS is predominantly a disease of men, whereas in sub-Saharan Africa, epidemic KS cases in women are beginning to approximate the number seen in men.

Patients typically present with mucocutaneous disease, though many may have coexisting visceral disease, most typically involving the lungs or gastrointestinal tract. cART has modified the presenting manifestations of KS from what was initially described in the early HIV epidemic (Nasti et al. 2003).

Mucocutaneous KS

The morphotypes and distribution of mucocutaneous KS appear to vary by demographic and geographic populations. In the United States, Europe, and Australia, epidemic KS classically presents as an erythematous patch, progressing to a violaceous plaque or macule, and often on to a larger nodule. These lesions can be distributed virtually anywhere on the body. The oropharynx is the most common mucosal surface involved, and the tumor typically involves the hard palate and less commonly the soft palate, gingiva, and tongue. Especially in sub-Saharan Africa, additional morphotypes of KS are seen, including fungating and infiltrative disease. Perhaps due to the tendency to present with more advanced disease or the challenges of diagnosing the disease among persons with darkly pigmented skin, the patch morphology is infrequently observed as a presenting manifestation of KS in the region, but may come to predominate following treatment. Also of note in sub-Saharan Africa are the differences in clinical presentations between women and men, with women more often having disease which involves the face and less commonly involving the lower extremities.

Although the diagnosis of mucocutaneous KS is often made clinically, even astute clinicians may incorrectly ascribe a different disease process to KS. Definitive diagnosis is rendered through biopsy, where histopathology reveals the classic spindle cells and, where available, in resource-rich regions in situ hybridization for HHV-8

proteins (most often LANA) can provide confirmation to the visual histology.

Visceral KS

Up to 40% of HIV patients presenting with KS had visceral disease identified in the early HIV pandemic in the United States, but this number has decreased substantially in the era of cART (Nasti et al. 2003). Visceral disease is not routinely or exhaustively sought in the workup of patients with cutaneous KS in either resource-rich or resource-poor regions, so the true prevalence of disease is not known. Presenting manifestations of visceral KS are most commonly hemoptysis or hematochezia, representing pulmonary and gastrointestinal disease, respectively. Early staging systems for KS, which were subsequently validated later in the HIV pandemic, confirm that visceral disease portends a poorer prognosis (Krown et al. 1997). Diagnosis of pulmonary KS is established by bronchoscopy – because biopsy of lesions may be associated with hemorrhage, the detection of HHV-8 by polymerase chain reaction has been proposed as both a sensitive and specific alternative. Conversely, diagnosis of gastrointestinal KS is best established through biopsy or reliance on morphologic appearance in the appropriate clinical setting. Involvement of lymph nodes is also common, and KS in inguinal lymph nodes is often associated with substantial edema of the lower extremities.

Pediatric KS

Epidemic KS among children is uniquely described in sub-Saharan Africa. Interestingly, disease in HIV-positive children (► [Children, Care and Treatment](#)) most commonly presents with lymphadenopathic involvement, and mucosal disease is less common (Gantt et al. 2010). The median presenting CD4 T-cell count is substantially higher than what has been reported in adults, and outcomes tend to be poor.

KS as a Manifestation of IRIS

The exacerbation or novel presentation of KS among persons initiating cART has now been well described in both the United States and sub-Saharan Africa, with the incidence ranging

from ~4% of persons in the United States (Achenbach et al. 2012) to one-third of persons in Uganda (Martin et al. 2009). The predictors of developing KS-IRIS and the complete clinical manifestations are still being identified, though cases of fatal KS-IRIS complicated by systemic inflammatory syndrome, multiorgan system failure, and death have been reported.

Associated Comorbidities

KS represents one of several HHV-8-associated diseases, including ► [multicentric Castleman disease \(MCD\)](#), ► [primary effusion lymphoma \(PEL\)](#), and the recently described Kaposi sarcoma-associated herpesvirus inflammatory cytokine syndrome (KICS) (Uldrick et al. 2010). Not surprisingly, immunocompromised patients with HIV may present with more than one of these illnesses. KS accompanies up to one-third of cases of MCD, and although PEL and KICS are too rare to develop accurate estimates of the frequency of co-presentation with KS, clinical reports suggest it commonly occurs.

Outcomes After Diagnosis of KS

KS is a disease that when treated early and aggressively is infrequently fatal, but as noted above responses to therapy are uncommonly complete, and advanced disease still is associated with a poor prognosis. In the current cART era in the United States, a diagnosis of mucocutaneous KS is associated with a 2-year overall survival of 85%, but visceral disease reduces the 2-year overall survival to 64% (Achenbach et al. 2011). In the pre-cART era in Uganda, less than 10% of patients with HIV-associated KS survived 5 years (Gondos et al. 2005), though more recent data suggests that survival approximates what has been described in the United States.

Conclusions

More than three decades after the resurgence of KS accompanied the HIV pandemic, the disease remains a common complication of HIV infection worldwide. Years of steady research have led to great leaps in the understanding of KS

pathobiology and facilitated better treatments for the disease, but significant gaps in knowledge preclude the development of highly effective and implementable prevention and treatment strategies. Continued efforts are needed to comprehensively reduce the morbidity of KS.

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Epidemiology of AIDS-Defining Malignancies

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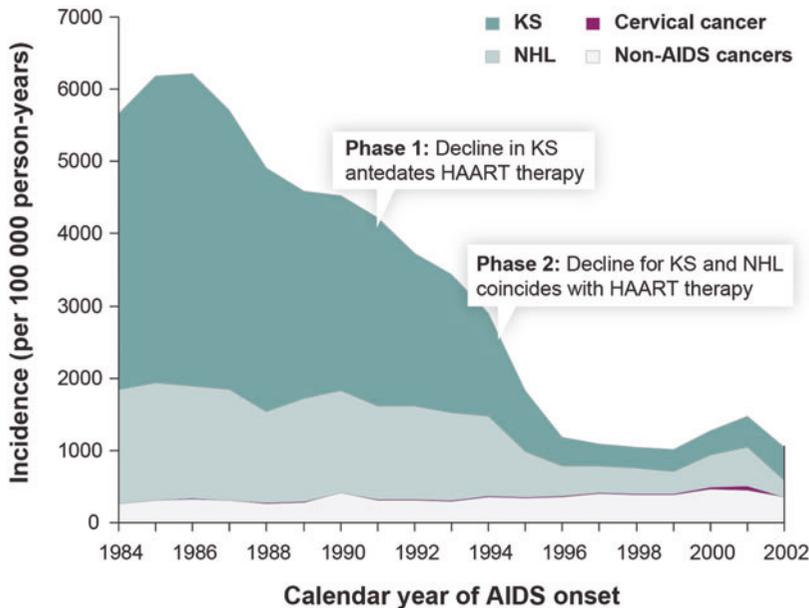
Definition

AIDS-defining malignancies are a subset of HIV-associated malignancies that include Kaposi

sarcoma, some forms of non-Hodgkin lymphoma, and invasive cervical cancer. KS along with PCP are among the first diseases that comprised the original definition of AIDS “in the United States.” Subsequently, non-Hodgkin lymphoma (1985) and invasive cervical cancer were added (CDC 1992). Compared to non-AIDS-defining malignancies, Kaposi sarcoma and non-Hodgkin lymphoma demonstrated the highest incidence in the early AIDS epidemic. As shown in Fig. 1, with the introduction of highly active antiretroviral therapy (HAART) in 1996, the incidence patterns of these cancers with the exception of cervical cancer dropped significantly. It is hypothesized that HAART restores immune function and delays progression to AIDS. However, even with the improved clinical outcome, HAART has not eliminated the risk of AIDS-defining malignancies. HIV-positive persons still remain at a substantially increased risk of developing these cancers as compared to the general population, and AIDS-related and non-AIDS-related cancers combined are the most frequent underlying causes of death in AIDS in the United States.

Epidemiology of Kaposi Sarcoma Before HAART

Prior to the AIDS epidemic, Kaposi sarcoma (KS) was a cancer with an incidence in white males of 0.3 per 100,000 in the United States (1973–1978) (Mbulaiteye et al. 2003). With the advent of the AIDS epidemic in 1981, a new form of KS, classified as “epidemic” or AIDS-associated KS, was identified. In the initial definition of AIDS, it was considered an AIDS-defining illness because epidemiologically these cases (characterized by young age at onset, high prevalence among men who have sex with men (MSM), and association with unexplained immunodeficiency) were distinct from the other three recognized patterns of KS (classic KS, elderly Mediterranean men; endemic KS, sub-Saharan Africa; and iatrogenic KS, associated with therapeutic immunosuppression) and they tended to occur in individuals with other manifestations of this new syndrome. Beginning with the first report



Epidemiology of AIDS-Defining Malignancies, Fig. 1 Cancer incidence among people with AIDS in the United States (1984–2002). Incidence is shown as a function of calendar year of AIDS onset for Kaposi sarcoma (KS), non-Hodgkin lymphoma (NHL), cervical cancer, and non-AIDS-defining cancers. Incidence estimates for each cancer are stacked on top of each other to depict the

proportion of total cancer incidence contributed by each cancer type. Analysis was restricted to the 2-year period 4–27 months after AIDS onset (Engels EA, et al., Trends in cancer risk among people with AIDS in the United States 1980–2002, *AIDS*, 2006, 20:1645–1654, pending permission from Wolters Kluwer Health through RightsLink)

of KS among MSM in 1981, incidence rapidly increased and peaked in 1985–1986. By 1989–1991, incidence was 8.9 per 100,000 with nearly 40–50% of men who have sex with men (MSM) presenting with KS as their AIDS-defining illness (Fig. 1; Mbulaiteye et al. 2003). KS became the most common AIDS-associated cancer in the United States in 1990–1995, and it was associated with a 53,000-fold higher risk compared to the general population (Engels et al. 2006). AIDS-associated KS generally presented with purple or black cutaneous lesions but also frequently with organ involvement, especially among those with advanced immunodeficiency. Survival prior to antiretroviral treatment was at most 12–18 months (Bower et al. 2006) and for the cases with extensive involvement of internal organs and concurrent opportunistic infections, prognosis was particularly poor.

Epidemiological studies documented an association of epidemic KS with high rates of sexual partner exchange, coincident sexually transmitted

infections (particularly oral gonorrhea), anal intercourse, fecal-oral exposure [particularly oral anal insertive sex (rimming)], fisting (insertion of the hand into the partners rectum), usage of nitrite (a smooth muscle relaxant used to facilitate anal sex), and contact of KS cases with a sex partner from the New York and San Francisco epicenter. These associations, plus a discordance in KS rates between MSM compared to those who acquired AIDS through parenteral [transfusion (4%) and injection drug use (10%)] and perinatally (3%) (Mbulaiteye et al. 2003), supported the hypothesis that a sexually transmitted factor independent of HIV was responsible.

Human herpesvirus 8 (HHV-8), also known as Kaposi sarcoma-associated herpesvirus (KSHV), was discovered by Chang and Moore in KS lesions, and this virus is now established as the causative agent of KS. Epidemiological analyses established that HHV-8 is a necessary but not sufficient cause of KS, based on cross-sectional and longitudinal studies of MSM that documented

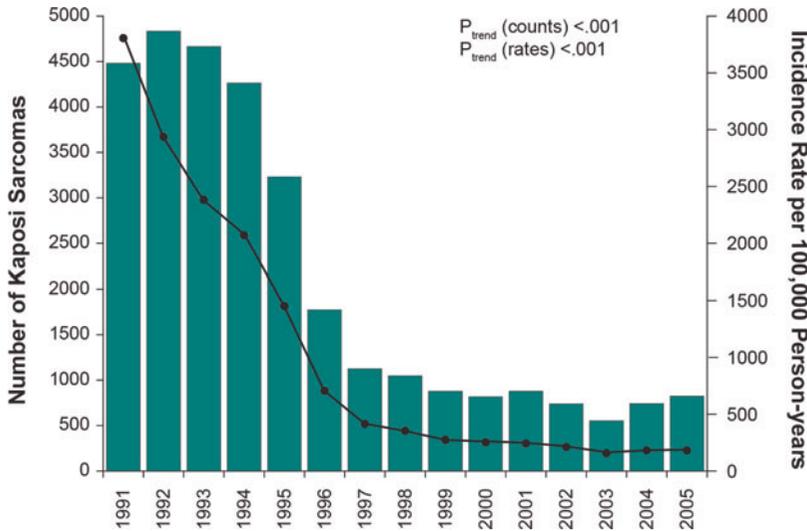
that all AIDS-associated KS cases were HHV-8 positive. Also, these studies revealed that a substantial fraction of MSM, especially in the New York and San Francisco epicenters, were carriers of the virus. Seroepidemiological studies further documented that not only was HHV-8 linked to AIDS-associated KS but that HHV-8 is also detected in cases with classic, endemic, and iatrogenic KS. By contrast, the prevalence of HHV-8 infection was low in blood donors in the United States. To better understand the complex epidemiological relationship between HIV and HHV-8, several epidemiological studies documented that anal-related sexual activities were independent risk factors for the acquisition of both viruses, confirming the hypothesis that co-epidemics of HIV and HHV-8 accounted for the emergence of AIDS-associated KS. These co-epidemics were most prominent in the New York and San Francisco epicenters, and this pattern explained the association with sexual contact with someone from New York or San Francisco as a risk factor for KS in populations outside of the New York and San Francisco areas. In summary, both HIV and HHV-8 share some overlapping risk factors, particularly fecal-oral exposure for HHV-8 and the closely related penile-anal exposure for HIV.

The AIDS-associated KS epidemic in Africa has a different epidemiological pattern, essentially reflecting the superimposition of HIV upon an existing endemic pattern of HHV-8 infection that drives the occurrence of endemic KS. Epidemiological data suggest that in this setting, HHV-8 was highly prevalent before the AIDS epidemic and like other herpesviruses is associated with oral exposure resulting from poor hygiene and practices such as pre-mastication of food during infant feeding common in the African setting. The sexual risk factors detected in the African KS setting (e.g., high rates of sexual partner exchange, commercial sex worker exposure, etc.) are associated with the acquisition of HIV infection rather than with the acquisition of HHV-8. This superinfection with HIV in an HHV-8-infected person and its attendant HIV-induced immunosuppression accelerates and amplifies the imposition of epidemic KS on a background of endemic KS.

Epidemiology of Kaposi Sarcoma After HAART

As shown in Fig. 1, the phase 1 decline in KS incidence commencing in 1986 likely represented the rapid expansion of AIDS beyond the original epicenters with high rates of HHV-8, changes in sexual behaviors, and modest impacts of early mono and dual antiretroviral HIV therapy. A more dramatic declination occurred in 1994–1996 (Fig. 1) in the United States coincident with the introduction of the highly active antiretroviral therapy (HAART), a pattern observed in other populations when effective treatment was implemented. In the Multicenter AIDS Cohort Study (MACS) of MSM, the incidence of KS declined by 87% in the post-HAART era (1996–2007) as compared to the pre-HAART era (1984–1995) (Seaberg et al. 2010). In the Swiss HIV Cohort study, the standardized incidence rates of KS declined from 1375 to 67 per 100,000 in 1985–1996 and 2002–2006 periods, respectively (Franceschi et al. 2010). Similarly, the incidence rate of KS declined significantly from 15.2 to 4.9 per 1,000 person-years in the 1992–1996 and 1997–1999 periods, in a collaborative study of 23 prospective studies from North America, Europe, and Australia (International Collaboration on HIV and Cancer 2000). HAART was also associated with improved survival of KS. The mortality rate from KS decreased fourfold for persons with AIDS and nearly 70% of persons survived up to 3 years (Simard et al. 2010; Spagnuolo et al. 2012).

Regardless of these improvements, individuals continue to present with AIDS-associated KS (Fig. 2). KS is currently associated with a 3,640-fold higher risk in HIV-infected individuals compared to the general population (Engels et al. 2006). KS has been documented in individuals with failing HAART, individuals with immune reconstitution inflammatory syndrome after initiating HAART for the first time, and those who have steady suppressed HIV viral loads and relatively high CD4⁺ T cells. Children diagnosed with AIDS before the age of 14 years have significantly elevated risk of developing KS in the post-HAART era as compared to the general



Epidemiology of AIDS-Defining Malignancies, Fig. 2 Cancer burden of Kaposi sarcoma among people living with AIDS in the United States during 1991–2005. **Bars** depict estimated counts (i.e., number of cancers) and **points connected by lines** depict the incidence rates standardized to the 2000 US AIDS population by age group,

race, and sex. Trends in cancer counts and rates were estimated with linear regression. Two-sided *P* values were calculated using the χ^2 test (Shiels MS, et al., Cancer Burden in the HIV-Infected Population in the United States, *J Natl Cancer Inst*, 2011, 103(9):753–762, by permission of Oxford University Press)

population. The current risk of AIDS-associated KS remains elevated, even though the risk has decreased significantly since the pre-HAART era.

Risk factors associated with KS in the post-HAART era primarily include low CD4 cell counts and high plasma HIV RNA levels. In particular, low CD4⁺ T cell counts (<50 cells/ μ l) were associated with an increased risk of KS for those taking HAART in the Swiss HIV Cohort (Franceschi et al. 2008). Additional risk factors specific to the post-HAART era include older age and immune reconstitution inflammatory syndrome (IRIS). HIV-infected individuals may be surviving longer and becoming at risk of KS independent of restored immunity from HAART. With IRIS, there is a transient increased incidence of KS after initiation of therapy independent of current CD4 counts. Starting HAART in a proportion of treatment-naïve individuals may stimulate a heightened inflammatory response that induces KS, perhaps in part, by provoking HHV-8 activation from latent to active gene expression. The relationship between HIV-related immune suppression and HHV-8 activation is dynamic, and understanding the relevance of the current risk

factors, such as age and IRIS, may be better understood at a molecular rather than population level.

More recently, host and viral cofactors are emerging as recognized factors in the development of KS. Molecular epidemiological studies suggest that polymorphisms in host genes involved with inflammation or immune responses are associated with a slightly increased risk of KS. Host factors include a positive association between KS and substitution of phenylalanine for glycine at position 13 in the HLA-DRB1 locus, suggesting a role for immunogenetic factors. Also, a specific polymorphism in the IL-8 promoter (TT is protective versus AT) is associated with both the occurrence and severity of KS. Additional host factors include a decrease in NK cells and immune activation measured by elevated cytokine levels.

A number of HHV-8 genes have functions that can promote tumorigenesis. Like other herpesviruses, HHV-8 has “picked up” host genes that encode for key cell cycle regulatory genes (e.g., human complement-binding protein, IL-6, BCL-2, cyclin-D, FLICE inhibitory protein

(FLIP)) and some DNA altering genes (e.g., dihydrofolate reductase, thymidine kinase, thymidylate synthetase, and DNA polymerase). These “accessory” genes are thought to be integral to KS oncogenesis on the molecular level. HIV-1 itself has a viral protein, Tat (trans-activating factor) that may synergize with HHV-8 to foster angiogenesis and disease progression and promote tumor survival. The potential synergistic effect of host and viral cofactors on KS progression may further disrupt immune control of HHV-8 and explain the more advanced clinical state of KS among HIV-1-infected individuals as compared to immunosuppressed organ transplant recipients.

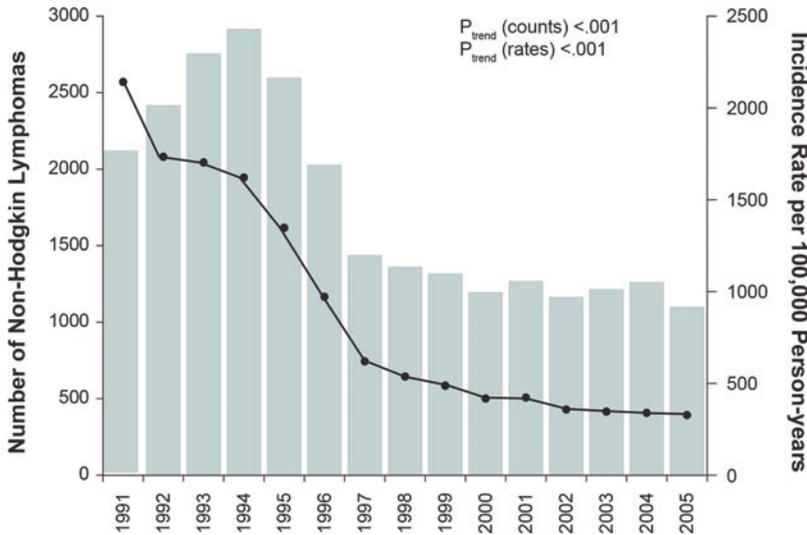
An emerging risk factor in the post-HAART era is ineffective treatment. Ineffective HAART may be the result of poor adherence resulting in insufficient immune restoration and the development of drug resistant strains. Ineffective therapy may also be associated with interrupted treatment. In the Swiss HIV Cohort study, the absence of antiretroviral therapy (ART) for 3 months was associated with an eightfold increased risk of KS (Franceschi et al. 2008). In a randomized clinical trial, KS incidence was higher among those with intermittent therapy as compared to those on continuous therapy (Silverberg et al. 2007). These relapses in HIV viremia may have a direct effect on viral and host interactions, ultimately altering host susceptibility to KS disease progression.

Epidemiology of Non-Hodgkin Lymphoma (NHL) Before HAART

NHL was the second most common cancer early in the AIDS epidemic and it was included as an AIDS-defining illness by the Centers of Disease Control (CDC) in 1985. The background prevalence of NHL in the United States prior to the AIDS epidemic was much higher than KS, so incidence trends were not as striking. In a period analysis, the incidence of NHL from 11 regions in the United States in the Surveillance, Epidemiology, and End Results (SEER) Program was 21.1 per 100,000 white men in 1995 as compared to

10.4 per 100,000 white men in 1973 (Mbulaiteye et al. 2003). For those with HIV infection, the incidence of NHL was 60–200 times higher as compared to HIV-negative individuals (Bower et al. 2006). 5–10% of all HIV-infected individuals were expected to develop lymphoma as either the first or subsequent AIDS-defining malignancy (Hamilton-Dutoit et al. 1991). Overall survival from NHL was worse than KS, less than a year (Bower et al. 2006).

AIDS-related lymphomas (ARL) are cancers of the lymphatic system. These NHL are predominantly of B-cell origin and intermediate- to high-grade malignancies and often have extensive extranodal involvement, such as the central nervous system. The prevalence of high-grade malignancy is much higher among AIDS patients (80–90%) as compared to HIV-uninfected individuals (10–15%) (Levine 1993). They occur late in HIV infection, and in general, their incidence is not affected by a transmission group or geographic region in the United States. Women have a slightly lower incidence of ARL, but that is similar to the gender distributions of lymphomas in HIV-uninfected individuals. The strongest risk factors are duration of HIV infection, low CD4⁺ T cell counts at lymphoma diagnosis, and having a prior AIDS-defining illness. Of the 35 different histological types of NHL, four are associated with AIDS-defining malignancies by the 1985 definition of the Centers for Disease Control: ► **Diffuse Large B-cell Lymphoma (DLBCL)** with centroblastic features; DLBCL with immunoblastic features; primary central nervous system lymphoma (PCNS); and Burkitt’s lymphoma (BL). The nomenclature for lymphomas has changed since then, and while it is hard to map the DLBCL in the 1985 nomenclature to the current lymphoma types, it is generally accepted that DLBCL of either the germinal center subtype or activated B-cell subtype can be considered AIDS defining. Three additional lymphomas that rarely occur in immunocompetent patients but are more specific to HIV infection are ► **primary effusion lymphoma (PEL)**, ► **plasmablastic lymphoma** (often of the oral cavity), and large B-cell lymphoma arising in HHV-8-associated ► **multi-centric Castleman’s disease**. About 70% of the



Epidemiology of AIDS-Defining Malignancies, Fig. 3 Cancer burden of non-Hodgkin lymphoma among people living with AIDS in the United States during 1991–2005. Bars depict estimated counts (i.e., number of cancers) and points connected by lines depict the incidence rates standardized to the 2000 US AIDS population by age

group, race, and sex. Trends in cancer counts and rates were estimated with linear regression. Two-sided P values were calculated using the χ^2 test (Shiels MS, et al., Cancer Burden in the HIV-Infected Population in the United States, *J Natl Cancer Inst*, 2011, 103(9):753–762, by permission of Oxford University Press)

ARL are of the DLBCL histological type which also includes variants or subtypes such as PCNS, PEL, and plasmablastic lymphoma.

► **Epstein-Barr virus (EBV)**, which had previously been identified as an etiologic agent of certain lymphomas, is a major contributor to AIDS-related lymphoma. EBV is ubiquitous in the general population and it has been associated with transplant-related lymphomas and primary immunodeficiency-associated lymphoma. Another oncogenic virus more recently linked to NHL is HHV-8, which like EBV, is specifically associated with a subset of AIDS-associated lymphomas. EBV occurs in 30% of centroblastic DLBCL, 90% of immunoblastic DLBCL, 100% of PCNS, 30–50% of Burkitt-like lymphoma, and 50% of plasmablastic lymphoma (Carbone 2003). Primary effusion lymphoma is associated with both EBV (about 80%) and HHV-8 (100%) (Carbone 2003). With varying prevalence of oncogenic viruses, no clear transmission patterns, and a multitude of host and viral risk factors, the only common risk among the ARL is HIV-related immunosuppression. Fortunately, for those who

were most susceptible to NHL, HAART significantly changed the incidence of the disease.

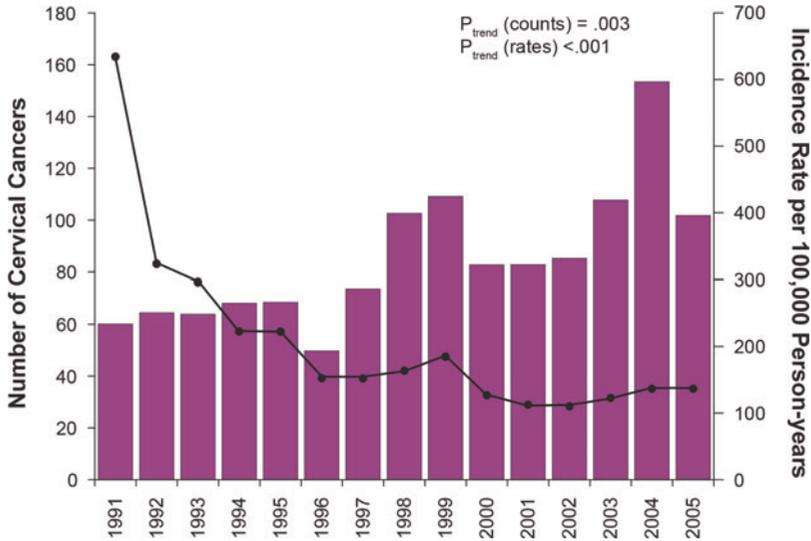
Epidemiology of Non-Hodgkin Lymphoma After HAART

Upon the introduction of HAART, the incidence of NHL declined in the United States, but not as dramatically as KS (Fig. 1). Further, unlike KS, which showed a biphasic decline, the decline for ARL was only observed during the 1994–1996 period when HAART was introduced (Fig. 3). In the Multicenter AIDS Cohort Study (MACS) of MSM, the incidence of NHL declined by 77% in the post-HAART era (1996–2007) as compared to the pre-HAART era (1984–1995) (Seaberg et al. 2010). In the Swiss HIV Cohort study, the standardized incidence rates of NHL declined from 952 to 98.4 per 100,000 in the 1985–1996 and 2002–2006 periods, respectively (Franceschi et al. 2010). Similarly, the incidence rate of NHL declined from 6.2 to 3.6 per 1,000 person-years in the 1992–1996 and 1997–1999 periods,

respectively, based on a collaborative study of 23 prospective studies from North America, Europe, and Australia (International Collaboration on HIV and Cancer 2000). In looking more specifically at the histological types that comprise ARL, HAART has been particularly effective at reducing the incidence of PCNS and immunoblastic DLBCL, but it appears to have less impact on the incidence of Burkitt's lymphoma. Both mortality and survival improved for NHL in the post-HAART era, but the changes were more gradual. NHL mortality rate decreased twofold for persons with AIDS and about 56% had an overall survival of 3 years with HAART and improved lymphoma therapies (Simard et al. 2010; Spagnuolo et al. 2012). HIV-positive individuals continue to present with NHL (Fig. 3) and are at a 23-fold increased risk compared to the general population (Engels et al. 2006). In several recent studies in the United States and Europe, NHL has emerged as the most common AIDS-associated malignancy. For example, in a record linkage study that evaluated the cumulative incidence of all cancers across the pre- and post-HAART eras, NHL surpassed KS as the most prevalent cancer in the post-HAART era (Simard et al. 2011). One risk factor in the post-HAART era driving this increase is a larger and aging AIDS population. The AIDS population has grown fourfold and is comprised of a higher proportion of individuals aged 40 years or older (Shiels et al. 2011). For those on HAART, older age (45 years+) is independently associated with a threefold increased risk of NHL (Polesel et al. 2008). Cases currently present with the same stages of disease as seen early in the epidemic, but they are older, less likely to have a prior AIDS diagnosis, and have a higher CD4⁺ T cell count at NHL diagnosis. As a result, current epidemiological studies have focused less on markers of immune suppression and more on the molecular markers of pathogenesis to better understand the risk factors in the post-HAART era.

Studies of archived pre-lymphoma samples from the Multicenter AIDS Cohort Study (MACS) document that several markers of immune function are altered years before the

AIDS-associated lymphoma development. CXCL13 (a chemokine promoting B-cell chemotaxis) is significantly elevated particularly among EBV-negative compared to EBV-positive cases (Husain et al. 2010). CD23 (a B-cell stimulatory factor), IgE, IL-6, CD27, and IL-10 as well as the IL-10 promoter 592 C/C genotype are all elevated a year or more before lymphoma development compared to age and immune status matched controls, demonstrating underlying markers of immune activation associated with future risk for HIV-associated lymphoma (Breen et al. 2011). An emerging risk factor in the post-HAART era is HIV viral replication as measured by cumulative or intermittent viremia (linked to treatment interruption) (Silverberg et al. 2007; Zoufaly et al. 2009). Recent epidemiological studies have found that HIV may upregulate activation-induced cytidine deaminase (AID) prior to NHL diagnosis. AID's normal function is to promote hypermutation of immunoglobulin genes associated with increasing affinity of the antibody during the normal development of memory B cells and to promote class switching of antibody isotypes (i.e., IgM to IgG). The genetic modifications induced by AID sometimes lead to mistakes, such as chromosomal translocations or point mutations in immunoglobulin and/or oncogenes. The chromosomal translocations of the c-MYC gene common in HIV-associated Burkitt's lymphoma may be related to this upregulation of AID. Other early genetic modifications in HIV-related lymphomas include alterations of the BCL-6, a transcription repressor gene, and point mutations or deletions in proto-oncogenes (Ras) and tumor suppressor genes (P53) (Carbone 2003). The decreased functionality of EBV-specific CD4⁺ and CD8⁺ T lymphocyte cells affects the cell-mediated responses necessary to control reactivation and replication of EBV-associated lymphomas. Any combination of these risk factors may have a multiplicative effect on developing NHL. While the pattern of HIV-associated lymphomas has changed in the post-HAART era (Fig. 3), there remains the paradox of emerging lymphoma risk particularly for some histological types not previously linked to HIV, such as certain T-cell



Epidemiology of AIDS-Defining Malignancies, Fig. 4 Cancer burden of cervical cancer among people living with AIDS in the United States during 1991–2005. **Bars** depict estimated counts (i.e., number of cancers) and **points connected by lines** depict the incidence rates standardized to the 2000 US AIDS population by age group,

race, and sex. Trends in cancer counts and rates were estimated with linear regression. Two-sided P values were calculated using the χ^2 test (Shiels MS, et al., Cancer Burden in the HIV-Infected Population in the United States, *J Natl Cancer Inst*, 2011, 103(9):753–762, by permission of Oxford University Press)

lymphomas. In summary, lymphoma remains a major cause of mortality among HIV-infected patients even in the HAART era.

Epidemiology of Cervical Cancer Before and After HAART

Invasive cervical cancer occurring in HIV-infected women was added as an AIDS-defining condition in 1993. Its inclusion at that time was somewhat controversial, as the incidence of cervical cancer was not substantially increased in AIDS patients. However, the increase in cervical cancer in HIV-infected patients has since become more apparent. It takes approximately 10–15 years for a cervical abnormality to become invasive, and during those years, it progresses through the stages of cervical intraepithelial neoplasia (CIN 1,2,3). The benefit of HAART in delaying the progression of HPV disease in HIV-infected women remains unclear. As seen in Fig. 4, the pattern of HIV-associated cervical cancer differs from the patterns for KS and NHL where in the

pre-HAART era, rates were exceptionally high and declined after the introduction of HAART. In the case of HIV-associated cervical cancer, the initially high incidence rate in the early 1990s may be attributed to a lack of regular screening and management of abnormal Pap smears in HIV-infected women. As screening and treatment of precursor lesions improved, the incidence rate stabilized. HIV-infected women compared to the general population maintained a similar risk of cervical cancer through the introduction of HAART (1990–1995, SIR:4.2; 1996–2002, SIR:5.3) (Engels et al. 2006). The 5-year cumulative incidence of cervical cancer in the post-HAART era (0.64%, 1996–2006) is similar to the pre-HAART era (0.63%, 1980–1989; 0.73%, 1990–1995) among those living with AIDS (Simard et al. 2011). Additionally, a collaborative study of 23 prospective studies from North America, Europe, and Australia found no change in the incidence of cervical cancer in the post-HAART era (International Collaboration on HIV and Cancer 2000). HAART appears to have had little or no impact on the incidence of cervical cancer so far,

and HIV-infected women remain at an elevated risk of developing the disease. However, it is possible that HAART may in the future be found to have effects on cervical cancer after a number of years.

For HIV-infected women who do develop cervical cancer, the disease is more aggressive, develops at a younger age with more advanced disease that relapses after treatment (Pantanowitz and Michelow 2010). AIDS-defining cervical cancer progresses more rapidly and has a worse median survival compared to cervical cancers among HIV-negative women (Bower et al. 2006). The aggressive nature of cervical cancer in HIV-infected individuals would suggest that HIV-related immunosuppression may play a role in disease progression. However, HAART has had little impact on the incidence or progression rates of cervical lesions (Bratcher and Sahasrabudde 2010). Declining levels of CD4⁺ counts have not been shown to be a strong risk factor for disease progression. This is further supported by the observation that there is no difference in the severity of neoplasia in women with asymptomatic HIV as compared to women with AIDS (Clarke and Chetty 2002). It is possible that oncogenic changes that result in genetically unstable precancerous lesions determine progression rates independent of any restored immunity from HAART. There is also the possibility that the inconsistent findings in the prior research have been biased by the variability in cervical detection methods, follow-up time, duration of HAART, and small numbers of incident cases of cervical cancer.

The strongest risk factor for cervical cancer is the persistent infection of high-risk types of the sexually transmitted virus, human papillomavirus (HPV). Human papillomavirus is comprised of at least 40 different genotypes that infect the genital tract, 15 of which are considered high oncogenic risk. Genotypes HPV 16 and HPV 18 carry the highest risk and account for nearly 70% of cervical cancers in the United States. HPV is highly transmissible particularly among younger women at sexual debut where an immunologically naïve host first experiences viral exposure. Risk factors associated with HPV acquisition include a lifetime number of sexual partners, frequency of sex, partner's sexual history and behavior, parity, hormonal

contraception, smoking, HIV, and other sexually transmitted infections. For most women, nearly 90% of the infections are cleared by cell-mediated immunity within 2 years. For those coinfecting with HIV, the course of HPV disease differs significantly.

HIV-infected women have a higher incidence, prevalence, and number of concurrent HPV infections than HIV-negative women, and this is compounded by the sexual risk factors shared between HIV and HPV. Cervical HPV infection persists longer in HIV-infected women and has a higher probability of progressing from low- to high-grade lesions. The regression rates of low-grade cervical lesions decrease from 60% in uninfected women to 27% in HIV-infected women (Clarke and Chetty 2002). The burden of HPV infections increases as CD4 counts decline and HIV-1 viral loads increase. It is believed that selective depletion of effective CD4⁺ T cells results in poor responses by local CD8⁺ T cells in promoting clearance or regression of HPV (Clarke and Chetty 2002). A decrease in Langerhans cells in the cervical epithelium where HPV resides results in fewer numbers of antigen-presenting cells needed to activate a cell-mediated response (Clarke and Chetty 2002). HIV may also release viral proteins, such as Tat, in the local environment that can enter cells and interfere with the repair of DNA double-strand breaks resulting in mutations and genetic instability (Nunnari et al. 2008). Tat may also activate gene transcription which may not be specific to the host or virus. Tat has been found to increase expression of early HPV genes that promote cell cycle progression (Clarke and Chetty 2002). Further studies of novel cofactors, such as HIV viral proteins, are needed to understand malignant transformation and development of cervical cancer. Any restored immunity from HAART may be too late depending on when therapy is initiated relative to the oncogenic events occurring with HPV.

Central to HPV's oncogenic potential are two viral gene products, viral proteins termed E6 and E7 that increase cell proliferation, immortalization, and transformation. The E6 protein, particularly from HPV 16 and HPV 18, is efficient at binding the tumor suppressor protein p53, leading to degradation. Loss of p53 prevents DNA repair

and apoptosis and allows cell cycle progression regardless of any DNA damage. The E6 protein also increases telomerase activity and promotes cell immortalization. The E7 protein binds and inactivates another tumor suppressor, retinoblastoma gene protein (pRB), resulting in activation of gene transcription and cell cycle progression. Unlike low-risk HPV types that are not associated with precancerous lesions, high-risk types are more likely to integrate HPV's episomal chromosome into the host DNA. This disrupts a regulatory gene, viral E2, resulting in overexpression of E6 and E7 proteins. Viral integration confers an advantage of cell cycle progression, but it is not in itself sufficient for malignant transformation. The majority of invasive cancers, particularly for HPV 18, have integrated HPV genomes, but there are still a proportion of invasive cervical cancers with episomal genomes. Recently, HPV DNA methylation has been described as a potential biomarker that may be able to distinguish the HPV infections that will progress to precancerous lesions (Clarke et al. 2012). HPV has conserved sites of CpG that when methylated by the host cell's DNA methyltransferase may, by mechanisms that are not completely understood, promote pathogenesis. Increased methylation in the capsid genes, L1 and L2, has been associated with increased risk of CIN2+ lesions (Clarke et al. 2012). However, methylation of the promoter and enhancer regions upstream of E6 is mixed, and further studies are needed (Clarke et al. 2012). As more studies explore the epigenetic changes in precancerous lesions, the role of HIV and the host's genetic background in enhancing HPV infection will be better understood. In summary, HIV is a strong risk factor for more aggressive cases of cervical cancer, but widespread use of HAART so far appears to have little effect on the incidence, progression, or mortality rates of cervical cancer.

Conclusion

Most studies evaluating the changes in morbidity and mortality of AIDS-defining malignancies across calendar time rely on record linkage studies. This is an effective way to evaluate the cumulative effect of changing treatment strategies on cancer

risk, given the power of the large sample sizes. A number of studies have shown that HAART on a population level has significantly decreased the rates of KS and NHL but has had little impact on cervical cancer. However, cancer is a leading cause of death "in the United States" among HIV-infected individuals. Further studies that evaluate HAART on the level of the individual are needed to identify molecular factors involved with the pathogenesis of these different malignancies. More specifically, studies need to evaluate the role of HIV and its viral proteins in driving lytic replication in KS, somatic hypermutations and chromosomal translocations in NHL, or early gene expression in cervical cancer. A common theme across all these malignancies is that they often present with more aggressive cases during HIV infection. Therefore, cohort studies that dive deeper into the molecular interactions will offer new insights into the continued burden of AIDS-defining malignancies and potentially lead to new therapies and prevention measures in the current era of HAART.

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Epidemiology of HIV-2 Infection in West Africa

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Definition

HIV-2 is mainly prevalent in West Africa, where an estimated 1–2 million people are infected. It is a rare infection elsewhere. From the eight known HIV-2 groups, only group A and B have established themselves as ongoing endemics in

populations. Guinea-Bissau is thought to be the nucleus of the HIV-2 epidemic. HIV-2 has been able to spread there, probably through iatrogenic spread and increased sexual risk behavior between 1950 and 1970. Few studies have investigated risk factors for acquiring HIV-2 infection, but sexual behavior and increased mobility have been associated with HIV-2 infection. In most populations, the highest HIV-2 prevalence is observed in women older than 45 years. Mortality is lower for HIV-2 than for HIV-1 infection, because many infected individuals have low viral loads. The Unusually low viral load is also the reason for the lower transmission rates compared to HIV-1. The HIV-2 epidemic in West Africa has been declining since the mid-1980s, while HIV-1 prevalence increased. The rise of HIV-1 may have impacted on the decline of HIV-2. HIV-2 remains a public health problem in West Africa, and understanding this virus may also help us to increase our understanding of both HIV-2 and HIV-1.

Introduction

In 1986, three years after HIV-1 was first isolated, a very similar virus was isolated from two AIDS patients from Guinea-Bissau and Cape Verde. This virus was found to be approximately 40% genetically homologous to HIV-1 (Guyader et al. 1987) and therefore was assigned a different name: HIV-2. The source of HIV-2 was the simian immunodeficiency virus (SIV) from sooty mangabeys, small monkeys that are indigenous to West Africa.

The most important difference between HIV-1 and HIV-2 is that a large proportion, perhaps around 50%, of HIV-2-infected persons controls the virus and remains asymptomatic, while an untreated HIV-1 infection almost always leads to AIDS. Those with HIV-2 who do experience disease progression develop similar symptomatology as HIV-1-infected people. The highest prevalence of HIV-2 has been reported in Guinea-Bissau in the 1980s: 8% in the adult population. By 2007 this had declined to 4% (van Tienen et al. 2010). Substantial declines in HIV-2 prevalence have

been observed in all countries with HIV-2 epidemics, so HIV-2 is an epidemic in decline. Yet, HIV-2 remains an important public health problem. Based on the reported adult prevalence by country and the adult population size of these countries, an estimated 1 to 2 million adults are infected (based on the highest published prevalence data from countries and World Bank data on population size by country). Thus, HIV-2 has caused and still causes a sizeable burden of morbidity and mortality. By studying the natural course of HIV-2 infections in individuals and of HIV-2 epidemics in populations, we may increase our understanding of the pathogenesis of HIV in general and find new ways of preventing and controlling these infections.

HIV-2 Groups

HIV-2 can be divided into eight groups (formerly referred to as subtypes) based on phylogenetic studies based on the genetic differences between the strains: group A to group H (Damond et al. 2004). Each group represents a separate cross-species transmission. HIV-2 group A is most prevalent in the western part of West Africa (e.g., the Gambia, Senegal, and Guinea-Bissau) and group B in the eastern part of West Africa (e.g., Cote d'Ivoire and Nigeria). HIV-2 groups C to H have not established themselves in populations, and only sporadic cases of these HIV-2 groups have been described in individuals from Liberia, Sierra Leone, and Cote d'Ivoire. The groups C, D, E, F, and H viruses were isolated from persons suffering from symptoms compatible with AIDS. Subtype G was isolated from an asymptomatic blood donor in Cote d'Ivoire. This suggests that most of the HIV-2 groups are pathogenic. It is not known why only groups A and B have successfully established epidemics.

Transmission

HIV-1 and HIV-2 share the same routes of transmission: sexual, mother to child, and via blood or

blood products. The rate of transmission is much lower for HIV-2. In HIV-2 infection, the concentration of virus in the blood (viremia) is generally several logs lower than in HIV-1 infection. A high proportion of people infected with HIV-2 – estimates vary between 30% and 60% – have very low levels of viremia (<100 copies/ml). When viremia is low, the concentration of virus in semen and cervical secretions is also low; therefore, a low viremia indicates a low transmission risk (Gottlieb et al. 2006). It has been estimated that the heterosexual transmission risk is one fifth of that of HIV-1 and that the risk of vertical transmission is one sixth of that of HIV-1 (4% vs. 25%).

Risk Factors

Two important considerations have to be taken into account when assessing the risk factors or associations with HIV-2. First, the risk factors are likely to have changed over time. Iatrogenic spread and blood transfusions may have played an important role during the initial spread, but probably did not contribute significantly to transmission after 1980. Second, due to its low incidence, few studies have been able to prospectively study risk factors, i.e., characteristics or behavior of people preceding HIV-2 acquisition.

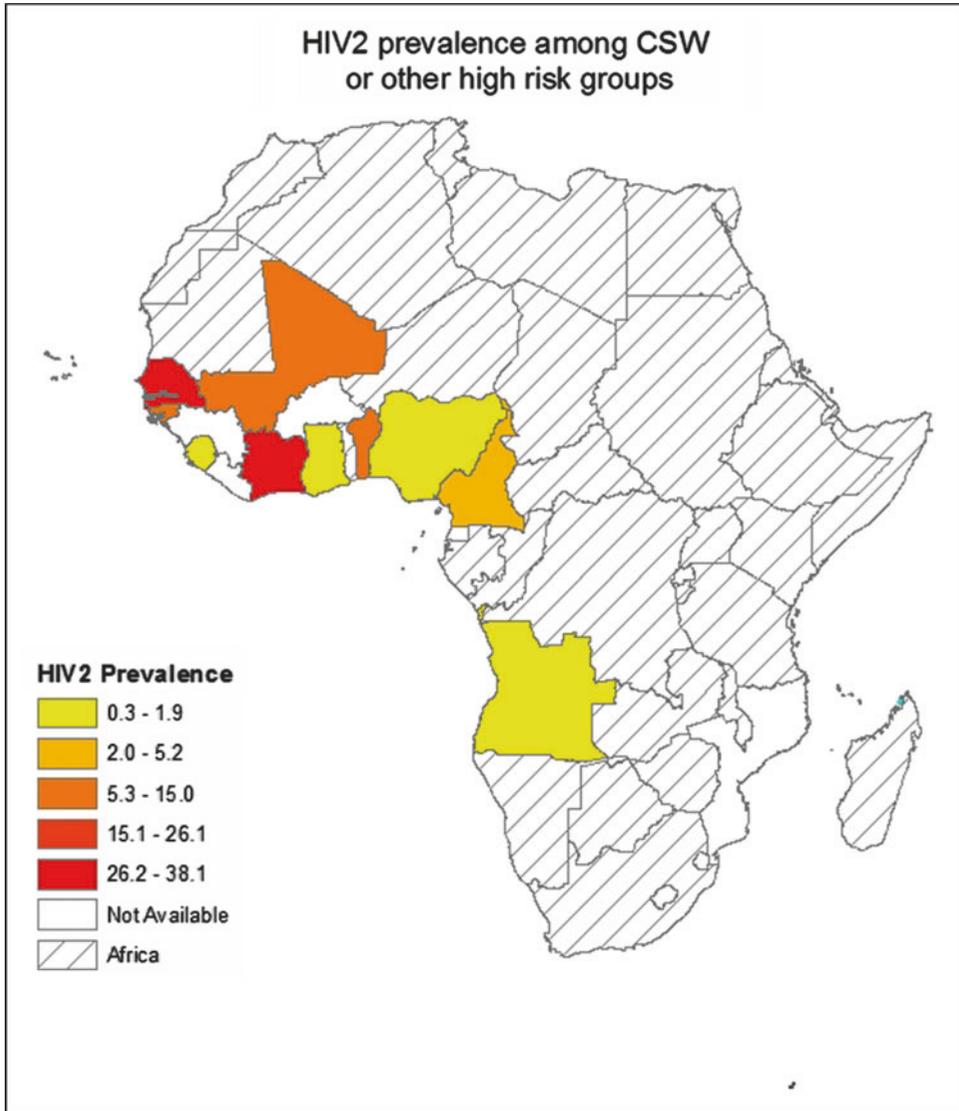
Cross-sectional studies found that HIV-2 infection was associated with having received multiple injections, having been hospitalized, and having undergone ritual excision (in women), suggesting iatrogenic spread. Also, having had sex with a white man, having had extramarital sex, or having worked as a female sex worker (FSW) were associated with HIV-2 in women, which is in line with heterosexual transmission (Poulsen et al. 2000). In studies among younger persons, and thus including more recent HIV-2 infections, a history of travelling and having had genital ulcers, vaginal discharge, or serological evidence of syphilis were associated with HIV-2 infection. These risk factors are also seen in HIV-1 infection and indicate sexual transmission.

Geographical Distribution of HIV-2

The first cases of HIV-2 infection were described in persons from West Africa. Large studies conducted in the late 1980s and the early 1990s confirmed that West Africa, and specifically Guinea-Bissau, was the center of the HIV-2 epidemic. Testing of archived blood samples collected in 1980 in Guinea-Bissau showed antibodies against HIV-2, indicating that the virus must have circulated in the population at that time. A few case reports suggest that some persons were already HIV-2 infected as early as the 1960s in Guinea-Bissau (Pepin 2011). Based on sequence data of the HIV-2 genome (notably of the *env*, *pol*, and *gag* genes), molecular clock analyses have estimated that the introduction of HIV-2 into the human population in West Africa occurred approximately between 1932 and 1962. HIV-1 (subtype CRF02_AG) was introduced later into West Africa, estimated between 1957 and 1986 (de Silva et al. 2012; Lemey et al. 2003).

After the discovery of HIV-2, several countries reported cases of HIV-2 infection and surveys among high-risk populations, pregnant women, and the general population were conducted (Figs. 1, 2, and 3, based on information from a review by de Silva et al. (2010) and updated with more recent data). Guinea-Bissau reported the highest prevalence of HIV-2, 8% in the adult population. The prevalence reached almost 20% in persons older than 45 years old. HIV-2 prevalence is higher in high-risk groups such as female sex workers, tuberculosis patients, or patients attending sexually transmitted diseases (STD) clinics (Fig. 1). For example, in the Gambia in 1993–1995, 22% of female sex workers were HIV-2 infected, while the prevalence among pregnant women was only 1%.

Guinea-Bissau was a colony of Portugal between the early sixteenth century and 1975. HIV-2 has been mostly reported from West Africa, but also in countries outside West Africa which have historical links to Portugal, i.e., Mozambique, Angola, Brazil, and India. The highest prevalence of HIV-2 in Europe has been found in Portugal, with approximately 2,000 cases

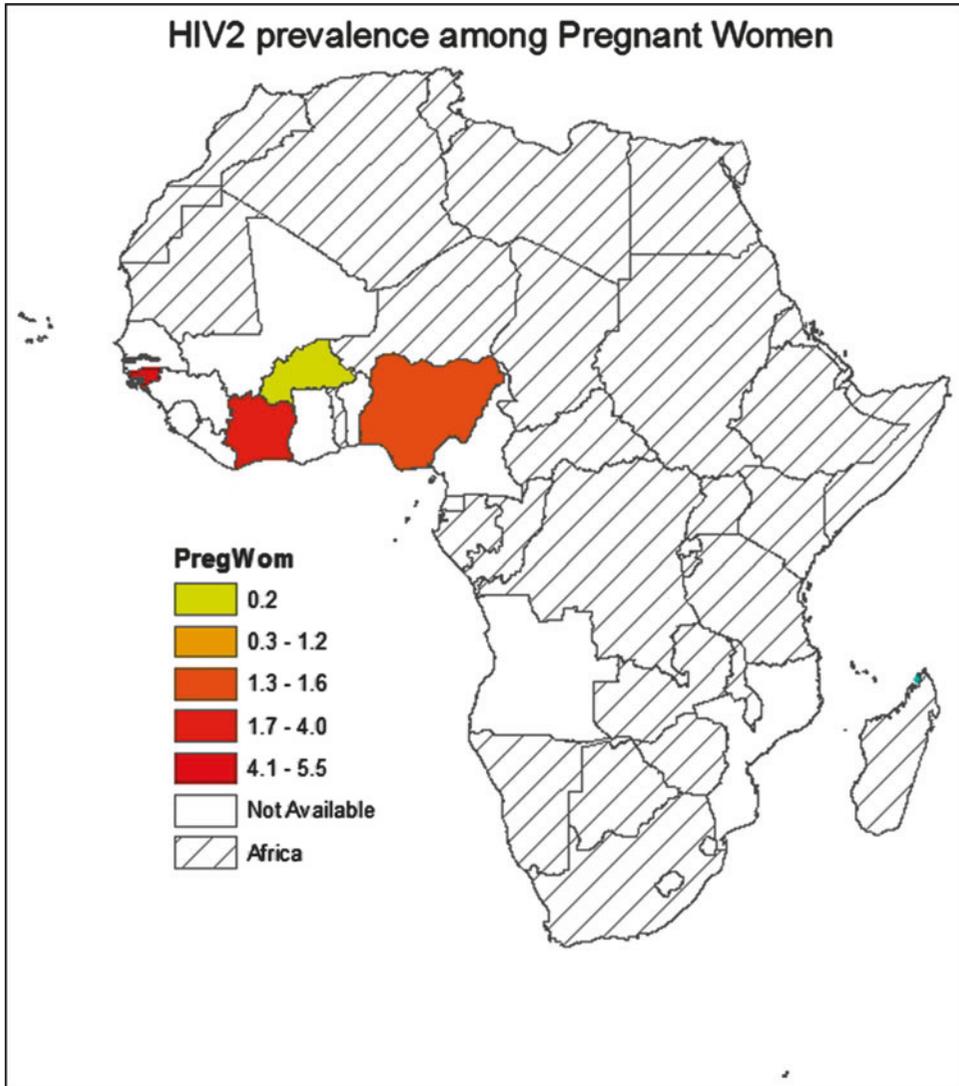


Epidemiology of HIV-2 Infection in West Africa, Fig. 1 Seroprevalence of HIV-2 among female sex workers or other high-risk groups in Africa, by country. Prevalences shown in this figure are the highest prevalences that have been reported for each country. HIV-1/HIV-2 dual infections are included in the HIV-2

prevalence. *White areas* (without stripes) indicate countries where HIV-2 infections have been reported, but no prevalence data are available (Thanks to Dr Mirjam Bakker (Royal Tropical Institute, Amsterdam, the Netherlands) for making Figs. 1, 2 and 3)

reported so far. HIV-2 was mainly found in repatriates and soldiers from the colonial wars in Guinea-Bissau, Angola, and Mozambique, in persons originating from West Africa, or in persons who had sexual partners from West Africa. Genetic studies of HIV-2 corroborate historical

links and epidemiological observations between countries: there is phylogenetic evidence for transmission of HIV-2 between persons from Senegal and Cote d'Ivoire and France and between persons from Guinea-Bissau and Cape Verde and Portugal.



Epidemiology of HIV-2 Infection in West Africa, Fig. 2 Seroprevalence of HIV-2 among pregnant women in Africa, by country. Prevalences shown in this figure are the highest prevalences that have been reported

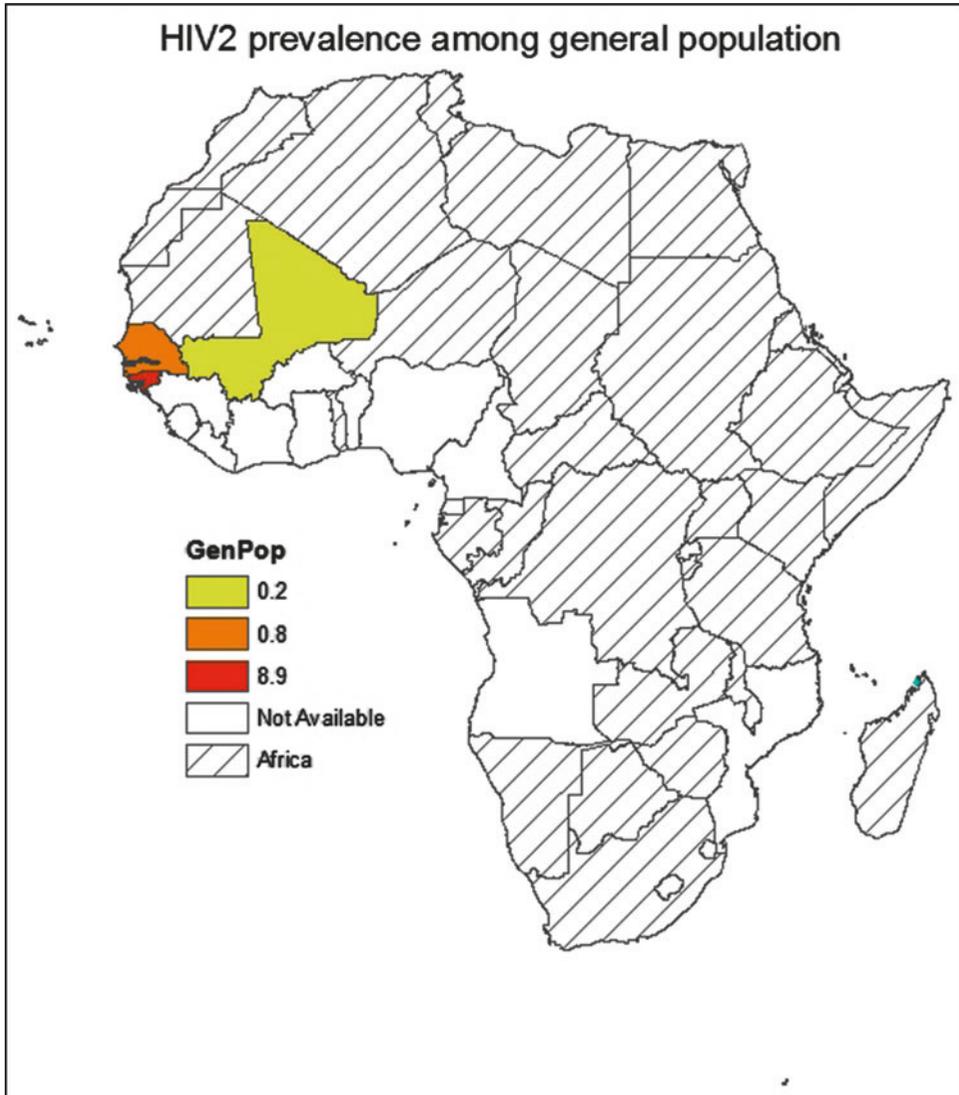
for each country. HIV-1/HIV-2 dual infections are included in the HIV-2 prevalence. *White areas* (without stripes) indicate countries where HIV-2 infections have been reported, but no prevalence data are available

The Spread of HIV-2 in Guinea-Bissau

Guinea-Bissau merits special attention as this country is thought to be the nucleus of the epidemic. Before the first cases of HIV-1 were detected in this country, HIV-2 was widespread. Why did a virus that is much less virulent and less transmissible than its cousin HIV-1 reach a prevalence of 8% in the late 1980s? Both in the capital, Bissau, and in a rural area, Caió, prevalence in the

general population was high (Fig. 3). Three main hypotheses have been postulated and investigated to explain this observation.

First, iatrogenic spread through large vaccination and treatment campaigns may have caused the spread of HIV-2. In the 1950s and 1960s, the Portuguese conducted large treatment campaigns for endemic diseases such as yaws, leprosy, and tuberculosis. In a survey among elderly persons in Bissau, conducted in 2005, having received



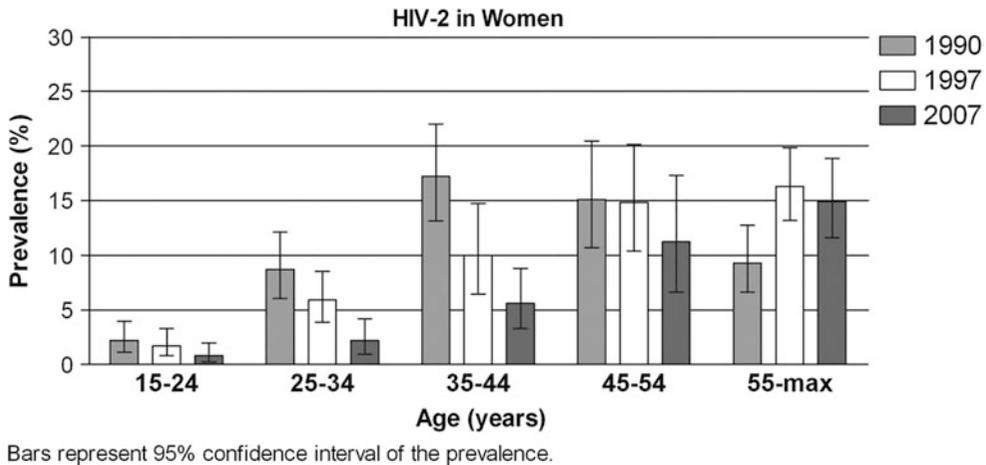
Epidemiology of HIV-2 Infection in West Africa, Fig. 3 Seroprevalence of HIV-2 among general adult population in Africa, by country. Prevalences shown in this figure are the highest prevalences that have been

reported for each country. HIV-1/HIV-2 dual infections are included in the HIV-2 prevalence. *White areas* (without stripes) indicate countries where HIV-2 infections have been reported, but no prevalence data are available

intramuscular treatment for tuberculosis and sleeping sickness were factors associated with HIV-2 infection (Pepin et al. 2006). Vaccinia (smallpox) scars were also associated with HIV-2 infection among persons in both urban and rural Guinea-Bissau (Aaby et al. 2006). Large vaccination campaigns with presumably insufficiently sterilized equipment (e.g., injection needles were used multiple times) are known to

have taken place in Guinea-Bissau before the country reached independence. Phylogenetic analyses of HIV-2 support increased transmission of HIV-2 in 1955–1970 (Lemey et al. 2003).

Second, the protracted War of Independence fought against Portugal between 1963 and 1974 is thought to have facilitated the spread of the virus due to increased sexual risk behavior, unscreened blood transfusions, and possibly



Epidemiology of HIV-2 Infection in West Africa, Fig. 4 HIV-2 prevalence among women by age in three serosurveys in Caio, Guinea-Bissau (van Tienen et al. 2010) (Reproduced with the permission of JAIDS)

increased use of unsterile needles in healthcare facilities.

A third hypothesis that has been proposed for the high initial prevalence of HIV-2 is that the virus may have been more virulent at the start of the epidemic than it currently is. According to this theory, the virus, or certain strains of the virus, became less virulent by adapting to the host. Similar suggestions have been made regarding HIV-1. However, the epidemic may not have been around for long enough to have had such an impact on HIV-2: many of the people who got infected at the time of its presumed higher virulence (the 1960s) were still alive and harboring the virus in the 1980s and 1990s. Also, phylogenetic studies indicate that genetically very similar viruses lead to very different disease courses in infected persons. This suggests that the most important determinant of the outcome of the infection is variation in the host response and not variation of viral strains.

Age and Sex Distribution

The age and sex distributions of people with HIV-2 infection are different from those of people with HIV-1 infection. In HIV-2, prevalence and incidence peak at older adult ages, whereas the peak incidence and prevalence of HIV-1 usually are observed in young adults (HIV-1 prevalence

peaks are now shifting towards older age due to the recent increasing coverage of ART). Figure 4 is an example of the HIV-2 prevalence by age group among women from a general population in Guinea-Bissau, by study year (van Tienen et al. 2010).

The age group with the highest prevalence is older for HIV-2 than for HIV-1 (e.g., for women in 2007 in Guinea-Bissau 45–54 years vs. 20–24 years). This is due to three phenomena: the HIV-2 epidemic is older, survival with HIV-2 is longer, and the transmission rate of HIV-2 is lower, all compared to HIV-1. A clear cohort effect can be seen in Fig. 4: the prevalence increases with age, and within each age group (except ≥ 55 years), the prevalence declines over time. This is compatible with a period of increased transmission in the past. In the case of Guinea-Bissau, this might be linked to the War of Independence. Because older women are more often infected with HIV-2 (a higher prevalence but also a higher incidence) and also more often coinfecting with HIV-1 or human T-cell lymphotropic virus type 1 (HTLV-1), another retrovirus, it has been suggested that older women may be more susceptible to retroviral infections. A better survival of HIV-2-infected women compared to men could also explain the higher prevalence in women. However, studies of mortality of HIV-2-infected people have not been able to confirm this.

Prevalence and Incidence: Rise and Decline

As mentioned previously, HIV-2 was circulating in West Africa before HIV-1 was introduced to this part of Africa. Antibodies against HIV-2 were found in archived blood samples from as early as 1980. Since the 1990s, decreases in prevalence of HIV-2 have been observed in countries where repeated surveys were done, while HIV-1 has risen. The more recent surveys show that HIV-1 is surpassing HIV-2 and is becoming the dominant virus in West Africa. Relatively few studies have reported HIV-2 incidence rates, and in the 1990s, it varied between 0.2 and 1.6 new infections per 100 pyo in Senegal and Guinea-Bissau in general adult populations and FSWs (van der Loeff and Aaby 1999). Both the prevalence and the incidence rate decreased significantly in the 2000s in Guinea-Bissau. It should be noted that these trends represent the natural course of the epidemic; antiretroviral treatment became available in Guinea-Bissau only after 2006, and rollout was slow.

The natural decline of the HIV-2 epidemic might help us understand how the HIV-1 epidemic could be halted as well. Successful antiretroviral treatment (ART) leads to undetectable viral loads and reduces the rate of transmission to almost nil. Thus, antiretroviral treatment programs are expected to lead to “treatment as prevention.” Although this is very promising, more actual studies and more modeling are necessary to estimate what is needed to reduce the reproductive number R_0 (the number of secondary cases in a susceptible population that arise from one infected person) of HIV-1 below 1. When the R_0 of HIV-1 is below 1, transmission would be too rare to sustain the epidemic and HIV-1 would slowly disappear. The declining HIV-2 epidemic could be used as a natural model in which people with undetectable viral load could be compared to people with HIV-1 on successful ART. Studying HIV-2 in detail could thus be used to explore the causes of the decline and so inform “treatment as prevention” programs for HIV-1.

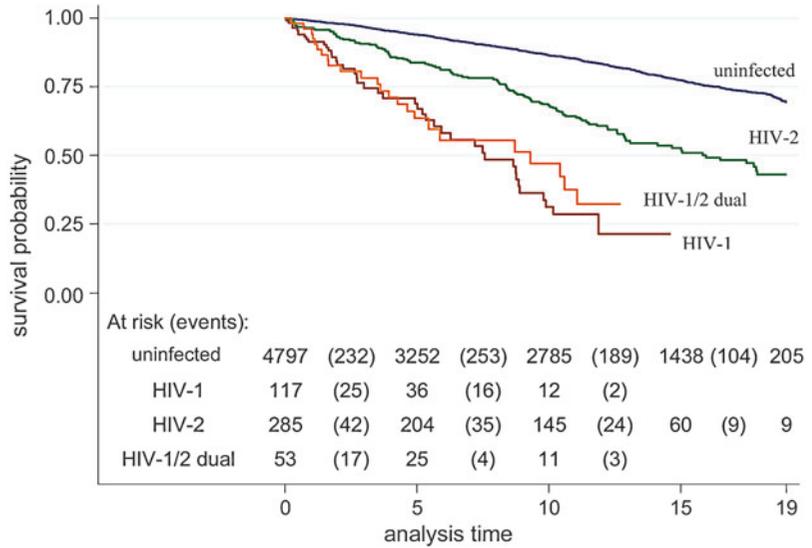
HIV-1 and the Decline of HIV-2

The decline in prevalence in West Africa and elsewhere is consistent with the generally lower viral load and therefore lower transmissibility of HIV-2. In addition, HIV-1 may have a role in the decline of HIV-2 as well. Two different mathematical models explored the contribution of the increase in HIV-1 to the decrease in HIV-2 prevalence. Both suggest that HIV-1 may hasten the decline in HIV-2 by removing susceptible hosts from the population, i.e., individuals at high risk of STDs who drive the spread of HIV-2, contract HIV-1, and die, so they cannot transmit HIV-2 anymore (Anderson and May 1996; Schmidt et al. 2008). Despite the decreased prevalence and incidence, new cases of HIV-2 still occur. This is mainly in older women and in married couples.

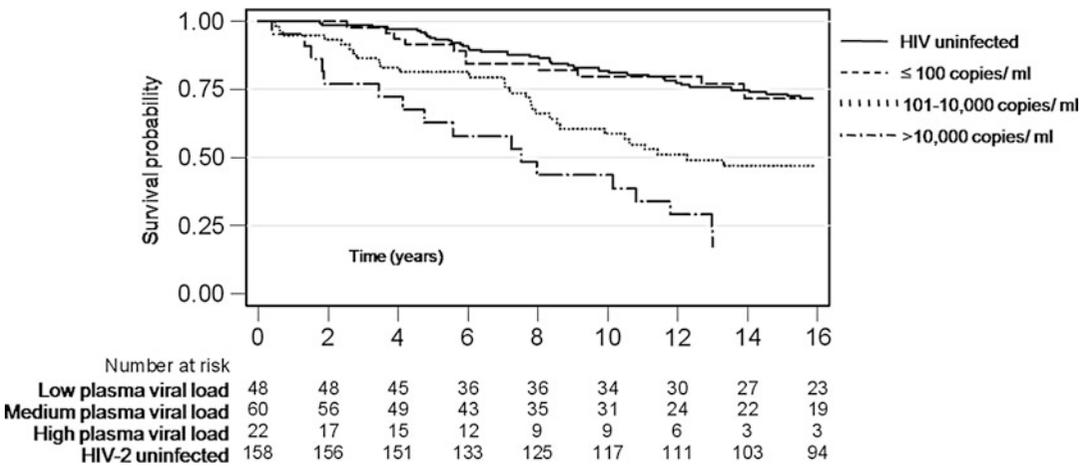
HIV-2 Mortality

The mortality rate of HIV-2-infected people is lower than that of HIV-1-infected people. The median survival after infection (if untreated) is about 11 years for HIV-1; for HIV-2 the median survival time has never been estimated as no seroincident cohort was large enough and followed long enough to establish this. However, based on modeling of CD4 counts and mortality data from a clinical cohort in the Gambia, it was found that half of the HIV-2 infected do not display any disease progression; for the other half, the median time to death was estimated to be 2.7 years longer than that for HIV-1 (Geskus et al. 2009). The mortality rate for HIV-2 infection has been estimated to be twice as high as that of uninfected persons (compared to a 10-fold increased risk of death for HIV-1 infection). In a large community study from Guinea-Bissau, the mortality hazard ratio varied from 1.2 among persons above 60 years of age to 9.1 among the people age between 15 and 29 years. An unadjusted Kaplan-Meier curve illustrates the difference in survival in a cohort study from Guinea-Bissau (Fig. 5) (van Tienen et al. 2011).

Epidemiology of HIV-2 Infection in West Africa, Fig. 5 Kaplan-Meier graph of survival probability by HIV-status, Guinea-Bissau, 1989–2009 (HTLV-1-infected subjects excluded) (van Tienen et al. 2011) (Reprinted in accordance with the Creative Commons Attribution License)



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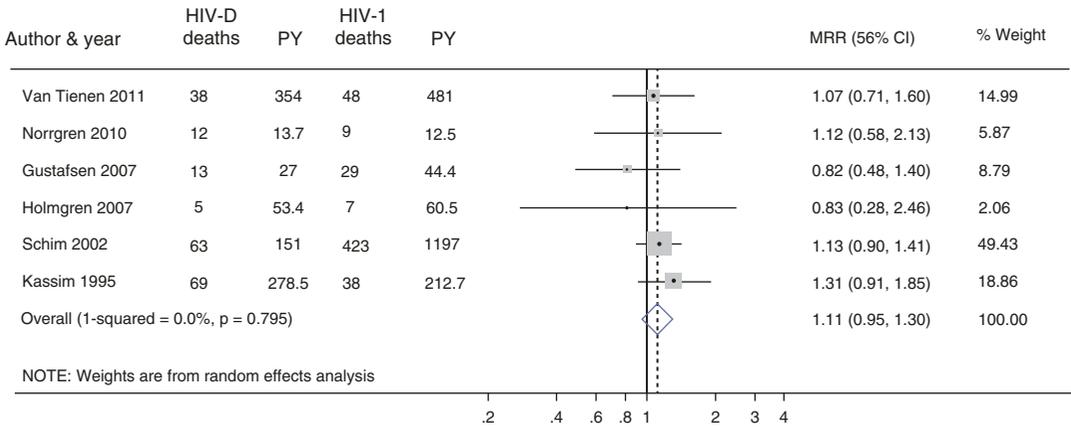
Epidemiology of HIV-2 Infection in West Africa, Fig. 6 Survival probability by viral load of HIV-2-infected and HIV-2-uninfected individuals in Caio,

Guinea-Bissau (Schim van der Loeff et al. 2010) (Reprinted in accordance with the Creative Commons Attribution License)

Thus, in elderly persons, mortality is hardly increased compared to the uninfected population. This is probably due to two factors: first, the background mortality among older individuals is higher than among younger persons and second, there is a survival bias of a group of older individuals who are nonprogressors, leading to a low mortality rate ratio.

The difference in mortality for both viruses is caused by the difference in viral loads. In general, the HIV-2 viral loads are lower than HIV-1 viral

loads. Strikingly, persons with undetectable (<100 viral copies/ml) viral loads (an estimated 30–50% of all HIV-2-infected individuals) have no significantly increased mortality compared to uninfected persons (Schim van der Loeff et al. 2010). In persons with high viral loads, the mortality is similar to that of persons infected with HIV-1 (Fig. 6). Several persons known to be infected with HIV-2 before 1990 were still alive more than 20 years later, but had no HIV-related symptoms.



Epidemiology of HIV-2 Infection in West Africa, Fig. 7 Forest plots showing the individual and combined mortality rate ratios (MRRs) of HIV-D versus HIV-1. (Matser et al. *AIDS* 2014) (Reprinted with permission from AIDS)

Immunological and virological studies have suggested that HIV-2 infection may inhibit the progression of HIV-1 in individuals dually infected by HIV-1 and HIV-2. A recent meta-analysis of studies comparing the mortality rates in HIV-2 and HIV-1 and HIV-2 dually infected individuals found no difference in mortality (Fig. 7) (Prince et al. 2013). Hence, based on epidemiological studies, it seems unlikely that HIV-2 has a mitigating effect on HIV-1 disease progression in dually infected people.

Conclusion

The HIV-2 epidemic has spread mainly in West Africa, and after a high prevalence in the 1980s, it is now an epidemic in decline. The declining epidemic could be used as a natural model to study the most important factors for halting the HIV-1 epidemic by early treatment (“treatment as prevention”). The epicenter of the epidemic is thought to be in Guinea-Bissau, where the highest prevalence has been reported. It is likely that through iatrogenic spread (mass vaccination and treatment campaigns) and a period of increased sexual risk behavior during the War of Independence, HIV-2 has been able to spread widely in Guinea-Bissau. Although the awareness of iatrogenic spread of infections has

increased, lessons can be learned from HIV-2, as it may serve as a model for HIV-1 “treatment as prevention.” Although most countries with reported cases have observed a decline in prevalence, HIV-2 remains a public health problem in West Africa with important excess mortality, and the infection poses diagnostic and therapeutic challenges for physicians, nurses, and healthcare systems.

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Epidemiology of Non-AIDS-Defining Malignancies

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Definition

AIDS-defining malignancies are those which are included in the case definition of AIDS, namely, Kaposi sarcoma, non-Hodgkin lymphoma, and cervical cancer. *Non-AIDS-defining malignancies* are those other malignancies whose incidence is increased in individuals with HIV infection. In some instances, the term can also refer to any malignancy occurring in an individual with HIV infection. More recently, as the incidence of AIDS has declined in people with access to antiretroviral therapy, the term “HIV-associated malignancy” has become more widely used to describe the same group of malignancies.

Introduction

In the early 1980s, a rare form of skin cancer, Kaposi sarcoma (KS), occurring at greatly increased rates among homosexual men, was a harbinger of the AIDS epidemic. Over the subsequent decade, when it became apparent that non-Hodgkin lymphoma (NHL) and cervical cancer occurred at increased rates in people with HIV, these two cancers were added to the case definition of AIDS when they occurred in an HIV-infected person. Collectively, KS, NHL, and cervical cancer are called AIDS-defining cancers and are separately considered in the entry “► [Epidemiology of AIDS-Defining Malignancies](#).”

Even early in the AIDS epidemic, it was recognized that other cancers also occurred with increased rates. In the mid-1990s, the introduction of highly active antiretroviral therapy (HAART)

changed the clinical face of HIV disease. In particular, it led to immune recovery and the much longer survival of people with HIV. In general, immune recovery was only partial, and subtle effects of long-term mild immune deficiency, including the occurrence of certain cancers, began to emerge.

In this entry, the spectrum of non-AIDS malignancies is defined, and then individual cancer types are examined in detail. These cancers are grouped as (1) cancers with a known infectious cause that occur at increased incidence in people with HIV, (2) other cancers occurring at increased incidence in people with HIV, and (3) cancer types that do not occur at increased incidence in people with HIV.

The Spectrum of Non-AIDS-Defining Malignancies

In the late 1990s, as the survival of people with HIV began to increase, it became more apparent that people with HIV were experiencing higher rates of a number of types of cancer than in the general population. This was identified within large-scale studies which linked population-based AIDS and/or HIV registers with cancer registries in several countries including the United States, Italy, and Australia. Increased rates of a wide range of cancers were identified. Initially, there was considerable debate about whether these cancers occurred at increased rates because of HIV-associated immune deficiency. The alternate explanation was confounding, because people with HIV also experience increased exposure to a number of other common carcinogens including tobacco smoke and sexually transmitted and blood-borne oncogenic viral infections. The answer to whether cancer was truly caused by HIV came in part from studying cancer patterns in solid organ transplant recipients. Solid organ transplant recipients are required to take lifelong immunosuppressive medications and so share long-term immune deficiency with people with HIV. In other respects, they do not share similar carcinogenic exposures. A study published in 2007 conclusively demonstrated that these two

populations have a broadly similar pattern of cancer occurrence, with increased rates of about 20, mostly infection-related cancer types (Grulich et al. 2007). The pattern of increased risk of cancer types is summarized in Fig. 1. More recently, cohort studies of people with HIV, which have included data on CD4-positive lymphocyte count and HIV viral load, have demonstrated that many of these cancers are associated with impaired immune function.

In the remainder of this entry, I will review data on the epidemiology of specific cancer types in people with HIV and their association with the level of immune function.

Infection-Related Cancers

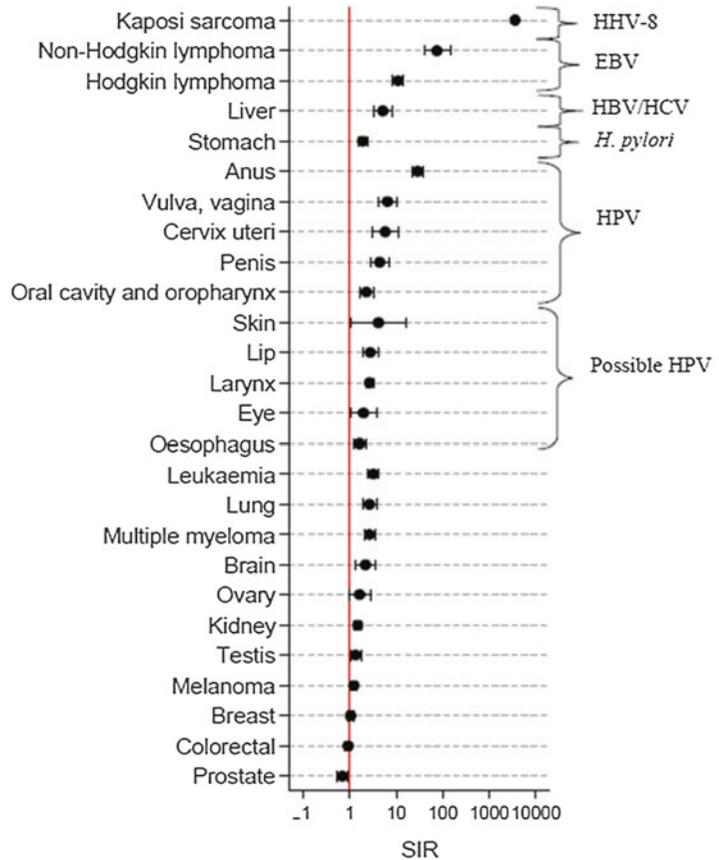
Cancers Related to Epstein-Barr Virus

The World Health Organization's International Agency for Research on Cancer (IARC) classifies Epstein-Barr virus (EBV) as carcinogenic in humans with respect to certain types of non-Hodgkin lymphoma (NHL, including Burkitt lymphoma, immunosuppression-related NHL, extranodal NK/T cell lymphoma of nasal type), Hodgkin lymphoma, and cancer of the nasopharynx (IARC 2009). Data on NHL are summarized in the entry "[► Epidemiology of AIDS-Defining Malignancies](#)" and are not further considered here.

EBV can be detected in 40–50% of cases of Hodgkin lymphoma in developed countries and is even more commonly detected in people with HIV (IARC 2009). Hodgkin lymphoma occurs about 11-fold more commonly in people with HIV than in the general population and is raised about fourfold in organ transplant recipients (Grulich et al. 2007). The increase in risk in people with HIV is not consistent across Hodgkin lymphoma subtypes and is confined to the mixed cellularity and lymphocyte-depleted subtypes. In recent years, rates of Hodgkin lymphoma in people with HIV have been relatively constant, and despite improvements in antiretroviral therapy and immune function, they have not declined. Three cohort studies with longitudinal measurements of CD4 lymphocyte count have described

Epidemiology of Non-AIDS-Defining Malignancies,

Fig. 1 Standardized incidence ratios of cancer types in people with HIV, grouped by infectious cause (Figure based on data presented in Grulich et al. 2007)



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an association between increasing rates of Hodgkin lymphoma and declining CD4 count (Guiguet et al. 2009; Reekie et al. 2010; Silverberg et al. 2011). At least part of the reason why rates of Hodgkin lymphoma have not declined in people with HIV in recent years is that the association with immune deficiency is much weaker than with NHL and Kaposi sarcoma.

Prospective studies have identified a strong relationship between serological markers of EBV infection and future risk of nasopharyngeal carcinoma (IARC 2009). As nasopharyngeal carcinoma is a rare cancer except in people originating from certain areas of Southeast Asia, there are relatively few data on the association with HIV infection. A US-based linkage study of AIDS and cancer registers described a twofold increase in risk, based on only 39 cases, and a sixfold increase in risk was reported in a Chinese cohort of people with HIV.

Cancers Related to Human Papillomavirus

The IARC classifies certain types of human papillomavirus (HPV) infection as carcinogenic in humans with respect to cancers of the cervix, vulva, vagina, penis, anus, oral cavity, oropharynx, and tonsils (IARC 2009). There is weaker evidence that HPV may be associated with cancer at some other sites, including cancer of the larynx (IARC 2009).

Globally, the most common HPV-associated tumor is cervical cancer. Cervical cancer is an AIDS-defining cancer and is reviewed in the entry “► [Epidemiology of AIDS-Defining Malignancies.](#)”

HPV can be detected in over 80% of cases of anal cancer (IARC 2009). Anal cancer occurs at approximately 30-fold increased rates in people with HIV and is also raised about fivefold in organ transplant recipients (Grulich et al. 2007). Risk is greatest in homosexual men with HIV, but

substantial elevations in risk (greater than tenfold) are also seen in men with other risk factors for HIV and in women with HIV (Chaturvedi et al. 2009). Some cohort studies have described an increase in anal cancer incidence in the HAART era, although a plateau in rates appears to have emerged in recent years. In some settings where homosexual men comprise a majority of people with HIV, anal cancer has become the most commonly occurring non-AIDS-defining malignancy (van Leeuwen et al. 2009). Anal cancer is not strongly related to current immune function, although a more moderate association with declining CD4 count has been described in two recent cohort studies (Reekie et al. 2010; Silverberg et al. 2012). Associations with prolonged duration below a CD4 cell count of 200 cells per microliter (Guiguet et al. 2009) and lower CD4 cell count nadir have also been described. The high incidence of this cancer in people with HIV and the lack of a decline in incidence in recent years have led to anal cancer becoming a substantial health issue in people with HIV. Preventive interventions, including the possibility of a screening test for early detection of anal cancer and its precursors, have received considerable research attention in recent years. However, the introduction of screening at a population level has been impeded by the lack of evidence of the efficacy of treatment of the preinvasive lesion and concerns about the accuracy and the complexity of the screening test.

Fewer data have been published on other HPV-related cancers, and only small numbers have been described in cohort studies of people with HIV (IARC 2009), making a description of the relationship with immune function impossible. For cancer of the vulva and vagina, HPV can be detected in about 40% and 70% of cases, respectively (IARC 2009). The cancers are increased about 6- and 23-fold in people with HIV and in organ transplant recipients, respectively (Grulich et al. 2007). HPV can be detected in about 50% of cases of penile cancer (IARC 2009), and incidence rates are increased 4- and 16-fold in people with HIV and in organ transplant recipients respectively (Grulich et al. 2007). Within the oral cavity, there is substantial

variation in the association of cancer types with HPV. The strongest association is with cancer of the oropharynx and tonsils. In recent series, 60% or more of cases have detectable HPV (IARC 2009). For oral cavity cancer overall, incidence rates are increased two and threefold in people with HIV and in organ transplant recipients respectively (Grulich et al. 2007). A US-based AIDS-cancer linkage study reported a modest 1.6-fold increase in oropharyngeal cancer in people with AIDS (Chaturvedi et al. 2009).

Cancers Related to Hepatitis B Virus and Hepatitis C Virus

The IARC classifies hepatitis B virus (HBV) and hepatitis C virus (HCV) as carcinogenic in humans with respect to hepatocellular carcinoma (liver cancer) and, for HCV only, for certain subtypes of NHL. There is weaker evidence that HBV may be related to NHL and that both viruses may be related to cholangiocarcinoma (IARC 2009).

Liver cancer is increased about five and twofold in people with HIV and organ transplant recipients respectively (Grulich et al. 2007). The risk of liver cancer is greatly increased in people with HIV who are coinfecting with HBV and/or HCV (Guiguet et al. 2009), and thus incidence is particularly high in injection drug users and hemophiliacs with HIV. Two cohort studies have reported a moderate association of increased liver cancer risk with impaired immune function (Clifford et al. 2008; Guiguet et al. 2009).

A US-based AIDS-cancer linkage study has recently reported that risk of cholangiocarcinoma was increased by about 40% in people with AIDS, but this did not reach statistical significance (Sahasrabudde et al. 2012).

Cancers Related to *Helicobacter pylori*

The IARC classifies *Helicobacter pylori* (*H. pylori*) as carcinogenic in humans with respect to gastric carcinoma and low-grade B cell mucosa-associated lymphoid tissue (MALT) gastric lymphoma (IARC 2009).

The incidence of stomach cancer is increased about twofold in people with HIV and in organ transplant recipients (Grulich et al. 2007). When examined by subsite within the stomach,

increased risk does occur at non-cardia sites, where *H. pylori* has been casually associated with cancer (Persson et al. 2012). There is some evidence that EBV may cause a proportion of cases of gastric cancer (IARC 2009), and this is another potential reason why the incidence of gastric cancer is increased in people with HIV.

In the US AIDS-cancer match, gastric MALT lymphoma occurred about sixfold more commonly in people with AIDS than in the general population (Persson et al. 2012). *H. pylori* eradication is frequently associated with complete remission of the MALT lymphoma (IARC 2009).

Merkel Cell Carcinoma

Merkel cell carcinoma is a rare but very aggressive skin cancer. In people with HIV, MCC occurs about 13-fold more commonly than in the general population, and it also occurs at greatly increased rates in organ transplant recipients and other immunosuppressed states including chronic lymphocytic leukemia. In 2008, it was discovered that a polyomavirus, now termed Merkel cell polyomavirus, is clonally integrated in Merkel cell carcinoma tissue (Feng et al. 2008). It is now recognized that Merkel cell polyomavirus is the cause of about 80% of cases of Merkel cell carcinoma.

Cancers Occurring at Increased Rates in People with HIV with No Accepted Infectious Cause

In this section, cancers that have been documented to occur at increased rates in people with HIV, but do not have a well-accepted infectious cause, are discussed.

Lung Cancer

The incidence of lung cancer is increased about 2.7-fold and 2.2-fold in people with HIV and in transplant recipients respectively (Grulich et al. 2007). Lung cancer is a common cancer in the general population of developed countries, and in many developed countries, lung cancer is the most common non-AIDS-defining carcinoma as assessed by the number of cases per year. It is

thus a very important cause of morbidity. Assessing the cause of this excess risk is made complex by the fact that people with HIV have a substantially raised prevalence of tobacco smoking. While tobacco smoking has been controlled for using multivariate techniques in several studies, it is difficult to confidently exclude the possibility of residual confounding by dose or timing of tobacco exposure (IARC 2009).

Another possible cause of the increased lung cancer incidence in people with HIV is an unknown pulmonary infection. In favor of this hypothesis is the fact that several cohort studies have found an association between lower CD4 count and increased lung cancer risk (Guiguet et al. 2009; Reekie et al. 2010; Silverberg et al. 2011), although one well-conducted cohort reported no association with immune function (Clifford et al. 2012). One study reported an association of lung cancer risk in people with HIV with recurrent pneumonia (Shebl et al. 2010), but another found no association of lung cancer with AIDS-related pulmonary disease. Irrespective of the cause, lung cancer is a major public health issue in people with HIV.

Conjunctival Cancer

Squamous cell cancer of the conjunctiva is a rare cancer that has been described as occurring at approximately 12-fold increase in people with AIDS in the United States and also occurs at increased risk in kidney transplant recipients. A marked increase in risk of this cancer has been described in African settings (IARC 2009). Both solar ultraviolet radiation and HPV have been hypothesized to contribute to the etiology of this cancer (Chaturvedi et al. 2009).

Nonmelanoma Skin Cancer

In most parts of the world, data on basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin are not recorded by cancer registries, and hence there are relatively few data on their occurrence in people with HIV. In solid organ transplant recipients, SCCs occur about 50 times more commonly than in the general population. In that population, SCC also behaves much more aggressively than in the general

population and is a substantial clinical management issue. It is clear that people with HIV are less predisposed toward SCC than are solid organ transplant recipients. Nevertheless, a recent cohort study in California reported that rates of SCC were increased about twofold, which was a similar increase to the incidence of BCC (Silverberg et al. 2013). In that study, SCC but not BCC was related to lower recent CD4 count. The association with immune deficiency is suggestive evidence that an infective agent may be related to SCC. There is suggestive but not definitive evidence that HPV may play a role.

Melanoma

The incidence of melanoma is increased marginally, by about 24%, in people with HIV, and by 2.3-fold in organ transplant recipients (Grulich et al. 2007). In kidney transplant recipients, the current receipt and intensity of immune suppression increase melanoma risk, suggesting that impaired immunity is related to melanoma risk. The absence of a substantially increased risk in people with HIV suggests that HIV-induced immune deficiency may be less closely linked to melanoma risk than the pharmacological immune suppression in transplant recipients.

Lip Cancer

The incidence of lip cancer is increased by 2.8-fold in people with HIV and is increased very markedly, by about 30-fold, in organ transplant recipients (Grulich et al. 2007). In organ transplant recipients, the increase in lip cancer probably reflects the very large increase in risk of SCC of the skin. In this population, increased risk is associated with current immune suppression, and risk rapidly returns to normal in kidney transplant recipients who stop receiving immune suppression because of kidney graft failure.

Esophageal Cancer

The incidence of esophageal cancer is increased by 1.6-fold in people with HIV and is increased about threefold in organ transplant recipients (Grulich et al. 2007). The increase in incidence in people with HIV appears to be similar for adenocarcinoma and SCC of the esophagus (Persson

et al. 2012). The increased alcohol and tobacco exposure of people with HIV is a potential explanation for these increased rates.

Laryngeal Cancer

The incidence of esophageal cancer is increased by 2.7-fold in people with HIV and is increased about twofold in organ transplant recipients (Grulich et al. 2007). Based on a small case series, HPV does not appear to be associated with this cancer in people with HIV. Increased tobacco is a likely cause of the increased occurrence in HIV disease.

Cancers that Do Not Occur at Increased Rates in People with HIV Infection

Given the large range of cancer types that occur at increased rates in people with HIV, it is notable that for several of the cancer types that occur commonly in the general population, incidence is not increased. These cancers include breast cancer, prostate cancer, and colorectal carcinoma.

Breast Cancer

In a meta-analysis of prospective research, breast cancer incidence was neither increased in people with HIV (meta-analysis SIR 1.03, 95% CI 0.89–1.20) nor in transplant recipients (meta-analysis SIR 1.15, 95% CI 0.98–1.36) (Grulich et al. 2007). Studies from earlier in the HIV epidemic described a decreased incidence of breast cancer compared to the general population. Incidence increased toward that of the general population in more recent years. A potential explanation for that pattern is that prior to effective antiretroviral therapy, women with AIDS received less breast cancer screening, and that with prolonged survival, screening rates are likely to have increased.

Prostate Cancer

In the meta-analysis described above, prostate cancer incidence was decreased about 30% in men with HIV. In transplant recipients, incidence was not significantly different to 1 (meta-analysis SIR 0.97, 95% CI 0.78–1.19) (Grulich

et al. 2007). In the United States, the decreased incidence of prostate cancer in people with AIDS was confined to the era when prostate-specific antigen was being used as a screening test. The deficit in incidence was confined to early-stage cancers and not present for advanced cancer. These findings suggest that decreased prostate cancer incidence is related to decreased cancer screening in men with AIDS.

Colorectal Cancer

In the meta-analysis described above, colorectal cancer incidence was not increased in people with HIV (meta-analysis SIR 0.92, 95% CI 0.78–1.08). In transplant recipients, incidence was increased by about 70% (Grulich et al. 2007). The reason for the increased incidence in organ transplant recipients is unclear.

Conclusion

As the HIV epidemic unfolds, the pattern of cancer occurrence in people with HIV has continued to change. A constant theme has been that people with HIV experience increased oncogenic exposure compared to the general population, and this has led to patterns of cancer that are often very distinct. Over the years, these increased exposures have changed but in most populations have included immune deficiency, tobacco and alcohol, and oncogenic blood-borne and sexually transmitted infectious agents. The occurrence of immune deficiency in millions of people has given us a unique opportunity to examine the role of infection in carcinogenesis. For many types of cancer, this has helped us delineate the role of infection in causing cancer. For a few others, most particularly for prostate and breast cancer, the absence of any increased risk in either people with HIV or organ transplant recipients has provided strong evidence that infection appears not to be an important cause.

The HIV epidemic is over 30 years old, and most people with HIV have acquired infection in early adulthood. With effective antiretroviral therapy, these individuals are living substantially longer, and a substantial aging of the HIV-infected

population is occurring. As a result, the large-scale interaction of the carcinogenic effects of mild immune deficiency with the much higher cancer incidence that is experienced in old age will occur. If people with HIV carry the relative risks of HIV-related cancer into older age, that will lead to a very large increase in the burden of HIV-related cancer. Such increases are already beginning to happen. However, to the extent that effective antiretroviral therapies can completely or nearly completely remove the oncogenic effect of immune deficiency, then such increases may be lessened. Further follow-up of large cohorts of people with treated HIV is required to clarify these issues.

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Epstein-Barr Virus (EBV)

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Definition

There are more than 100 viruses in the herpesvirus family, which is divided into three subfamilies, alpha, beta, and gamma, based on the genome structure and biological behavior of the viruses. Epstein-Barr virus (EBV) is one of the eight herpesviruses currently known to infect humans and is a member of the gammaherpesvirus subfamily, genus *Lymphocryptovirus*. Like all herpesviruses it can establish latent infections and persist for the lifetime of the host. In some individuals it behaves more as a commensal than a pathogen, while in

others it can cause significant and even life-threatening disease.

Introduction

All viruses are problematic for those who are infected with the human immunodeficiency virus (HIV), but the herpesviruses, which are often carried by the vast majority of individuals worldwide and are typically fairly well controlled by a functioning immune system, pose a particular concern. EBV, like its fellow oncogenic gammaherpesvirus, ► [Kaposi's sarcoma-associated herpesvirus](#), can contribute significantly to the morbidity and mortality of HIV. In the developing world primary infection with EBV, which is orally transmitted in saliva, usually occurs in the first few years of life. In the developed world infection generally occurs somewhat later in the second decade or beyond. Few individuals anywhere, however, escape infection by midlife and in none has elimination of the virus been documented.

Biology of the Virus and Colonization of the Host

EBV shares the structural characteristics of all herpesviruses. It has a relatively large linear double-stranded DNA genome of approximately 175 kb, which encodes more than 80 genes. The genome is tightly housed in a proteinaceous icosahedral capsid and is wrapped in a lipid envelope carrying multiple copies of eleven different membrane glycoproteins. An additional space between the envelope and the capsid includes proteins and RNAs capable of modifying virus and host cell function. These are collectively known as the tegument (Kieff and Rickinson 2007).

EBV has two major target cells, B lymphocytes and epithelial cells, and it appears to behave very differently in each. The prevailing dogma in the field is that, in vivo, virus primarily establishes long-term latency in B cells and primarily replicates in epithelial cells.

In an uncomplicated, asymptomatic, primary infection, incoming virus, either cell-free or

associated with lymphocytes found in saliva, accesses B cells in the lymphoid tissue of Waldeyer's ring, the tonsils, and adenoids that surround the pharynx (Thorley-Lawson and Gross 2004; Hislop et al. 2007). This occurs perhaps directly or perhaps subsequent to an initial round of replication in a mucosal epithelial cell. B cell infection does not immediately lead to productive replication, but instead the virus genome circularizes and remains extrachromosomal and all initial transcripts are devoted to maintaining the episome and changing the behavior of the cell.

This type of latent infection is easily modeled *in vitro* by infection of peripheral B cells and their immortalization into the lymphoblastoid cell lines that are so frequently used for study of human tissue. It involves expression of a full panoply of latency proteins, the latency III phenotype or growth program, including six EB nuclear antigens or EBNA, EBNA 1, EBNA2, EBNA3A, EBNA3B, EBNA3C, and EBNA-LP; the latent membrane proteins, latent membrane protein 1 (LMP1) and latent membrane protein 2 (LMP2); two sets of small, abundant untranslated RNAs, EBER1 and EBER2; and some miRNAs. These collectively drive the cell to become a proliferating blast cell in which the latent virus episome is replicated by host polymerases (Kieff and Rickinson 2007). In one prevalent model of colonization of the host (Thorley-Lawson and Gross 2004), they are considered responsible for initiating the passage of the infected cell down a pathway that parallels that of an antigen-stimulated B cell during a normal immune response. Unlike in the lymphoblastoid cell line *in vitro*, where continued expression of the latency III phenotype maintains the cell in a proliferative state, a switch in promoter usage is then thought to limit expression to the latency II phenotype in which a subset of latency proteins including EBNA1, LMP1, and LPM2 are expressed. This mimics positive selection by antigen stimulation, rescues the cell from the default of apoptosis, and enables it to emerge as a long-lived virus-carrying memory B cell. Memory B cells can circulate in the periphery and seed distant sites. Protein expression in the memory B cells can then be further limited to the

latency I phenotype, in which only EBNA1 and some miRNA are expressed. EBNA1 tethers the genome to the chromosome, which is required for its maintenance in dividing cells (Frappier 2012). In addition to these phenotypes, a resting cell may express no virus proteins at all. This means that while an emerging immune response, primarily a CD8⁺ T cell response (Long et al. 2011), can control cells that express EBV-encoded proteins, resting memory cells remain invisible to the immune system and persist as the long-term reservoir of virus in the infected host. Sporadic terminal differentiation into plasmablasts in Waldeyer's ring triggers reactivation of virus into the lytic cycle, leading to the ultimate death of the cell and production of virus. At this point virus can reenter an epithelial cell and undergo amplification for transmission in saliva to a new host or for reinfection of a B cell and replenishment of the latent reservoir, completing the ongoing cycle of persistence; infectious virus is thus continually found in saliva in healthy carriers (Jiang et al. 2006). In individuals treated over very long periods with valacyclovir, which inhibits lytic virus DNA replication mediated by the viral DNA polymerase but does not affect the latent episome, the frequency of latently infected memory cells is reduced. It has, however, been estimated that it would take at least 11 years of daily therapy to eliminate the latent reservoir entirely (Hoshino et al. 2009).

Despite its assumed occurrence *in vivo*, full lytic replication resulting in production of new virions has not yet been achieved *in vitro* in epithelial cells. In these cells, if EBV persists at all, which it frequently does not, the virus becomes latent. Because of this experimental limitation, lytic replication of EBV has primarily been studied by reactivating virus in latently infected B cell lines. A variety of stimuli including cross-linking of surface immunoglobulin, which may mimic events leading to terminal differentiation triggered by antigen cross-linking or addition of transforming growth factor beta 1, phorbol esters, or histone deacetylase inhibitors, can be used to induce virus reactivation in a variable proportion of cells. Thus a picture of the entire life cycle of the virus at the cellular level comes not from a

continuum but from piecing together early events involving virus entry, which can be studied in both cell types and reactivation from latency.

Infection of a B cell is initiated by attachment of the virus through the binding of one of the envelope glycoproteins, gp350, to the cellular complement receptor type 2, CR2/CD21 (Hutt-Fletcher 2007). The virus is then endocytosed into what becomes a thin-walled vesicle or endosome, and its envelope fuses with the endosomal membrane to deliver tegument and capsid into the cytosol. Fusion of virus and cell is triggered by an interaction between envelope glycoprotein gp42 and cellular human leukocyte antigen (HLA) class II. Glycoprotein gp42 exists as part of a trimeric complex with glycoproteins gH and gL (gHgL) to which it is thought to transmit the activating trigger. Current models (Connolly et al. 2011) propose that the activation of gHgL via gp42 in turn activates the core virus fusion machinery comprised of gHgL and glycoprotein gB. Glycoprotein gB is thought to be the ultimate fusogen, but it requires gHgL for function.

As on a B cell, epithelial cell infection may be initiated by binding of glycoprotein gp350 to CR2, which is expressed on some, though not all, epithelial cells. Infection of a CR2-negative epithelial cell *in vitro* can be more efficient if cells are overlaid with virus-producing B cells (Imai et al. 1998) than if cell-free virus is used, but the major players in attachment and fusion are probably the same. In addition to gp350 binding, attachment can also occur as result of binding of the dimeric complex of gHgL to one of three integrins, $\alpha v\beta 5$, $\alpha v\beta 6$, or $\alpha v\beta 8$. These same integrins then directly trigger the core machinery of gHgL and gB to mediate fusion with epithelial cells, which lack constitutive HLA class II expression (Chesnokova et al. 2009). The use of two different triggers of fusion has allowed the virus to evolve an ingenious strategy for trafficking alternately between B cells and epithelial cells during the cycle of persistence. The virion contains both dimeric gHgL complexes and trimeric gHgLgp42 complexes. Only the trimeric complexes can support fusion with B cells because of the required interaction between gp42 and HLA class II. Only dimeric complexes can support fusion with an

epithelial cell because of the required interaction between gHgL and an integrin, which is blocked by the presence of gp42. In a B cell with replicating EBV, some trimeric complexes interact with HLA class II in the endoplasmic reticulum and are lost to the HLA class II trafficking pathway. Virus emerges rich in dimeric complexes and is about fivefold more infectious for an epithelial cell than a B cell. Conversely, virus emerges from an HLA class II-negative epithelial cell with more trimeric complexes and can be as much as one hundredfold more infectious for a B cell than an epithelial cell (Borza and Hutt-Fletcher 2002).

Once in the cytoplasm of the cell, the virus ultimately travels to the nucleus where the genome and some virus proteins enter through the nucleopore and the episome is established. The events that follow initiation of lytic reactivation then follow a pattern similar to those of other herpesviruses that enter a productive lytic cycle directly. Gene expression is divisible into three general classes, immediate early, early, and late. The immediate early proteins Zta and Rta, encoded by the BZLF1 and BRLF1 genes, are transactivators. Expression of these immediate early proteins is followed by the activation of expression of early proteins, which include enzymes required for DNA synthesis, and finally late proteins, which are principally the structural components of the new virion (Kieff and Rickinson 2007). Assembly of the DNA-containing capsid occurs in the nucleus, and it is assumed, primarily by analogy with work done with other herpesviruses, to follow an envelopment-deenvelopment pathway of egress (Mettenleiter 2002). It is believed that the capsid first acquires a primary envelope as it buds through the inner nuclear membrane; loses it as it fuses with the outer nuclear membrane; acquires tegument proteins and a secondary envelope as it buds into a compartment of the secretory pathway, possibly the trans-Golgi; and is released from the cell by exocytosis.

The latently infected resting memory B cell can evade immune recognition by virtue of expressing few or no EBV proteins. The virus has also, however, evolved a series of approaches to blunting both innate and adaptive immune responses to

lytic cycle proteins. Three early proteins, pBNLF2A, pBILF1, and pBGLF5, have been identified as variously binding to the TAP transporter and interfering with peptide delivery to HLA class I, binding to HLA class I itself and blocking its export, and effecting a general shutoff of host protein synthesis, which limits the expression of new HLA proteins. Perhaps to compensate for the loss of HLA protein, which could activate natural killer (NK) cell recognition, one of the EBV miRNAs can in turn reduce expression of the NK activating ligand MicB. The immediate early protein, Zta, downregulates HLA class II by targeting inhibiting transcription of the class II transactivating protein, and the glycoprotein gp42 can interfere with HLA class II recognition by CD4+ T cells (Long et al. 2011).

Disease Associated with Primary Infection

Primary infections with EBV, particularly those occurring before adolescence, are almost always asymptomatic or at least unrecognized. However, as many as 70% of those in whom infection first occurs during adolescence or later in life develop infectious mononucleosis (Odumade et al. 2011). This syndrome is generally thought to be an immunopathology and to reflect a more intense version of what occurs during an asymptomatic infection, though why it occurs more frequently in adolescence than in infancy remains unclear. The incubation period is estimated at about 6 weeks, which, together with the fact that mononucleosis does not occur epidemically, has made the early events difficult to document. Once the classic symptoms of fever, sore throat, fatigue, and cervical lymphadenopathy appear, sometimes accompanied by eyelid edema, splenomegaly, and liver abnormalities, one of the pathognomonic features is a lymphocytosis including large atypical lymphocytes. These atypical cells are activated CD8+ T cells, and in mononucleosis caused by EBV, they are directed at controlling latent and lytically infected cells. Responses to epitopes derived from lytic cycle proteins dominate. The onset of symptoms coincides with the

activation and proliferation of the CD8+ T cells, and they, with attendant cytokines and perhaps an increase in the number of NK cells in the periphery, are thought to be primarily responsible for the clinical symptoms (Hislop et al. 2007; Odumade et al. 2011). Diagnosis is then typically based on these clinical symptoms, on the presence of a lymphocytosis and also on the production of heterophile antibodies, which appear transiently during the acute phase. These antibodies are measured by the monospot test in which agglutination of the red blood cells of sheep or other species is assessed. Heterophile-negative infectious mononucleosis does, however, occur, and diagnosis may need to be further refined by examining specific antibody responses. Acute infection is typically associated with high levels of antibodies to early lytic (EA) and late lytic antigens (VCA), in particular immunoglobulin M (IgM) antibodies, and absence of antibodies to EBNA1, which become more readily detectable in months following acute disease (Odumade et al. 2011).

Uncomplicated infectious mononucleosis resolves as cells expressing EBV antigens decline in number, with an accompanying decrease in the number of reactive activated CD8+ T cells. There are, however, two extremely rare disorders that can follow primary infection (Hislop et al. 2007). One occurs in patients with a genetic disorder, X-linked lymphoproliferative disease (XLP), in which there is a mutation in the SH2D1A gene encoding SLAM-associated protein (SAP). SAP deficiency produces multiple immunologic abnormalities including defects in NK and T cell effector functions. Following EBV infection there is an exaggerated NK and CD8+ T cell response, but one which fails to control infected cells. High levels of cytokines are produced, macrophages become activated, and a hemophagocytic syndrome can develop and prove fatal. The second disorder is not familial but occurs more commonly in children in Southeast Asia than elsewhere. It appears to result from an unusual entry of EBV into T cells and NK cells. High levels of cytokines are again produced, and virus loads remain very high in peripheral blood. A potentially fatal viral-associated hemophagocytic syndrome may develop, or the disease may rather present with

chronic or recurrent mononucleosis symptoms accompanied by large expansions of EBV-positive NK and T cell clones. In vitro studies of infected T cell lines have demonstrated the down-regulation of SAP by LMP1, suggesting that the underlying pathogenesis of the disorder may resemble that of XLP.

Diseases Associated with Long-Term Carriage of Virus

For the vast majority of infected individuals, long-term carriage of EBV has little apparent consequence. EBV-induced infectious mononucleosis does not recur, and although virus shedding continues indefinitely, it is controlled and has no deleterious effects. The virus is, however, potentially oncogenic. It has after all evolved, to profoundly change the behavior of latently infected cells, and has been implicated in the development of long list of cancers (Rickinson and Kieff 2007). Some of these, including ► [plasmablastic lymphoma and leiomyosarcoma](#), which are almost exclusively seen in HIV-infected individuals, ► [aids-associated Burkitt's lymphoma](#), ► [AIDS-associated Hodgkin's lymphoma](#), and ► [immunoblastic lymphoma](#), are linked in some way to immune suppression. Others such as endemic and sporadic Burkitt's lymphoma, Hodgkin's lymphoma, NK/T cell lymphoma, undifferentiated nasopharyngeal carcinoma, and gastric carcinoma are not. Some are always EBV associated; some have only a partial association.

The role that the virus plays in immunoblastic lymphomas in the context of iatrogenic immunosuppression is possibly the most straightforwardly etiologic. Posttransplant lymphoproliferative disorders occurring early after transplant-related immunosuppression, most of which are immunoblastic lymphomas, can begin as polyclonal expansions of EBV-infected B cells in uncontrolled latency III and may respond to reduction in immunosuppression, suggesting that they are driven by EBV gene expression (Cohen 2005). They are most common in EBV-seronegative recipients, consistent with the assumption that it is failure to control initial

infection of a B- and a failure of the infected cell to transition into the resting memory B cell compartment cell (Thorley-Lawson and Gross 2004). Many such tumors, however, particularly those that arise several years after transplantation, are monoclonal and may carry mutations and chromosomal abnormalities. The vast majority are still EBV positive, but the role of the virus is less certain. This is also the case for the systemic EBV-associated AIDS-associated lymphomas, which, although they are associated with immune deregulation, can arise in patients with relatively high CD4 counts. In general, their incidence has not dropped as profoundly as certain other tumors, such as Kaposi sarcoma, with the advent of highly active antiretroviral therapy (HAART) (Appley et al. 2000). However, some EBV-associated lymphomas, such as primary central nervous system lymphoma, are generally associated with profound immunosuppression and are rarely seen in HIV patients receiving HAART.

Endemic Burkitt's lymphoma is the tumor in which EBV was originally identified. It is a tumor typically seen in children in East Africa in areas of holoendemic malaria, essentially always carries latent virus, and generally expresses a latency I phenotype. Sporadic and AIDS-associated Burkitt's lymphomas, found elsewhere, are less consistently associated with EBV. The principal underlying cause of all Burkitt's tumors is believed to be the c-myc translocation that they all carry. They are typically B cells that have undergone somatic hypermutation, a process during which any B cell is more vulnerable to the occurrence of inappropriate translocations. EBV upregulates the activation-induced deaminase required for hypermutation and translocation and can counterbalance the apoptotic effects of c-myc overexpression (Gromminger et al. 2012). EBV, HIV, and malaria are also all potent B cell drivers, and this is one possible contribution that each may make to the development of the tumor (Magrath 2012).

About 50% of non-AIDS-associated Hodgkin's lymphomas are EBV positive, and the risk for the development of Hodgkin's lymphoma is increased about fourfold following infectious mononucleosis. Many Hodgkin's lymphomas

and most EBV-positive lymphomas, which typically expressed the latency II phenotype, have nonproductively rearranged immunoglobulin genes, and it has been suggested that the role of EBV may be to rescue such a cell, which otherwise would be lost to apoptosis because of the absence of antigen stimulation (Thorley-Lawson and Gross 2004).

Extranodal nasal NK/T cell lymphomas and undifferentiated lymphoma are relatively rare tumors, most common in East Asia, and are typically also associated with EBV (Nava and Jaffe 2005). As noted above infection of NK and T cells is not typically seen in uncomplicated infection with EBV, and these cells are not known to harbor virus in generally healthy individuals. However, just as infection of NK and T cells in the context of viral-associated hemophagocytic syndrome has potentially life-threatening outcomes, the EBV-positive NK/T cell lymphomas are particularly aggressive. It is perhaps of interest that both are most common in the same part of the world.

Essentially all undifferentiated nasopharyngeal carcinomas (NPC) carry latent EBV, often with a latency II phenotype. It is generally agreed that EBV is an essential risk factor for the disease, but the view that is developing is that it may play the role of a tumor promoter rather than a tumor initiator (Lo et al. 2012). Although the cancer occurs worldwide, it is most common in ethnic Southern Chinese, in Eskimos of Alaska and Greenland, and in some populations in North Africa. In Southern China, the annual incidence rate in men, in whom the disease is more prevalent, is more than 20 per 100,000. NPC also shows a familial distribution which has facilitated studies of its etiology. The current model of tumor development is that genetic abnormalities, perhaps exacerbated by exposure to carcinogens, facilitate infection and latency of EBV. EBV then contributes further to disease as a result of expression of RNAs and proteins that influence the cell environment, perhaps effect epigenetic changes, and lead to outgrowth of a faster growing clone (Lo et al. 2012). High titers of antibodies to lytic cycle proteins presumably reflective of a burst of lytic replication precede development of disease by several years and are prognostic (Zeng

et al. 1985). The association of EBV with gastric cancer is less strong with only about 10% of cases being positive, but on a global scale this amounts to possibly the largest tumor burden associated with the virus. Again, it is generally believed that EBV is playing some role in the development of the cancer and emphasis has been put on the epigenetic changes that it can induce, particularly, CpG island methylation.

Finally, it should be noted that there is one, and currently only one, disease associated with lytic replication of EBV, namely, oral hairy leukoplakia (OHL). OHL was first recognized at the beginning of the AIDS epidemic. It is a feature of substantial immunosuppression and is infrequently seen outside the context of full-blown AIDS. It is an epithelial lesion, resembling thrush in appearance and is typically seen on the lateral borders of the tongue. In patients receiving HAART, it is rarely seen (Nokta 2008).

Vaccines and Therapeutics

To date there is no licensed vaccine for EBV, although there is a general consensus that there is a need for both a preventive and a therapeutic vaccine. One phase 2 trial of a soluble form of the glycoprotein gp350 has been performed (Sokal et al. 2007). It failed to prevent infection but did significantly reduce the incidence of infectious mononucleosis in those who were infected. It has been suggested that T cell epitopes should be included in any improved vaccine to eliminate B cell infections (Long et al. 2011), although a recent study of the efficacy of a combination of gp350 and EBNA3 proteins from the very closely related lymphocryptovirus in rhesus monkeys did not support this (Sashihara et al. 2011). Use of gp350 alone was the most effective approach in this model, and it significantly reduced virus load. Clearly more work is needed to explore additional candidate proteins.

EBV replication is sensitive to valacyclovir and related drugs, but no EBV-associated disease, beyond OHL has been shown to be amenable to treatment with them. Considerable progress, however, has been made toward generating

EBV-specific T cells for therapy of posttransplant lymphoproliferative disease, and efforts are ongoing to extend the approach to treatment of other tumors expressing EBV antigens. The main challenge has been to overcome the potentially immunosuppressive environment of the tumor and the fact that there are fewer CD8⁺ epitopes in those tumors that express the latency II phenotype (Long et al. 2011).

Conclusion

Like many of its herpesvirus brethren, EBV is a very successful virus that has evolved primarily for peaceful coexistence with its host. Even the best of relationships can go wrong, however, and, given the vast numbers of people who carry EBV, even relatively rare outcomes can have significant consequence. The ever-growing list of diseases associated with long-term carriage gives pause for concern. Several of the tumors arising in individuals at known risk, transplant recipients or populations at risk for NPC, are potentially amenable to early diagnosis by monitoring for increased DNA loads in blood or, in the case of NPC, altered antibody profiles, and these kinds of preemptive measures are ongoing. There has been no big effort to this point to interfere with infection *ab initio*, but this may be changing. Prevention of infection with any virus capable of establishing latency is extremely challenging, as experience with HIV vaccines has shown us. Unlike HIV, EBV is, however, antigenically very stable, so the prospects for success are brighter.

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Faith Based Interventions

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Definition

Religion and spirituality have social, cultural, and historical importance in most societies. Indeed, some argue that religion and spirituality are at the heart of being human and are an important aspect of socialization. Nevertheless, until recently, religion and spirituality were at best ignored and at worst denigrated by social scientists and health professionals (Koenig et al. 2001). Acceptance of religion- and spirituality-related research has been fueled by empirical evidence supporting the efficacy of faith-based health promotion interventions and the relationship between dimensions of religion and numerous mental and physical health outcomes (Koenig et al. 2001; DeHaven et al. 2004; Campbell et al. 2007; Yeary et al. 2012). In the area of sexual health, dimensions of religion and spirituality have been associated with several HIV risk behaviors

(Koenig et al. 2001). However, most HIV-related religion-health research is cross-sectional, and the faith-based HIV risk-related interventions that have been conducted are primarily among Protestant Christian study populations in the United States. As a result, the specific ways that positive (e.g., religious support and religious coping) and negative (e.g., stigma and social isolation) dimensions of religion and spirituality affect HIV risk behaviors are poorly understood.

Advantages of Faith-Based HIV-Related Interventions

The Centers for Disease Control and Prevention and the 2010 National AIDS Strategy for the United States have both called for greater involvement of faith communities in addressing HIV in the United States (Sutton and Parks 2013). Likewise, international organizations such as the Joint United Nations Program on HIV/AIDS, the United Nations Children's Fund, the World Health Organization, and the United States Agency for International Development have also appealed to faith-based organizations to help address HIV (Woldehanna et al. 2005). There are multiple reasons for requesting greater involvement from faith-based organizations. Partnering with religious organizations often adds credibility to community-based health research (Campbell et al. 2007). Faith-based organizations can provide a unique and opportune venue to recruit

participants and carry out community-based health promotion research because of their stable memberships, regularly held meetings, and available resources (Campbell et al. 2007). Furthermore, studying the role of religion and spirituality in health promotion may be particularly important for research efforts targeting health disparities in minority and underserved populations (Campbell et al. 2007) since many faith-based interventions in these communities frequently enjoy enhanced cultural relevance (DeHaven et al. 2004). These benefits are believed to be due to the community's extant view of local faith-based organizations as sources of spiritual support and social outreach and religion's role as a source of personal strength and cultural cohesion (Levin et al. 2005).

Core Elements of Faith-Based Interventions

Most faith-based HIV interventions share common fundamental elements. A critical element of several faith-based interventions is the use of a community-based participatory research (CBPR) approach to support collaborative partnerships between the faith community and the researchers (Francis and Liverpool 2009; Williams et al. 2011). The involvement of members of the faith-based organization in intervention development, implementation, evaluation, and even dissemination can be advantageous to all parties. Collaboration can help to establish trust, build effective and equitable partnerships, create open and honest dialogues, and allow researchers to gain a better understanding of the attitudes and norms of the faith community, while members of the faith community gain a better understanding of research methodology and a sense of ownership in the intervention and its outcomes (Francis and Liverpool 2009; Williams et al. 2011). Following CBPR principles may also result in a more effective and sustainable faith-based intervention, greater participation by the faith-based organization, and greater cultural understanding and sensitivity among the researchers (Francis and Liverpool 2009; Williams et al. 2011).

Another core element of faith-based HIV-related interventions is the use of cultural targeting, defined as "the use of a single intervention approach for a defined population subgroup that takes into account characteristics shared by the subgroup's members (p. 136)" (Kreuter et al. 2003). Thus, culturally targeted faith-based HIV prevention, testing, and support messages should be adapted to be applicable to the beliefs and values of the faith community. Incorporating content that is culturally, socially, and historically relevant to the characteristics and religious beliefs of the community will enhance the intervention's relevance and comprehension among participants (Campbell et al. 2007; Williams et al. 2011). Cultural targeting of the intervention design, materials, and content naturally flows from the use of a collaborative and equitable approach to research. Lastly, the use of nonjudgmental and non-stigmatizing language and content is essential to any HIV-related intervention. Compassion and support are often the hallmarks of faith-based interventions because compassion and support for the sick and vulnerable are central tenets of most religions and faith-based organizations. However, early in the collaboration, partnering researchers may need to help faith leaders address lingering stigma surrounding HIV within their communities of faith (Francis and Liverpool 2009).

Definitions of Faith-Based Interventions

According to DeHaven et al. (2004), an intervention is faith-based only if it arises from a church's health ministry or a special interest group (DeHaven et al. 2004). In contrast to this strict criterion, Lasater et al. (1997) describe four levels of faith-based interventions. The first level requires the church to be used as the recruitment site for the intervention; the second level requires that the intervention be delivered at a church; the third level includes members of local churches in intervention delivery; the fourth level includes spiritual elements in the health message of the program (Lasater et al. 1997). Most published faith-based HIV-related interventions fulfill the requirements of being faith-based according to

these definitions (Williams et al. 2011). Faith-based interventions may also vary by the extent to which they incorporate religious or spiritual elements into their intervention content (e.g., interventions being faith-based versus church-placed), which can range from matching observable characteristics on printed materials to adapting intervention content to comply with core cultural values and sociohistorical factors (Resnicow et al. 1999).

Barriers and Challenges to Faith-Based Interventions

The primary challenge to implementing faith-based HIV interventions is reconciling religious doctrines with HIV risk behaviors, including illegal drug use and unprotected sex outside of a monogamous relationship or marriage. Many religious traditions emphasize abstinence until marriage and consequently may be resistant to endorsing sexual education programs that include other risk reduction strategies. However, empirical findings support the use of comprehensive sexual education curricula as a more effective method of reducing HIV risk (Underhill et al. 2007). Researchers must appreciate the difficult position of faith leaders who seek to host interventions in their places of worship; researchers may need to be willing to compromise and revise intervention content and project goals accordingly. Other strategies that may help navigate this sensitive negotiation process include providing faith leaders with knowledge about the HIV epidemic in their community and similar communities and actively collaborating with faith leaders throughout the intervention so they feel a sense of ownership in the intervention and its outcomes. Researchers can also address this challenge by developing faith-based interventions that allow participants to use a personal construction of spirituality and faith rather than utilizing formal religious tenets. An example of a faith-based intervention that does this is discussed below (Margolin et al. 2006).

Stigma and homophobia are also barriers to effective faith-based interventions. Gay, lesbian,

bisexual, and transgender individuals have reported experiencing stigma and homophobia at places of worship, which may limit their access to the support and prevention efforts offered by religious communities and may inadvertently increase vulnerability to HIV (Sutton and Parks 2013). Additionally, stigma surrounding relationship types and dynamics that are unfamiliar and/or unapproved by the faith community continue to be barriers to HIV interventions in faith-based settings. For example, religious communities may face uncertainty in handling sexual relationships outside of traditional marriage, sexual relationships with more than one partner, same-sex relationships, and relationships that do not conform to typical gender roles. When working with faith communities, stigma and homophobia must be addressed in the formative phases of intervention development. Innovative solutions can be devised when researchers and faith leaders have open and honest dialogues about these difficult topics and work to reach compromises that promote the sexual health of the population of interest. Furthermore, extending these conversations to all religious leadership and congregants will help reduce stigma and normalize discussions of HIV in their community (Nunn et al. 2012). Another strategy is to collaborate with local public health agencies to provide faith-based intervention participants with evidence-based materials about HIV risk reduction. This will allow faith leaders to frame intervention content in a manner that is congruent to their religious values while allowing participants to receive a comprehensive sexual health message (Francis and Liverpool 2009). Framing HIV risk reduction in a culturally appropriate manner may help to reduce stigma. For instance, conceiving of HIV risk reduction in terms of social justice or public health rather than sexuality, or framing support for people living with HIV or at risk for HIV in religious values of compassion and caring for the sick, may make the message more acceptable to the faith community (Francis and Liverpool 2009; Nunn et al. 2012).

Another barrier to effective faith-based interventions is that religious communities may not believe that their members are at risk for

HIV. Many faith communities remain silent about the presence of extramarital relationships, concurrent partnerships, same-sex relationships, drug and alcohol use, and risk of sexually transmitted infections in their community. Such silence is a long-standing tradition in some communities that deem such behavior as sinful, and social norms opposed to discussing such topics in a place of worship further support this silence. Without innovative strategies to overcome this challenge, researchers may have a particularly difficult time partnering with these faith communities. However, researchers can encourage such faith communities to begin to talk about these difficult topics by providing information about HIV risk in their community, culturally tailoring HIV-related messages and programs in a manner that is appropriate for the faith community, and presenting the leadership of the faith-based organization with diverse options about how they can address HIV in their community (i.e., HIV prevention, HIV testing, and/or supporting people living with HIV) (Nunn et al. 2012). In addition to the challenges listed above, mistrust between churches and researchers, reciprocal misunderstanding of values and norms (e.g., appropriate language and content for a faith-based setting versus the need for a control condition), competing agendas and priorities (e.g., evangelism versus evaluation), the difficulty of integrating the complex topic of religion and spirituality into any health promotion intervention, and the perpetual lack of resources act to heighten the barriers that limit the development and dissemination of faith-based interventions that address HIV (Margolin et al. 2006; Williams et al. 2011).

Special Ethical Considerations of Faith-Based Interventions

Plante (2007) recommends four ethical concerns that should be addressed when integrating faith into health promotion: respect, responsibility, integrity, and competence (Plante 2007). *Respect* for religious beliefs and values is critical in every HIV-related intervention. However, while conducting faith-based interventions, researchers

and faith leaders alike must examine their own religious and spiritual beliefs and ensure that they do not impose their beliefs onto intervention participants. Although researchers are not required to agree with the faith traditions of the study population, it is critical that they be respectful of the faith traditions of the study population. This is a particularly delicate matter when dealing with behaviors and populations that are frequently not accepted by the faith community. Collaboration with members of the faith community and local faith leaders and extensive formative research will help ensure that intervention participants are being respected and that the intervention is appropriate for the population of interest. Additionally, the use of CBPR approaches will help assure that disputes between faith community beliefs and researcher recommendations are appropriately vetted by all stakeholders involved in the intervention. Researchers considering conducting faith-based HIV-related research can also engage in thorough reflective analysis of personal religious beliefs prior to conducting such research. Reflection should be aimed at addressing judgmental attitudes toward differing faith beliefs and establishing an open dialogue about ways to understand and respect the faith beliefs of others.

The use of faith to culturally adapt health promotion interventions demonstrates *responsibility* to the religious beliefs of the study population. Conversely, ignoring the faith of individuals at risk for and living with HIV has the potential to be ethically irresponsible, especially if the incorporation of faith-based elements will enhance the relevance, effectiveness, and sustainability of the intervention. Researchers are responsible for acknowledging the importance of faith beliefs and should use faith as an innovative tool for culturally adapting HIV-related interventions. Similar to strategies to ensure respect, responsibility to the study population is enhanced through formative research with members of the faith community and potential participants. Likewise, beliefs that increase HIV risk behaviors should be acknowledged and addressed in a sensitive manner.

Integrity demands that researchers conducting research in communities of faith must not try to

usurp the role of faith leaders in that community. The role of the researcher is to collaborate with faith leaders and congregants to create HIV interventions that are appropriate for that community. Most HIV researchers are not trained in theology, philosophy, or religion and therefore should not overstep their contribution to the faith-based adaptation of the HIV intervention. This is particularly important for researchers that are members of the same or similar religious group as the community they are working with. Researchers must be honest regarding their limitations. Similarly, faith leaders must not force their religious beliefs on intervention participants.

Lastly, *competence* on how to appropriately address HIV-related topics in a faith-based setting cannot be assumed. Researchers desiring to create faith-based interventions should take advantage of the growing literature on the topic, attend conferences and workshops on the topic, and most importantly seek out partnerships with faith leaders that are willing to collaborate on these topics. Furthermore, researchers should carefully examine themselves for potential biases against certain religious beliefs.

Examples of Faith-Based HIV Interventions

Three published faith-based interventions can provide examples of the diversity of faith-based interventions and ways that faith elements can be incorporated into HIV-related interventions. These interventions were selected because they were current, provided outcome data, and were descriptive in their use of faith-based elements. While the three major topics that faith-based interventions typically address are HIV prevention, HIV testing, and supporting people living with HIV, the interventions described below address only HIV prevention and HIV testing. Although there were several reports on the work of faith-based organizations, particularly the Catholic Church, to provide financial, spiritual, emotional, and physical support to people living with HIV, few provided outcome data and therefore were not included in this discussion.

Spiritual Self-Schema (3-S) Therapy was a spirituality-focused clinic-based randomized-control psychotherapy intervention designed to promote drug abstinence and HIV prevention behavior among methadone-maintained individuals with negative or unknown HIV serostatus (Margolin et al. 2006). Therapists used a framework based on the Buddhist Noble Eightfold Path and promoted mindfulness exercises and meditation to support participants' weakening of their addict self-schema and development of their spiritual self-schema. Unlike the addict self-schema, the new spiritual self-schema would be motivated to abstain from drugs and reduce risk of HIV transmission. A Buddhist framework was chosen because it was compatible with cognitive behavioral therapy and multiple belief systems and allowed participants to personally define their own spiritual beliefs. Seventy-two participants received either standard care or 3-S therapy. Compared to the standard of care participants at post-treatment, controlling for demographic variables and pretreatment risk behavior, participants who received 3-S therapy reported greater motivation for HIV prevention and were eight times less likely to report HIV risk behavior during the prior month (Margolin et al. 2006).

YOUR Blessed Health (YBH) was a community-initiated multi-level intervention that aimed to improve HIV risk reduction skills and behaviors and increase HIV awareness and knowledge among African American youth ages 11 to 25 years old by improving the capacity of religious leadership to address HIV/AIDS and sexual risk behavior (Griffith et al. 2010a, b). Several activities were used to accomplish this goal, including HIV awareness group sessions for youth, group sessions for adults, extensive training for religious leadership, church-wide HIV awareness and stigma reduction activities during weekly services, and community-wide HIV awareness activities. A community-owned and community-managed approach to CBPR was used to design, implement, and evaluate the intervention. In keeping with CBPR and ethical principles described above, each of the 42 faith-based and community-based participating organizations was allowed to tailor the YBH intervention as they

saw fit. Typically, adaptation was focused around the structure, frequency, and timing of the sessions. Although qualitative findings were promising, there were no significant differences in HIV-related outcomes among intervention participants. However, researchers and community partners count the real success of the project as their ability to build capacity within their faith community and to initiate changes in community norms.

Taking It to the PEWS (TIPS) is another example of a CBPR intervention (Berkley-Patton et al. 2010). Rooted in the Black Church Week of Prayer for the Healing of AIDS in Kansas City, the TIPS program was a community-initiated and collaboratively developed intervention to increase HIV awareness and testing and reduce HIV-related stigma within African American communities and churches. Church partners selected these topics as the focus of the TIPS program because they were considered less controversial and potentially less divisive than sexual risk reduction (i.e., condom use). A detailed needs assessment and an equitable and collaborative partnership with faith leaders were integral to the intervention. Intervention materials and activities were religiously tailored, church leadership served as interventionists, and the program was designed to fit in with already-ongoing church activities. Twelve Kansas City churches participated in the TIPS program, reaching approximately 3,400 church and community members. Process evaluation findings using focus group and implementation data were promising. Church members that reported high TIPS exposure had significantly lower HIV stigma, greater positive feelings about church involvement in HIV, and greater readiness to be tested for HIV.

Conclusion

Despite barriers and challenges, faith communities and researchers have readily collaborated in the development and implementation of several interventions that relate to HIV prevention including sexual risk reduction, substance abuse treatment, injection drug use risk reduction, HIV

testing, and support for people living with HIV/AIDS. There is a need for more faith-based HIV prevention research and outcome-focused religion-HIV risk-related research. Although relatively few in number, faith-based interventions offer an innovative approach to addressing HIV that has the potential to enhance the effectiveness of community-based research efforts.

Cross-References

- ▶ [HIV Prevention and African Americans](#)
- ▶ [HIV Prevention Efforts Within Substance Use Disorder Treatment Settings](#)

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Family Interventions

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Definition

This chapter describes family-based HIV prevention interventions and research on their efficacy. Both domestic and international interventions are discussed.

Risky sexual behaviors that can lead to HIV infection are influenced both by the characteristics

of an individual and the characteristics of their interpersonal, social, and cultural environment. The recognition of these layers of influence has prompted the development of ► **multilevel and structural approaches to HIV prevention** that go beyond targeting only the individual. For adolescents, the family can be a particularly significant external system of influence, characterized primarily by parent-adolescent relationships and interactions (Bastien et al. 2011; DeVore and Ginsburg 2005). Research has shown consistent links between parenting behaviors and characteristics and adolescents' sexual risk taking (DeVore and Ginsburg 2005; Kincaid et al. 2012).

The definitions of “families” and “parents” are quite broad and flexible in the HIV prevention literature; generally these terms identify the primary caregiver(s) for an adolescent regardless of biological relationship, with emphasis on the role and relationship to the adolescent. Caregivers can be biological parents, grandparents, adoptive parents, guardians, other extended family members, or even an older sibling and are referred to as “parents” in this chapter. Likewise, a “family” may be a parent-adolescent dyad or a large household whose members share responsibility for an adolescent's well-being. Regardless of family structure, the central goal of family interventions is to build and support family systems that can protect adolescents from contracting HIV.

The family provides a unique and promising point of intervention, as parents are in a position to serve as teachers, role models, sources of support, and monitors of their adolescent's behavior (Pequegnat and Bell 2012). Working at the family level can therefore generate change across these multiple roles. Further, the family unit is generally a constant structure in adolescents' lives and can wield ongoing influence long after an intervention ends. The consistent presence of the family may protect against the common problem of waning intervention effects over time, a major concern in the field of HIV prevention. Family-based interventions are particularly advantageous because they provide information and skills to multiple people who are connected with one another, rather than relying only on an individual's ongoing self-motivation to maintain behavior change.

Parenting and Sexual Risk Behaviors

Specific parenting practices have been shown to prevent or reduce adolescent sexual risk behaviors, a key component of HIV prevention. Parents' monitoring of their adolescent's activities, whereabouts, and relationships has emerged as one important protective factor against risk sexual behavior; adolescents who are monitored closely are less likely to engage in risky sexual behaviors, including unprotected sex and early sexual debut. Most studies of parent-adolescent communication have also reported that higher-quality communication between adolescents and their parents is protective against risky sexual behaviors, particularly when the communication includes frequent, informed, and comfortable discussions about sex. In addition, parents' warmth and involvement in their adolescent's activities have been identified as positive parenting practices that reduce high-risk sexual behavior. For adolescents with specific risk factors for HIV infection, such as living in poverty, being part of a marginalized group, or having a psychiatric illness, the family unit is an especially promising source of protection, as broader community support systems can be less accessible and may provide fewer resources to these subpopulations.

Aims of Family-Based Interventions

As parents have the potential to directly impact adolescent risk behavior, family-based interventions to prevent HIV offer promise to reduce HIV incidence. Multiple family-based prevention interventions have been developed, most within the past 15 years, often with the objective to ► [Delayed Sexual Debut](#) among younger adolescents and to decrease risk behavior of sexually active teens (Downing et al. 2011; Pequegnat and Bell 2012).

The mechanisms of HIV prevention vary across interventions. Some family-based interventions focus primarily on improving knowledge and attitudes about HIV within the family, while others include more active behavioral components to build parenting skills that are associated

with reductions in sexual risk behavior. Most often, family-based interventions aim to improve parent-adolescent communication by expanding the content and increasing the frequency of conversations parents have with their adolescent about sexual risk and HIV (Bastien et al. 2011). Effective communication maximizes the synergistic impact of knowledge and parenting techniques, as transferring information and values to adolescents is likely to be more successful if parents are able to provide support, warmth, involvement, and effective monitoring as well.

Structure and Strategies of Family-Based Interventions

The first step in most family programs is often to give parents accurate and appropriate information pertaining to HIV prevention and sexual risk behaviors. The interventions then seek to build parents' comfort with the material and confidence to initiate conversations with their adolescents. Some interventions have relied on multimedia formats, such as audio CDs or videotapes, to deliver information to parents in the home setting. These multimedia strategies serve to allow parents to complete much of the intervention privately and at their convenience. However, most family-based interventions bring together multiple families in group sessions to deliver didactic information.

The length of family-based interventions varies greatly, and there is little consensus about optimum intervention exposure. Most interventions are delivered in multiple sessions, ranging from three to more than a dozen, often occurring once per week. Some programs target only the parents, while some include the adolescents as well. In interventions that involve both, parents and adolescents are often initially separated to deliver more targeted education on topics such as effective disciplining practices for parents or peer pressure for adolescents. Parents and adolescents are then brought together for discussion and/or skills practice. The separate groups can allow for more candid discussions about sex and HIV by confirming basic knowledge, answering

initial questions, and reducing discomfort – before adolescents and parents are asked to discuss these topics as a large group.

Successful family interventions are characterized by their participatory nature, engaging participants in interactive activities beyond the presentation of didactic material and discussion. Participatory activities for adolescents may include practicing condom use skills or engaging in role-play to refuse unwanted sex; parents may role-play to practice conversations with adolescents, particularly in interventions that aim to improve parent-adolescent communication and relationships. Participatory family interventions often have the most integrated designs, equally emphasizing both education and practice.

Some interventions have targeted either mothers or fathers specifically, as well as either daughters or sons. The gender of the parent and adolescent is not necessarily matched; for example, an intervention may aim to help mothers reduce risk behaviors of their sons through understanding their developmental challenges (Jemmott et al. 2000). In general, gender-specific interventions have been developed primarily for mothers, or female caregivers, because male caregivers have been more difficult to engage. Efforts to involve fathers are growing, however, by developing specific interventions for fathers and engaging them in family interventions more generally.

Interventions Tailored for Specific Populations

Perhaps even more than other types of interventions, family-based interventions must be tailored, as family structures and interaction styles can vary significantly across contexts, cultures, and populations (e.g., substance users). Further, sexual behaviors are often tied closely to value systems, religious beliefs, and cultural and social norms. The settings in which interventions are delivered should be selected appropriately for the intended recipients. In response to varied needs across subgroups, tailored interventions have been developed for specific populations both in the United States and internationally.

Ethnic Minorities. In the United States, tailored family-based interventions have been developed for several specific populations, with the largest number for African American and Latino families. Tailoring interventions for different ethnic groups allows the intervention content to go beyond the basic HIV prevention messages and skills to address some of the contextual challenges faced by specific populations. For example, an intervention for African American families in the rural south may integrate a focus on racial socialization (Murry et al. 2011). *Familia Adelante*, an intervention for Mexican-American families developed in 1993 and most recently implemented in 2003 among urban families in California, concentrated specifically on acculturative stress with the aim of reducing overall family stress and thus improving adolescent substance use behavior, attitudes toward HIV risk behavior, and psychosocial coping (Cervantes et al. 2011). Targeted interventions can also include discussion of HIV rates, norms for sexual behavior, and most common modes of transmission in that specific population.

Adolescents with Specific Risk Factors. Interventions have been developed for populations of adolescents who have specific risk factors that can increase their chances of contracting HIV, particularly substance use and mental health problems. One rationale for these types of programs is that similar family-level characteristics may underlie various adolescent risk behaviors and contribute to psychological distress. Thus, rather than focusing only on the adolescent's specific risk behavior or mental health symptoms, these interventions target family-level characteristics. For instance, a community-based study in low-income areas of Baltimore, MD, found that emphasizing parental monitoring – which is shown to be protective against HIV risk behavior – provided protection against adolescent substance use in both the short and long term (Wu et al. 2003). *Project STYLE* is an intervention for adolescents in treatment for mental health problems. The school-based intervention aims to improve family communication about sex while also addressing the coercive patterns of communication that can often develop in families affected by mental health concerns

(Donenberg et al. 2012). Efforts are also underway to develop interventions specifically for families of gay, lesbian, and bisexual youth.

International Settings. Outside of the United States, most family-based intervention development has been focused in developing countries, particularly in sub-Saharan Africa, where HIV rates are the highest. Family-based interventions have been developed or adapted specifically for these settings, as familial and cultural norms, as well as the factors contributing to HIV risk, vary by setting.

In many parts of sub-Saharan Africa, extended family members are integral to the family system. Being part of these larger family units is often a main source of identity, and family members may share resources freely with one another. In rural areas in particular, members of extended families and multiple generations of a family may live together and share material resources and household responsibilities (e.g., care of children and adolescents, farming). These familial norms influence the effective design and implementation of family-based interventions.

Cultural values and religious beliefs are especially important to consider when working in international settings. Examples include norms around gender roles, with males holding decision-making power, or religious beliefs that strongly discourage condom use. Culture and religion are also natural sources of support on which interventions can build if they are recognized as community strengths.

Some US family-based interventions have been effectively adapted for an international setting. The *CHAMP (Collaborative HIV/AIDS Prevention and Adolescent Mental Health Project; Baptiste et al. 2006)* intervention, originally implemented in Chicago, was adapted for South Africa. CHAMP is a family-based program that targets improved parenting skills and youth social problem-solving, among other aspects of family well-being. To tailor CHAMP, developers conducted ethnographic research in KwaZulu-Natal, South Africa, and identified changes needed in the adapted program (e.g., addition of a session on bereavement; changing the structure to a cartoon-based format rather than written).

Families Matter! is an intervention for 9- to 12-year-olds and their caregivers first adapted for Kenya from a US-based program (*Parents Matter!*). Adaptation was guided by a needs assessment involving focus groups with community members and consultation with local experts in HIV prevention (Poulsen et al. 2010). In this case, formative research found the original content to be largely relevant but changed the format of some of the materials and changed the name to include “families” to be more inclusive of all types of caregivers. *Families Matter!* is now being adapted for other countries as well.

Children and adolescents orphaned by HIV/AIDS in sub-Saharan Africa require specific consideration in developing family-based interventions. Orphans are a particularly vulnerable group, with some studies finding that they exhibit higher levels of risk behavior and/or mental health problems that can increase HIV risk during adolescence or in the future (Operario et al. 2011; Thurman et al. 2006), as well as mental health problems that can increase HIV risk behavior (Cluver and Gardner 2007; Puffer et al. 2012). Extended family members often assume responsibility for a child or adolescent after the death of their parents, though families living in low-resource communities often do not have or allocate the resources to accommodate these children’s needs. HIV prevention interventions for orphans have often focused on increasing the financial and material resources allocated to orphaned children and adolescents, such as providing their new caregivers with grants to reduce the extra financial burden, providing cash transfers, or setting up savings accounts for orphans themselves (Cluver et al. 2013; Han et al. 2013). Economic interventions can help close the resource gap and thus decrease orphans’ vulnerability to HIV.

Evaluating the Effects of Family-Based Interventions

The effects of family-based interventions are typically measured by (a) reduction of sexual risk

behaviors, (b) improved knowledge or attitudes about sexual risk behavior and HIV, and (c) improved family interactions and characteristics that have been linked to safer behaviors.

Within behavioral outcomes, some family-based interventions report increased condom use, often measured by whether or not the adolescent used a condom the most recent time they had sex. Intervention studies have also documented reduced frequency of adolescents engaging in a variety of sexual behaviors (from kissing to intercourse). For adolescents who are not yet sexually active, some interventions reported delayed sexual initiation beyond the expected age-given norms for their context.

Interventions have also achieved improved knowledge about HIV among adolescents and parents and increased confidence to use risk reduction skills among adolescents. Adolescents report feeling more able to refuse unwanted sex and more confident that they can use condoms, both in terms of understanding how to use them and how to initiate conversations about condom use with partners. Adolescents in some interventions also report increased intentions to engage in less risky behavior in the future.

Parents in family-based interventions have exhibited significant improvement in use of positive parenting behaviors, including improved monitoring of adolescent behavior and increased warmth and support in their interactions with their adolescent. Perhaps the most common parenting outcome is improved parent-adolescent communication about topics related to sexual behavior and HIV. Parents and adolescents report more frequent discussions on these topics, and parents report higher self-efficacy to provide accurate information about HIV to their adolescent and to initiate these discussions.

The ultimate behavioral impacts of these improved parenting skills are unclear. For instance, while some research documents clear links between communication and adolescent sexual behavior (Bastien et al. 2011), other research shows no direct connection between the two (Downing et al. 2011). The effects of improved parenting also may differ by gender and other

characteristics. For example, some evidence suggests that while certain aspects of parenting, including monitoring, warmth, and emotional connection, are protective overall, improved monitoring is more effective for boys while the latter two qualities are more beneficial for girls (Kincaid et al. 2012). Thus, the impacts of changing parenting strategies and family interactions are complex, and more research is needed to determine which family characteristics are most important to target and for whom.

Limitations and Future Directions

Perhaps the most difficult aspect of studying the effects of family-based interventions is measurement. Accurately measuring whether a behavior has successfully been prevented or changed requires long-term follow-up, which is often not possible or feasible. The alternative is to measure behaviors that are associated with and/or thought to precede sex, such as kissing or spending time alone with the opposite sex. However, it is unclear whether these behaviors are truly indicators of future sexual *risk* behaviors.

Measuring behavior, both of the adolescent and parents, is difficult given reliance on self-report measures. Participants often understand how parents or adolescents “should” behave, which can lead to over-reporting of positive parenting strategies among parents and safer sex behaviors among adolescents. Measuring biological outcomes is an alternative strategy that would significantly improve confidence in intervention outcomes in terms of actual changes in sexual behavior. Biological indicators, such as rates of sexually transmitted infections or pregnancy, are very seldom used, though this is a clear future direction for the field. Finally, family relationships and interactions are complex, and current measures do not adequately capture the family dynamics that surely play a large role in the behavioral changes that are observed in intervention trials. Understanding these more complex interactions would be invaluable to improving future intervention development.

Conclusions

The evidence base for family-level interventions for HIV prevention is growing, and interventions are showing promising outcomes, particularly in increasing condom use and self-efficacy to engage in safer sexual behavior among adolescents. Family-based interventions also have been shown to improve parenting practices, including monitoring and warmth, as well as parent-adolescent communication. Multiple interventions have been developed and tailored for specific at-risk populations, including adolescents living in developing countries where HIV prevalence is high and adolescents with risk factors such as substance use and mental health problems. Research to evaluate the effectiveness of family-based interventions would be strengthened by the development and validation of measures to more accurately assess adolescent and parent behaviors, as well as the longer-term effects of interventions to prevent future risk behavior and HIV infection.

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Female Condoms

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Definition

This chapter will introduce the female condom, review its basic properties, present progress in, and barriers to, its public health promotion, and identify priorities for future promotion. It will present the history, efficacy, and acceptability of the female condom; implementation and evaluation of female condom promotion programs; and implications for future action. The reader is also referred to the comprehensive overview of the female condom by Gallo et al. (2012).

Urgent Need for Female Condom Promotion

Worldwide, one-half of people living with HIV are women (UNAIDS 2010). In the countries of sub-Saharan Africa, infection is more likely among women than men. For every ten men infected, 13 women are infected (UNAIDS 2010).

Among women, heterosexual intercourse is the primary route of HIV, as well as sexually transmitted infection (STI) transmission (UNAIDS 2010). At the same time, pregnancy is also a salient goal and, under some circumstances, concern of women. However, the passive nature of the female gender role in heterosexual relationships, in many countries, can make women's initiation of safer sex practices problematic. In male-dominated relationships, women's discussion of safer sex can elicit hostility and, even, rejection or abuse, from the male partner (Wingood and DiClemente 2000; Gender role 00062). Taken together, these factors make the promotion of female-controlled methods for dual protection – with respect to HIV and STI transmission as well as contraception – an urgent priority.

Currently, breakthroughs in antiretroviral treatment (ART), treatment as prevention (Cohen et al. 2011), vaginal microbicides (Abdol Karim et al. 2010; Pre-exposure Prophylaxis (PrEP): oral and topical (microbicides), Prevention of HIV 00378), and pre-exposure prophylaxis (PrEP) (Grant et al. 2010; Post-exposure Prophylaxis 00329), have led the global public health community to embrace the goal of an AIDS-free generation. This is extremely heartening. However, until economic, political, and health care infrastructure conditions can support universal scale-up, it is imperative to maximize the reach of currently feasible, accessible, and effective HIV prevention methods. Until PrEP becomes an established, safe, available, and affordable prevention option, the male condom is the only widely accessible means of HIV/STI prevention. Yet, it is limited by being a male-controlled method – within an epidemic that, globally, affects men and women nearly equally (UNAIDS 2010). The female condom is the only female-controlled method that has both contraceptive efficacy and high likelihood of STI prevention efficacy (e.g., Lytle et al. 1997).

Description of the Female Condom

The first female condom (FC1) was a flexible polyurethane cylinder, which covers the vaginal canal – between its inner ring, fitting the cervix, and its outer ring, fitting the opening of the vagina. FC1 was developed and manufactured by the Female Health Company, in Chicago, and approved, by the United States Food and Drug Administration (USFDA), for contraception and HIV/STI protection, in the event that a male latex condom was not accessible, in 1993. The second female condom (FC2), also from the Female Health Company, superseded the FC1, in 2005 – improved by its synthetic latex composition, lower cost, and quieter use. It carries the approval of the USFDA (as of 2009); CE Mark, endorsing for use in the European Union; approval by the World Health Organization Female Condom Technical Review committee; and recommendation for use by United Nations agencies. Currently, it is available, in over

100 countries, through public health initiatives, and in 20 middle and high-income countries, for purchase (Gallo et al. 2012). Three other female condoms are in different stages of development or marketing and distribution. The Reddy Condom (Medtech Products, Chennai, India) and the Cupid Female Condom (Cupid Ltd., Mumbai, India) are both latex condoms that contain inner sponges to better secure insertion; both have CE Mark, but not WHO Female Condom Technical Review approval. The Woman's Condom is a product of the Program For Appropriate Technology in Health (PATH) (Seattle, USA), an international, nonprofit, nongovernmental organization (NGO), which has been at the forefront of advancing new health technology and systems, including the female condom. The Women's Condom is a thin polyurethane film pouch, inserted within a capsule, and then released when the capsule dissolves; foam helps steady the condom, along the vagina. The Women's Condom was licensed to Dahua Medical Apparatus Corporation (Shanghai, China) in 2008; it obtained its CE Mark in 2010 and its Shanghai Federal Drug Administration (FDA) approval in 2011. Work is in progress to seek USDFA approval.

The female condom offers several important benefits for women, over the male condom (HIV Prevention and Women 00311). Most importantly, it is female-controlled. In addition, it has the following advantages: it can be inserted and worn 24 h prior to sexual encounter – allowing for greater spontaneity during sex. It covers more area of the female genitalia – and, thus, better protection from infections transmitted via skin or mucosal membranes (Gallo et al. 2012). It can produce greater sensation for both the woman and her male partner. As non-latex, it can be used with oil-based lubricants.

Use of the Female Condom

Like the male condom, correct female condom use requires a series of steps; without which efficacy can be compromised. These include checking expiration date; checking for condom damage; placing inner ring against cervix, to insert; twist

the outer end of the condom, before pulling the condom out, to remove, etc.). In fact, across studies, rates of female condom “failures,” consisting of lapses or errors in such steps, have been estimated to occur during 2.5–25% of uses (Gallo et al. 2012). It should be noted that male condom use is also susceptible to these lapses as well. It should also be noted that WHO has developed standards for reuse of female condoms (i.e., including disinfecting, washing, and drying) – up to five uses – to address this practice, especially in resource-poor environments (WHO 2002). However, in the absence of supporting evidence of efficacy, this is not recommended.

Efficacy of the Female Condom

Contraceptive Efficacy

There is converging evidence from prospective observational studies of the substantial contraceptive efficacy of the FC1 female condom. Studies may distinguish pregnancy rates for two patterns of use, over a 6-month or 1-year periods: “perfect” and “typical.” A pioneering study (Bounds 1992) obtained a 15% pregnancy rate, for typical use, over a 1-year period, in the UK. Farr et al. (1994) obtained (6-month) pregnancy rates of 22.2% (typical use) and 9.5% (perfect use) for women in Latin America and of 12.4% (typical use) and 2.6% (perfect use) for US women. Trussell (1998) obtained (6-month) pregnancy rates of 3.2% (typical use) and 0.5% (perfect use) for women in Japan. Both the FC1 and FC2 product descriptions cite pregnancy rates of 21% (typical use) and 5% (perfect use) for the female condom, and of 18% (typical use) and 2% (perfect use) for the male condom. There are no randomized clinical trials from which to obtain direct, contemporaneous comparative data.

STI/HIV Prevention Efficacy

There is converging, though not uniform, evidence from laboratory, observational, and intervention research of the efficacy of the FC1 female condom for STI prevention.

Several studies have examined the efficacy of FC1 for STI prevention. It should be noted that,

while highly promising, these sometimes used crossover, sometimes prospective comparison, and, only sometimes, randomized control designs.

Crossover Designs Galvao et al. (2005) used a crossover design – to randomize Brazilian women in a family planning clinic to two conditions (i.e., instruction to use two female condoms followed by the use of two male condoms and instruction to use two male condoms followed by the use of two female condoms). Analysis used the presence of prostate-specific antigen (PSA) in post-intercourse vaginal specimens as the primary outcome, as a biomarker for semen contact. The rate of PSA detection was greater in the female condom than in the male condom specimens. Another crossover study (Valappil et al. (2005) used similar crossover conditions with ten female and male condoms. The rate of PSA detection was comparable in both female condom and male condom specimens. It should be noted that, in crossover studies, self-reports of use problems (e.g., slippage) were greater for female condoms than for male condoms. However, as Gallo et al. (2012), as well as others, observe, self-reported problems decline as experience increases. Two, or even ten, condom uses may provide too brief exposure to gain proficiency and comfort with the female condom.

Prospective (Nonrandomized) Comparison Designs Soper et al. (1993) compared two groups of women with trichomoniasis in public clinics in four US cities – who either agreed to use the female condom or received treatment as usual. At 45-day follow-up, reinfection rates were equivalent in the two groups. Macaluso et al. (1999) also compared two groups of women in US public clinics – who either agreed to use female condoms (i.e., as alternatives when male condoms are not accessible) or received male condoms. Among consistent condom users, the rate of gonorrhea and chlamydia were comparable in both conditions. In a quasi-experimental design, French et al. (2003) provided counseling and either female condoms or male condoms to women, respectively, presenting on alternate weeks, to a Philadelphia STI clinic. At 6–12-month follow-up,

women in the female condom condition had a lower rate of positivity on a composite measure of infection with at least one of the following STIs: chlamydia, gonorrhea, syphilis, or trichomoniasis. However, the rate did not significantly differ with that of the male condom condition.

Randomized Trials In a randomized design using brothel as the unit of randomization, Fontanet et al. (1998) compared female sex workers in Thailand in two conditions – who either received counseling around male condoms or around a hierarchical message (i.e., combining male condoms and female condoms, as an alternative when male condoms are not accessible). At 24-week follow-up, women in the hierarchical message condition showed a decrement in the rate of positivity on a composite measure of infection (i.e., including gonorrhea, chlamydia, trichomoniasis, or genital ulcer disease), in comparison to those in the male condom condition. However, this did not reflect a statistically significant difference. In a randomized design of a structural intervention, using plantation as the unit of randomization, Feldblum et al. (2001) compared women in Kenya in two conditions – community-level campaign, counseling, and provision of male or female condoms versus campaign, counseling, and provision of male condoms only. At 6-month and 12-month follow-ups, the two conditions were comparable in rates of positivity on a composite measure (i.e., including gonorrhea, chlamydia, or trichomoniasis). It should be noted that secondary analysis highlighted provider reluctance about the female condom as an important obstacle to efficacy in the combined condom condition (Welsh et al. 2001).

While the above discussion has focused on the efficacy of female condom interventions on biological STI markers, their impact on condom use uptake is a crucial part of this efficacy. In addition to female condom provision, these interventions are based on cardinal behavioral science principles – that efficacious HIV safer sex interventions for women must include (cognitive, behavioral, and interpersonal) skills building for correct condom use, decision-making, and negotiation with male partners. It should be noted that

increments in protected intercourse occasions are an underlying finding of the above interventions (Fontanet et al. 1998; French et al. 2003; Artz et al. 2000).

It can be concluded that, thus far, research has provided support for the equivalent efficacy of female and male condoms to confer STI protection. However, this research points to a few possibly crucial gaps in the work to date. First, the research suggests that interventions might be more efficacious – if they afforded more experience with the female condom, perhaps over a longer period, and perhaps with more ongoing troubleshooting and problem-solving with providers, as consumers gain real experience. Second, the research also points to a critical, unresolved problem in female condom promotion: substantial, although variable, acceptability among consumers, both female and male, but low uptake. Third, the research highlights low acceptability among clinic providers, who are a potential primary link to female condom use in consumers and who would be important targets for female condom promotion programs.

Acceptability and Uptake of the Female Condom

Acceptability is an essential component in making the link between the existence of a robust, accessible, and affordable female condom and its uptake. The female condom acceptability and uptake research makes some important distinctions. First: among consumers of various types of related clinic services (e.g., STI clinic, family planning clinic, HIV clinic, and others), a preponderance of studies show that short-term acceptability of the female condom is high (UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training In Human Reproduction 1997). Second: however, acceptability in the general population is far lower. For example, in the 2002 US National Survey of Family Growth (Mosher et al. 2004), 1.9% of childbearing aged women reported ever using the female condom. Third: acceptability and uptake in high HIV seroprevalence, developing

countries (e.g., Brazil, South Africa, and others) exceed that in developed countries (e.g., the USA, the UK) (Warren and Philpott 2003). It has been reasoned that, in the former, the immediately palpable toll of HIV is a potent driver of this.

Acceptability is a multilevel phenomenon – which is operative at the consumer, provider, systems, and policy levels. Below existing knowledge of factors (i.e., barriers and promoters) underlying attitudes toward female condoms, at the consumer and provider levels, are summarized:

Consumers Short-term acceptability studies conducted in a wide range of settings (UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training In Human Reproduction 1997) demonstrate that the female condom is acceptable to diverse populations of both women and men, with the proportion trying the method ranging from 37% to 96%. A consistent finding is that women report that the female condom enhances their safer sex bargaining power within their sexual relationships (e.g., Gollub 2000). However, when the gendered power inequity in a relationship is too daunting, this is a powerful barrier to female condom use. In addition, physical aspects of the female condom (e.g., length, difficulty manipulating it to insert or remove, discomfort, and others) that – at least initially – can be awkward or challenging, can – before sufficient familiarity – be deterrent to use (Hirky et al. 2003). Cost and limited accessibility can be another barrier to use.

Providers Provider attitude toward female condoms is a pivotal variable in consumer uptake of female condoms. Studies of female condom acceptability among providers demonstrate rates that markedly lag behind those of consumers. In a study of 27 ART programs in Africa, Asia, and South America, only 32% reported providing female condoms, while 96% provided male condoms (Spaar et al. 2010). In a study of providers in four types of clinics in New York State, while the majority acknowledged the equal efficacy of the female and male condoms for pregnancy and disease protection, they also expressed reluctance to recommend the female condom to their clients.

They attributed this to the lack of familiarity and inexperience with it, as well as the lack of access. They also expressed some of the same concern with its physical aspects, as did consumers (Mantell et al. 2003).

Structural Barriers to Female Condom Promotion

Widespread promotion of the female condom has faced numerous barriers in the political and social environment. In 2008, female condoms accounted for only 0.8% of condoms distributed in sponsored programs (UNFPA 2008). In the USA, these barriers have included negative press, limited advertising and marketing, higher cost relative to the male condom, limited distribution in the public health system, and inadequate training of providers.

Implementation and Evaluation of Female Condom Promotion Programs

In order to bridge the divide between acceptability and uptake, the complex interplay of consumer, provider, health service delivery system, and policy factors must be incorporated into intervention (Social Contextual Factors in HIV Prevention 00295). HIV prevention science has evolved to address broader contextual factors that affect sexual risk behavior (Blankenship et al. 2000). Internationally, a variety of structural interventions have been mounted to promote female condoms, including mass media campaigns, free distribution programs in the public sector, and training of health care providers. In Washington DC, a citywide female condom distribution campaign was determined to be cost-effective – in deterring 23 infections – after the distribution of its first 200,000 condoms (McNeil 2012).

However, comprehensive multilevel structural interventions – that integrate consumer, provider, and systems approaches – have the maximum potential to advance female condom uptake. Four examples of system-wide interventions provide evidence of this potential. In South

Africa, a national female condom introduction program implemented free condom distribution in multiple venues (e.g., public sector clinics, community-based organizations, social marketing outlets, commercial pharmacies, etc.) and provider training in female condom counseling in eight (of nine) provinces (Mqhayi et al. 2003). In Brazil, a comprehensive initiative, including community advocacy, provider training in female condom use counseling, integration of female condoms into existing public sector programs, and consistent access to female condoms. In Nigeria, a combined free condom distribution and provider counseling program in six family planning clinics, increased purchase of condoms and other barrier methods (i.e., from purchases at 2% of visits, at outset, to 9% of visits, 18 months later), primarily accounted for by purchase of female condoms (Adeokun et al. 2002). In New York State, Exner et al. (2012) conducted a randomized clinical trial of a system-level intervention to promote female condom-positive organizational policy, counseling, and client use – in 44 agencies receiving funds from the NYS AIDS Institute. The intervention consisted of (1) Agency level: female condom, provision, female condom program and policy toolkit, and 12 months of (telephone) technical support, and (2) Counselor level: 1-day female condom training workshop and 12 months of (telephone) technical support. At the counselor level, several significant effects were obtained: increase in proportion of heterosexual women and men counseled on the female condom, increase in female condom knowledge, and increase in self-efficacy to conduct female condom counseling and in positive attitude toward the female condom. At the client level, a few significant effects were obtained: increase in client intention to use female condoms and increase in female condom knowledge.

Conclusion

Gender inequities in power, along with restrictive and polarized gender roles, make it difficult for many women to negotiate safer sexual relations. In developing countries, and in some areas of

developed countries, women's economic and social dependence on men may further limit women's relationship power. Gendered power also creates the potential for sexual coercion and other forms of intimate partner violence against women. For women in primary relationships, negotiating condom use may be even more difficult than in casual partnerships. These factors highlight the urgency of promoting the female condom – as a female-controlled method of protection against HIV and STIs – while also affording women another contraceptive option.

Findings from several effectiveness studies have indicated that the female condom is at least as effective as the male condom in preventing STIs. Converging studies show that short-term acceptability of the female condom is high among diverse groups of women and men in many settings. Yet, even with indications of high efficacy, effectiveness and acceptability, the female condom has not been as widely used as expected. As noted in the WHO's discussion of introducing new contraceptive technology, acceptability and uptake are driven by complex interactions among individual, partner, provider, and health system factors. Multilevel, multi-component, structural interventions that also target broader social and environmental factors are needed to have maximal impact on female condom access, acceptability, and uptake.

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Female, Male and Transgender Sex Workers, Epidemiology of HIV/AIDS

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Definition

Sex work is a nonjudgmental term that connotes the sale or exchange of consensual sexual services between adults. As it is work, sex work should be considered through a labor rights framework. Specific labor rights language is outlined in the “International Labour Organization (ILO) Recommendation concerning HIV and AIDS and the World of Work” (No. 200). The Recommendation promotes fair and safe working conditions for all workers, including sex workers, working under all forms or arrangements at all workplace types in

both the formal and informal sectors (International Labour Office 2010). It also seeks to ensure universal access to stigma-free HIV prevention, treatment and care services among all workers, and the full protection of the human and labor rights of all people living with HIV in the workplace.

Background

Within the understanding of sex work as work, it is important to recognize the diversity of types of workers, work settings, and exchange dynamics that exist. Sex workers may be female, male, or transgender persons, and they may receive money or goods in exchange for sexual services on a regular basis or occasionally (UNAIDS Guidance Note on HIV and Sex Work, UNAIDS 2009, Updated 2012). Sex workers and their clients also come from diverse socioeconomic, cultural, and religious backgrounds, as well as sexual orientations. Sex work may take place in a formal sex work venue such as a brothel, informally in bars or other entertainment establishments, or the street, or in the context of other public or open spaces. Sex workers and their clients increasingly communicate via the Internet and mobile phones. Additionally, sex work may or may not involve a third party who facilitates the negotiation of sexual services and profits from them, beyond the sex worker and their client.

There are limited data on the numbers of persons engaged in sex work across settings. However, one secondary analysis calculated the proportion of adult women engaged in sex work across different geographic regions. In sub-Saharan Africa, this proportion ranged from 0.4% to 4.3%. In Asia, the proportion of adult females engaged in sex work ranged from 0.2% to 2.6%. In Eastern Europe, it was from 0.4% to 1.4%, and in Western Europe it was from 0.1% to 1.4%. In Latin America and the Caribbean, the proportion of women involved in sex work was 0.2–7.4% (Vandepitte et al. 2006). Few data are available regarding how many men and transgender people are engaged in sex work.

Epidemiology of HIV Among Sex Workers

Globally, female, male, and transgender sex workers all bear a disproportionately high burden of HIV across geographic settings and epidemic settings (Beyrer et al. 2015).

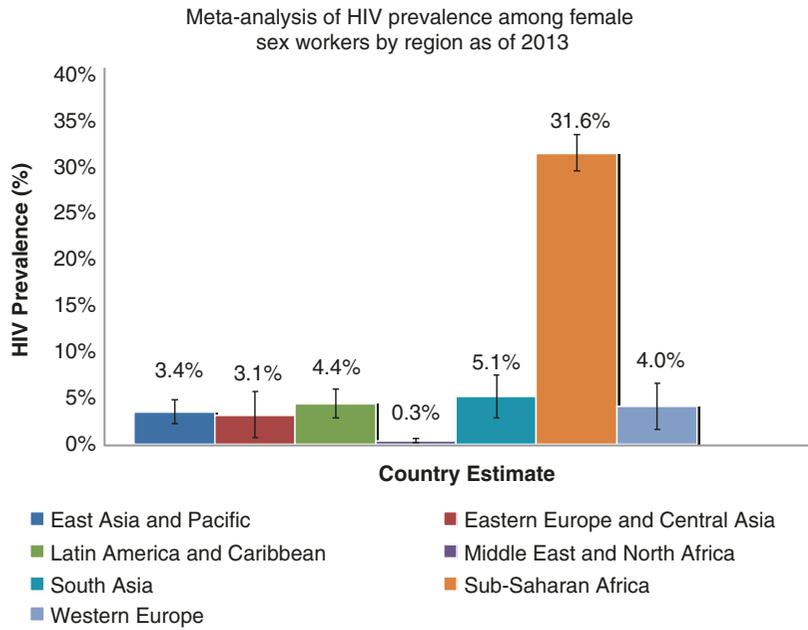
Female Sex Workers

A systematic review was conducted on the epidemiology of HIV among female sex workers in low- and middle-income countries using data from 2007 to 2011 from over 50 countries and representing about 100,000 female sex workers. This review documented a pooled global HIV prevalence of 11.8%. The authors also found that female sex workers have 13.5 times greater odds of being infected with HIV than adult women in the overall population (Baral et al. 2012; Kerrigan et al. 2013).

This review was updated in 2013 and expanded to include higher income countries (Beyrer et al. 2015). A total of 79 countries and over 437,000 female sex workers were included. As seen in Fig. 1, findings from that analysis show that the sub-Saharan African region continues to have the highest pooled HIV prevalence among female sex workers at 31.6%. Additionally, all countries where female sex workers had an HIV prevalence of 50% or greater were located in Southern Africa. The regional HIV prevalence among female sex workers in South Asia was 5.1%, followed by 4.4% in Latin America and the Caribbean, 4.0% in Western Europe, 3.4% in East Asia and the Pacific, 3.1% in Eastern Europe and Central Asia, and 0.3% in the Middle East and Northern Africa (Beyrer et al. 2015).

Despite sex workers' increased risk for HIV across geographic and epidemic settings, there has been little research conducted on the experiences and needs of sex workers living with HIV. A recent global review of HIV treatment outcomes among female sex workers found that current antiretroviral therapy (ART) use among HIV-infected female sex workers was only 38%. Ever use of ART among HIV-infected female sex workers in high-income countries was 80% versus 36% in low- and middle-income countries. Based

Female, Male and Transgender Sex Workers, Epidemiology of HIV/AIDS, Fig. 1 Meta-analysis of HIV prevalence among female sex workers by region as of 2013 (Beyrer et al. 2015)



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on available data, the review found that 57% of female sex workers on ART were virally suppressed (Mountain et al. 2014).

An analysis of population attributable fractions of HIV estimated that 15% of HIV in the female adult population in 2011 was attributable to occupational exposure during sex work. This same analysis estimated that approximately 106,000 HIV-related deaths occurred among female sex workers globally, with the vast majority of those deaths (98,000) occurring among sex workers in sub-Saharan Africa (Pruss-Ustun et al. 2013).

Male Sex Workers

In 2013, 27 of 192 countries reported data to the United Nations General Assembly (UNGASS) on HIV prevalence among male sex workers. Seven countries reported an HIV prevalence of greater than 20%, ten between 10–20%, and ten less than 10%. Data available from five African countries showed a median HIV prevalence of 12.5% among male sex workers. In ten European countries, the median HIV prevalence among male sex workers was 8.9%, as reported between 2007 and 2013. Additionally, a heavy burden of HIV among male sex workers in North America has also been

documented through various studies, with prevalence ranging between 5% and 31% (Baral et al. 2015).

Transgender Sex Workers

Data on transgender sex workers are even more limited. A meta-analysis of 25 studies from 14 countries (*n* = 6405) found an overall crude HIV prevalence among transgender sex workers of 27.3%. This was significantly higher than other transgender women (15%) in the review (Operario et al. 2008).

Structural Risk of HIV Among Sex Workers

Sex workers may experience heightened risk for HIV infection due to biomedical, behavioral, and structural factors. For example, they may experience HIV risk due to behavioral factors such as multiple and concurrent sexual partners, lack of ability to negotiate consistent condom use, and untreated sexually transmitted infections. However, structural factors are increasingly understood as the most significant factors driving sex

workers' increased risk for HIV (Shannon et al. 2015). For example, sex work is criminalized in some form in 116 countries (Global Commission on HIV and the Law 2012). Even in settings where sex work is not explicitly illegal, laws and policies are often indirectly used to negatively impact sex workers. Laws and policies prohibiting carrying condoms, public loitering, or "indecent" behavior laws are frequently used to harass and arbitrarily arrest sex workers and/or extort bribes from them (Open Society Foundations 2012). Criminalization and punitive policies related to sex work have been found to be associated with inconsistent condom use and increased HIV risk. Laws and policies that penalize and marginalize sex workers may also exclude them from participating in the formation and implementation of national HIV responses (Decker et al. 2015).

In addition to legal and policy issues, sex work is often highly socially stigmatized, and many sex workers face various forms of stigma and discrimination from community members, family and friends, government agencies, and healthcare facilities. Sex workers may also experience stigma and discrimination related to their gender, sexual orientation, HIV status, socioeconomic position, race/ethnicity, and/or substance use behaviors. Linked to these multiple forms of stigma, widespread human rights abuses, including high levels of physical and sexual violence, have been documented among sex workers in numerous settings including at the hands of state (e.g., police) and non-state actors (e.g., sexual partners, community members). These socio-structural factors interact to create significant barriers to the health and human rights of sex workers, including obstacles to HIV prevention, treatment, and care (Decker et al. 2015).

Modeling conducted for diverse settings such as Canada, India, and Kenya has also estimated that addressing specific key structural factors such as violence, police harassment, safer work environments, and decriminalization could avert 33–46% of incident HIV infections among female sex workers over the next decade. These analyses found that the single most important intervention that could impact HIV among sex workers would

be the decriminalization of sex work, which would have a substantial effect across both generalized and concentrated HIV epidemics (Shannon et al. 2015).

Community-Led Responses to HIV Among Sex Workers

Research findings point to a number of promising interventions to address the burden of HIV among sex workers across epidemic settings. In particular, approaches that address the structural context of sex workers' risk for HIV infection, as described above, have shown particular promise. For example, community empowerment-based responses have been shown to be effective in reducing sex workers' risk for HIV and sexually transmitted infections (STIs). A recent systematic review and meta-analysis examined the effectiveness of community empowerment-based approaches to preventing HIV among female sex workers in low-income and middle-income countries. Meta-analysis results revealed that community empowerment approaches among sex workers were associated with a 32% reduction in HIV prevalence as well as significantly decreased odds of gonorrhea, chlamydia, and high-titer syphilis. The odds of syphilis were reduced by almost half with a community empowerment approach. Additionally, this approach was associated with an approximately threefold increase in the odds of consistent condom use between sex workers and their clients across geographic settings (Kerrigan et al. 2015).

Community-led approaches involve a process by which sex workers themselves take collective ownership of programmatic initiatives to achieve the most effective HIV outcomes and to address social and structural barriers to their health and human rights. Such approaches often start by promoting cohesion within the sex worker community through peer-led activities, then mobilizing the collective power of sex workers to improve access to social and material resources including HIV services. Community empowerment approaches among sex workers have been recognized as a UNAIDS Best Practice for more than a

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Fig. 2 Essential elements of comprehensive, community-led responses to HIV among sex workers (WHO et al. 2013)



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decade and now underpin multiple UN policy documents regarding the HIV response among sex workers including normative guidelines (WHO et al. 2012) and a comprehensive program implementation tool for female, male, and transgender sex workers (WHO et al. 2013).

The “Implementing Comprehensive HIV/STI Programs with Sex Workers” tool starts with the recognition of community empowerment as the central component of a comprehensive programmatic approach to HIV among sex workers integrating biomedical, behavioral, and structural interventions as seen in Fig. 2. The tool then outlines five other essential programmatic elements including: addressing violence against sex workers; community-led services such as peer education and drop-in centers where sex workers can come together and organize; condom and lubricant programming; clinical and support services for fundamental prevention, treatment, and care; and program management and organizational capacity building (WHO, UNFPA,

UNAIDS, NSWP, The World Bank, UNDP 2013).

While the tool provides concrete recommendations and examples from successful programs, its use and components must be tailored to the diverse needs of female, male, and transgender sex workers across diverse sociocultural settings. For example, in geographic settings where sex work and injection drug use may intersect to create higher risk, such as the epidemics of Eastern Europe and Central Asia, ensuring that sex workers have access to tailored harm reduction services is critical to preventing HIV transmission.

Biomedical HIV Prevention Interventions Among Sex Workers

Mathematical modeling has shown that biomedical interventions such as pre-exposure prophylaxis (PrEP) and treatment as prevention could

significantly reduce HIV incidence among sex workers in South Africa, where HIV among sex workers remains quite high, demonstrating reductions of 40% over a 10-year period (Bekker et al. 2015). Additionally, improving coverage rates and virologic suppression through ART scale-up efforts among female sex workers in Kenya has also been shown through modeling to have a substantial effect, averting up to 34% of HIV infections over the next decade (Shannon et al. 2015). While biomedical interventions hold significant promise to address the HIV heightened risk of sex workers, it is critical that these services be part of a holistic package of services based on the stated needs of the sex worker community.

Related to this point, in other modeling analyses researchers demonstrated the potential impact of integrating universal access to HIV treatment within a community empowerment approach among female sex workers in Kenya, Thailand, Brazil, and Ukraine. When equitable access to HIV treatment for sex workers was paired with the expansion of a comprehensive community empowerment approach, modeling demonstrated that up to 31,200 new infections among female sex workers could be averted. These analyses also showed that by taking comprehensive (combining biomedical, behavioral, and structural elements), community empowerment-based approaches to HIV among female sex workers to scale, these countries can significantly reduce HIV incidence among sex workers and the overall adult population (Wirtz et al. 2014).

Achievements and Gaps in Implementation

Sex worker programs have had significant successes promoting the consistent use of condoms, perhaps more than those for any other affected population (Bekker et al. 2015). The number of countries reporting condom use at last commercial sex has risen steadily over the years as a result in part of the expansion of community-led peer education and condom distribution. Based on available UNGASS data, 44 countries reported higher

condom use at last sex between female sex workers and their clients in 2012 as compared to 2009: 85% versus 78% (UNAIDS 2013). Modeling of data from South Africa suggests that condom promotion activities may have already reduced HIV incidence in South African sex workers and their clients by up to 70% (Bekker et al. 2015). While these are positive trends, there are still significant ongoing gaps in addressing the risk for HIV among sex workers. To date the percent of global donor funding allocated to HIV services for sex workers has often been just a few percentage points of the total allocations made (UNAIDS 2013). This level of investment does not match the scope of sex work or the burden of disease faced by sex workers.

There are also important coverage gaps. A global review of HIV among sex workers found that across eight focal case study countries less than 50% of sex workers in those settings had access to basic prevention services (Kerrigan et al. 2013). While UNGASS indicators may show relatively high levels of having “received a condom” in the last year or “know where to test for HIV,” such program indicators do not adequately capture the broader health and human rights challenges faced by sex workers.

As community-led approaches to the HIV response among sex workers take root and are expanded, indicators of progress also need to expand to include measurement of structural intervention components as well as the processes and outcomes of community empowerment over time (WHO et al. 2013). These indicators may include measures of sex worker leadership, organizations, governance, decision-making, resource mobilization, control over funds, and engagement with the wider society to reduce sex work-related stigma at the community level, as well as potential shifts in laws and policies that disempower sex workers and create barriers to their health and well-being (Narayanan et al. 2012).

Additionally, barriers to scale-up and implementation of evidence-based, community-driven responses to HIV among sex workers have been shown to exist at various levels, including global discourse and donor investment priorities, national laws and policies criminalizing sex

work, and intersecting forms of social exclusion including sex work-related stigma, discrimination, and violence at the local level (Kerrigan et al. 2015).

Knowledge Gaps and Implementation Science

Moving forward in the response to the HIV epidemic among sex workers, we must continue to implement, expand, and evaluate the effectiveness of comprehensive, rights-based policies and programs in varied settings and among all types of sex workers including female, male, and transgender. Critical implementation science questions must also be addressed regarding the process and impact of community-led initiatives as they incorporate novel biomedical approaches, including ART-based prevention strategies.

Examples of important topics and operations research questions may include how to better integrate HIV and other health (reproductive health, substance use, etc.) and social services to serve sex workers' needs and improve HIV outcomes, how to reduce societal stigma regarding sex work at the community level, how to ensure that sex workers are appropriately screened and referred to tailored violence prevention and care services, and how to best promote the financial security and economic empowerment of sex workers.

In these efforts, sex worker organizations seek to lead intervention implementation as well as play a central role in the science of implementation by documenting their own experiences and efforts to respond to HIV while promoting and protecting their human rights. Several ongoing South-to-South initiatives have evolved in this regard working to document and then transfer community-led, programmatic lessons learned from one lower- or middle-income context to another. This has included distilling and sharing lessons on community mobilization from the internationally recognized sex worker-led responses to HIV in India to the generalized epidemics in sub-Saharan Africa where HIV prevalence among sex workers continues to be unacceptably high (Kerrigan et al. 2015).

Conclusions

Available evidence indicates that the greatest impact on the HIV risk of sex workers will be achieved through decriminalization of sex work and the implementation and scale-up of a comprehensive, community-led response to HIV. This response will include strategic combinations of biomedical, behavioral, and structural interventions that are context specific. Significant resources and advocacy are needed to shift the field toward a rights-based approach that responds to the multifaceted needs and realities of sex workers.

Cross-References

- ▶ [Combination Approaches to HIV Prevention](#)
- ▶ [HIV Prevention and Women](#)

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Fusion

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Definition

- Virus-cell fusion and cell-cell fusion are the primary means by which the human immunodeficiency virus (HIV) enters and replicates in human target cells. The glycoproteins gp120 and gp41 make up the outer coat (envelope) of HIV. Typically, an HIV virus particle, or virion, infects its host target cell by binding its envelope glycoprotein to a cellular receptor and ► **CXCR4, Co-receptors**, i.e., molecules that are present on the cell surface for ligand binding. Thus, HIV enters the host cell by fusion between viral and cytoplasmic membranes (direct membrane fusion) or between viral and endosomal membrane (endocytosis). The cytoplasm consists of the whole cellular contents, excluding the nucleus, whereas the endosome is a membrane-enclosed compartment of the endocytic pathway. The functions of endosomes include:
 - The transport of molecules internalized from the plasma membrane to the lysosome (a cell organelle containing digestive enzymes capable of degrading the constituents of living matter)
 - The transportation of microbes into the cytoplasm for infection

For an HIV virion in an infected cell to infect another target cell, two modes of fusion are observed: cell-cell fusion, where fusion occurs between membranes of the HIV-infected cell and target cell, and cell adhesion where binding between an HIV-infected cell and an uninfected target cell occurs with the assistance of cell adhesion molecules. Therefore, membrane fusion is a key step for HIV entry into the host cell to enable the delivery of its genetic material.

Virus-Cell Fusion

Virus-cell fusion is the primary means by which HIV delivers its genetic material into the human host cell, e.g., CD4⁺ T cells. As noted above, the outer coat of the HIV virus consists of two prominent envelope glycoproteins, a surface subunit gp120 and a transmembrane subunit gp41, and both envelope glycoproteins are utilized by HIV to fuse its membrane with that of the target cell.

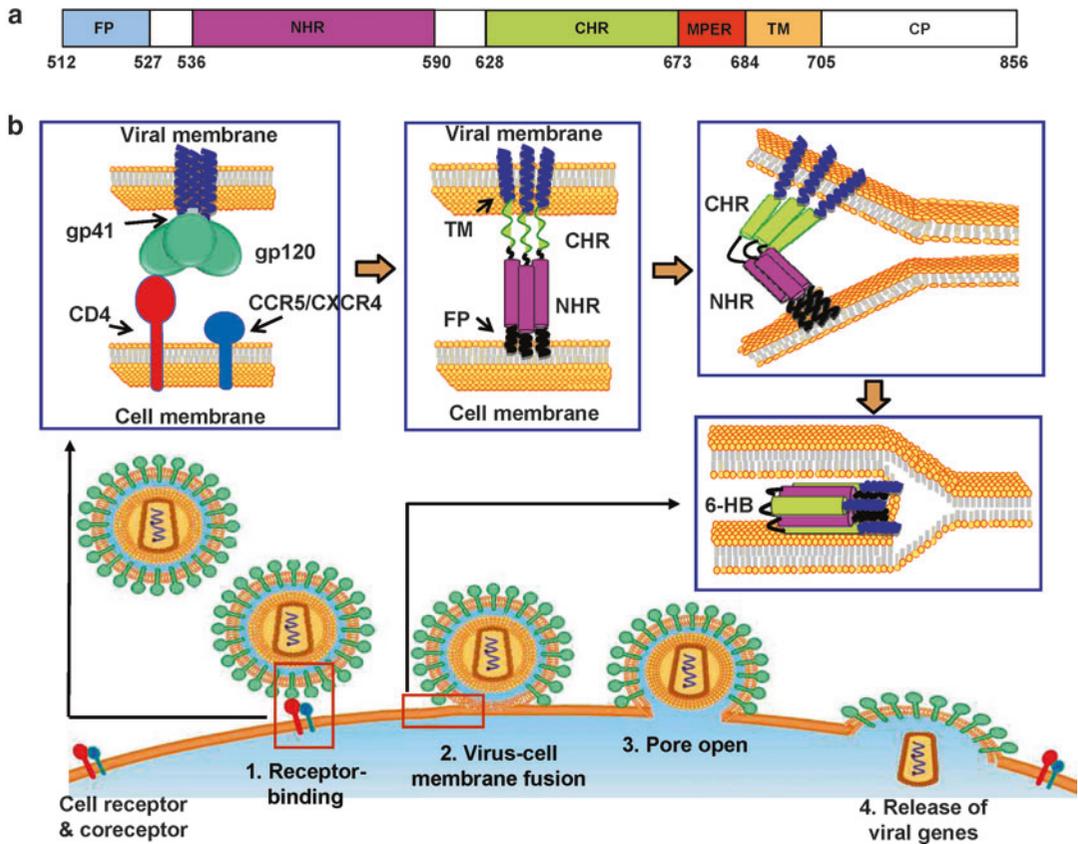
The gp120 subunit is responsible for virus binding and host tropism, i.e., the way in which different viruses preferentially attack specific host species or specific cell types in their hosts. The gp41 subunit mediates the actual viral fusion process. The gp41 molecule consists of an extracellular domain (ectodomain), a transmembrane domain (TM), and a cytoplasmic domain (CP). The ectodomain is composed of several functional regions, including a fusion peptide (FP), a N-terminal heptad repeat (NHR), a C-terminal heptad repeat (CHR), and a membrane-proximal external region (MPER) (Fig. 1a). A heptad repeat is a structural motif consisting of a repeating pattern of seven amino acids, which forms the basis for coiled-coil helix bundle. It is the HIV-1 gp41 subunit protein that plays a key role in mediating the fusion between the viral envelope and the target cell membrane by either direct membrane fusion or endocytosis, as described below.

Fusion Between Viral Envelope and Cytoplasmic Membrane (Direct Membrane Fusion)

The simplest and most common way for HIV and many other enveloped viruses to enter target cells is

through direct membrane fusion. In this scenario, the viral envelope membrane fuses with the target cell membrane, and the viral capsid, which contains the genetic material of the virus, is then delivered into the target cell (Hernandez et al. 1996).

More specifically, before the start of membrane fusion, gp41, which is sheltered by gp120, maintains its native, metastable conformation with its FP buried inwards. Next, the viral gp120 binds to the CD4 molecule, which is the primary receptor for HIV-1, on the target cell. This causes a structural change of gp120, allowing it to bind with a specific chemokine (small proteins secreted by cells) receptor on the cell surface, either CCR5 or ► [CXCR4, Co-receptors](#). CCR5 serves as the coreceptor for the macrophage-tropic or CCR5-using (R5) viruses, while ► [CXCR4, Co-receptors](#) is the coreceptor for T cell line-tropic or CXCR4-using (X4) viruses (Berger et al. 1999). After binding takes place, the viral transmembrane subunit gp41 is released from its metastable state and changes its conformation. The previously buried FP at the N-terminus of gp41 springs out toward the target cell membrane and inserts itself into the cytoplasmic membrane in either oblique or lateral direction. At this time, the N-terminal and C-terminal regions of the gp41 ectodomain (NHR and CHR, respectively) come into play, and the viral membrane is forced into proximity with the cell membrane. To accomplish this, interaction between the aforementioned NHR and CHR helices forms a thermodynamically stable six-helix bundle, which consists of three NHR helices that form a trimeric coiled coil with the exposed hydrophobic grooves and three CHR helices that bind to hydrophobic grooves in an antiparallel orientation. This event leads to the release of energy that, in fact, brings the viral and target cell membranes into close proximity for hemifusion of the two proximal layers of membranes (Chan et al. 1997; Weissenhorn et al. 1997). Finally, the two most distant layers of the membranes fuse together, driving the formation of the fusion pore, through which the viral capsid is released into the cytosol (the fluid component of the cytoplasm) of the target cell, which starts a new replication cycle of the virus (Fig. 1).



Fusion, Fig. 1 Schematic representation of the HIV-1 gp41 and the model of fusion between HIV-1 envelope and cytoplasm membrane of the target cell. (a) Schematic view of the HIV-1 gp41 molecule. *FP* fusion peptide, *NHR* N-terminal heptad repeat, *CHR* C-terminal heptad repeat,

MPER membrane-proximal external region, *TM* trans-membrane domain, *CP* cytoplasmic domain. (b) HIV-1 fusion with plasma membrane mainly includes four major steps: (1) receptor binding, (2) virus-cell membrane fusion, (3) fusion pore opening, and (4) release of viral genes

Fusion Between Viral Envelope and Endosomal Membrane (Endocytosis)

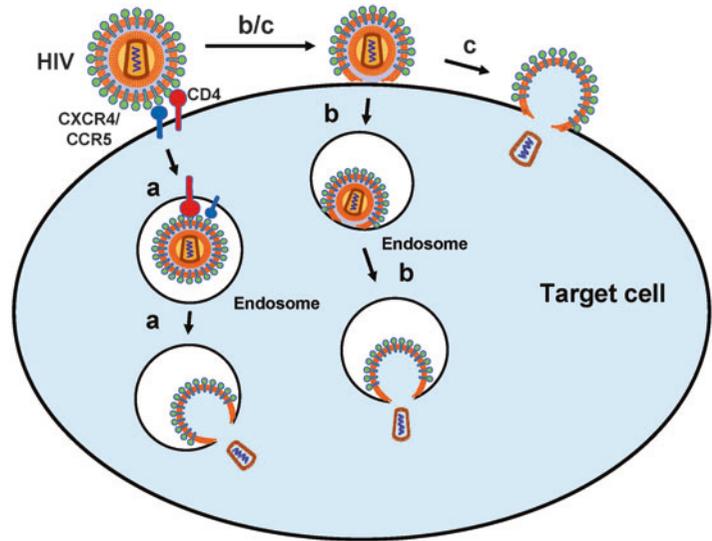
Endocytosis is the process by which a cell absorbs molecules (e.g., proteins) or particles (e.g., virions) from outside the cell by engulfing the molecules or particles with its cell membrane. Endocytosis is also an important pathway for some enveloped viruses (influenza), to enter target cells in a pH-dependent manner.

HIV can enter host cells through direct membrane fusion, as described above, while it has been shown that HIV entry cannot be inhibited by lysosomotropic reagents that completely block the entry of pH-dependent viruses. Therefore, it has been widely accepted that viral entry does not involve endocytosis. However, recent studies

have shown that endocytosis is indeed an alternative pathway for HIV to enter the target cell (Mercer et al. 2010).

After the HIV virion attaches to the CD4+ target cell via the interaction between gp120 and the CD4 receptor, gp120 may or may not bind with the coreceptor, CCR5 or \blacktriangleright CXCR4, Co-receptors, before endocytic uptake of virus particles. While some viral particles may have been destroyed in the matured endosome, others may be able to fuse their envelope membranes with endosomal membranes, resulting in the release of the viral capsids from the endosome into the cytosol (Miyachi et al. 2009) (Fig. 2a). After the virion binds to CD4 and a coreceptor, still another model indicates that its gp41 succeeds in forming

Fusion, Fig. 2 Multiple models of HIV-cell fusion. (a) Endocytosis after virus-cell binding. (b) Endocytosis after virus-cell fusion. (c) Direct membrane fusion and pore formation



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a coiled-coil six-helix bundle, pulling the viral and target cell membranes together for fusion. In this model, the virion enters the endosome and releases its genetic material through the fusion pore in the endosomal membrane (Miyachi et al. 2009) (Fig. 2b). However, the molecular mechanisms controlling the endocytosis of HIV have not been clearly determined, and the viral and cellular cofactors that are involved in the endocytotic process are not fully understood.

Cell-Cell Fusion (Syncytium Formation)

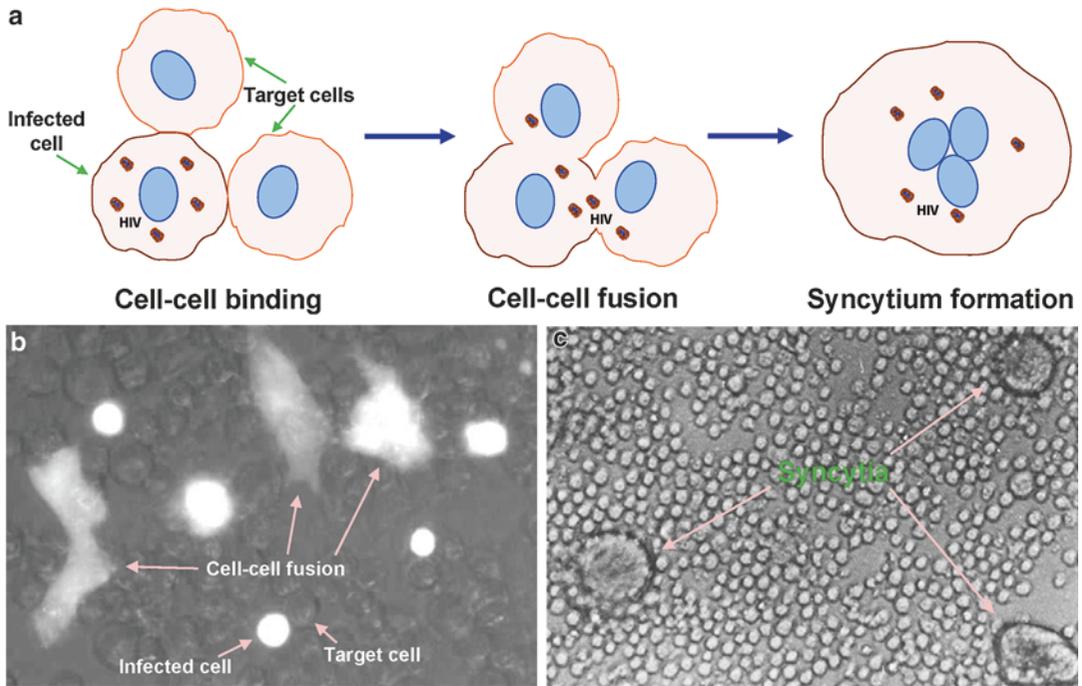
Cell-cell fusion is a natural process that occurs in physiological conditions, such as embryogenesis and morphogenesis, and the differentiation of muscle, bone, and trophoblast cells, as well as pathological conditions, e.g., cancer metastasis. Cell-cell fusion is also an effective way for cell-to-cell spread of many enveloped viruses, such as herpes simplex virus (HSV), respiratory syncytial virus (RSV), and HIV (Hernandez et al. 1996).

The syncytium is a giant cell with multinuclei resulting from multiple cell fusions of cells with a single nucleus. It can form under physiological conditions, e.g., the formation of skeletal muscle, or under pathological conditions, such as generation of binucleated and metastasizing tumor cells. Syncytia can also form when the host cells are

infected with some of the enveloped viruses, such as RSV and HIV. At the late stage of HIV-induced cell-cell fusion, syncytia appear when one or two HIV-infected cells fuse with several uninfected target cells.

In this case, after HIV replication, the cellular-produced HIV envelope glycoproteins gp120 and gp41 are transported to the cell surface. These HIV-infected cells can then bind to uninfected CD4⁺ T cells through the interaction between the viral envelope glycoproteins on the surface of the infected cells and the CD4 receptor and coreceptor (e.g., ► **CXCR4, Co-receptors**) on the surface of the uninfected cells (Dimitrov 1997). Similar to virus-cell fusion, the gp41 molecule on the cell surface changes conformation by inserting its FP into the uninfected cell membrane and forming a six-helix bundle, resulting in the fusion of the infected and uninfected cells. With the opening and expansion of the fusion pore in the cell-contact zone, the viral genetic material is easily transported into the uninfected cell. If one HIV-infected cell fuses with several uninfected cells, a syncytium forms at the late stage of cell-cell fusion (Fig. 3a).

HIV-induced cell-cell fusion and syncytium formation can be observed under a microscope. HIV-infected cells are labeled with a fluorescent dye and mixed with the uninfected CD4⁺ target cells. If a fluorescence-labeled HIV-infected cell



Fusion, Fig. 3 HIV transmission via cell-cell fusion. (a) Schematic representation of HIV-mediated cell-cell fusion and syncytium formation. (b) Microscopic view of an

HIV-infected cell fusing with a target cell. (c) Microscopic view of HIV-mediated syncytium formation

fuses with one or more uninfected cells, the fluorescent dye in the labeled HIV-infected cell diffuses into the uninfected target cells, resulting in decreased fluorescence intensity and increased size of the fluorescence-positive cell (Fig. 3b). Therefore, the fluorescence-labeled HIV-infected cells, both fused and unfused with uninfected cells, can be easily distinguished and counted under a fluorescent microscope (Lu et al. 2003). About 2–3 days after coculture of HIV-infected cells and uninfected cells, significant formation of syncytia containing multinuclei resulting from the fusion of multiple cells can be observed and quantified under an inverted microscope (Fig. 3c).

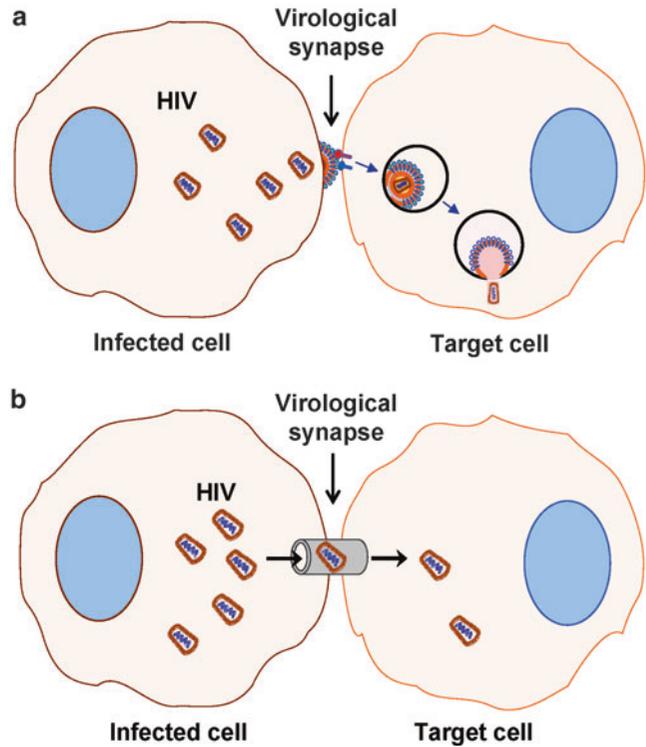
HIV Cell-to-Cell Transmission via Virological Synapse

A synapse is generally defined as a junction structure between neurons that permits a neuron to pass chemical or electrical signals to another neural cell, designating “chemical synapse” or “electrical

synapse,” respectively. Later, an “immunological synapse,” which is the interface between an antigen-presenting cell, a type of immune cell (such as dendritic cell) that can display an antigen to other immune cells (e.g., T cells) to boost immune response, and lymphocytes, a type of white blood cell involved in the immune response, was discovered as a molecular machine controlling T cell activation (Grakoui et al. 1999). Subsequently, an intercellular adhesive structure that formed between infected and uninfected CD4⁺ T cells, called a “virological synapse,” was identified (Jolly et al. 2004). In the virological synapse, the viral envelope glycoproteins, gp120/gp41, and the cellular receptors, CD4/▶ CXCR4, Co-receptors, were found to colocalize at the site of cell-cell contact, suggesting that the virological synapse may be involved in virus assembly or transmission.

Using a quantitative 3D video microscopy and fluorescent clones of HIV to visualize cell-to-cell transfer of HIV, researchers observed that an infected CD4⁺ T cell came into contact with an

Fusion, Fig. 4 Cell-to-cell transmission of HIV via virological synapses. (a) HIV spreads across a virological synapse between an HIV-infected cell and an uninfected CD4⁺ cell via endocytosis. (b) HIV travels along a “membrane nanotube” to enter the target cell



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uninfected CD4⁺ T cell to create a virological synapse at their junction. The fluorescent viral particles in the infected T cell then moved toward the synapse and entered into the uninfected cell within a few minutes (Hubner et al. 2009). Mature HIV virions have been observed to be transmitted across the virological synapse through direct membrane fusion, whereas immature virions may be taken up into the target cell by endocytosis and then fuse from within the endosomal compartment when maturation is complete (Fig. 4a). Besides assembling a single large-scale virological synapse containing pairs of conjugated cells, HIV can mediate the formation of “polysynapses” targeting multiple cells for more effective cell-to-cell transmission of HIV. In rare circumstances, HIV can also travel along an intercellular tubular structure termed “membrane nanotube” that joins the infected and uninfected T cells through a “micro-synapse” to enter into the target cell (Fig. 4b).

The virological synapse plays a key role in the presence of an adhesive junction between infected and uninfected cells, which is sufficiently stable to allow the transfer of virus to the target cell. The

high affinity binding of gp120 to the CD4 receptor molecule may be the initial force to hold the interacting T cells together, while the subsequent stable adhesive junctions may be maintained by integrin-ICAM interactions. Integrin is an adhesive protein that promotes interactions between a cell and its environment, while ICAM is a glycoprotein on the cell surface that mediates adhesive interaction important for the immune response. It is believed that cell-bound HIV transmission through a virological synapse is more than a 1,000-fold more effective than transfer of cell-free virus (Hubner et al. 2009). Therefore, direct cell-to-cell transfer through a virological synapse may be more efficient than that accomplished via cell-cell fusion, as illustrated above.

HIV Fusion as a Drug Target: HIV Fusion Inhibitors

Both virus-cell fusion and cell-cell fusion can serve as targets for the development of anti-HIV drugs. In the early 1990s, two groups discovered

that the synthetic peptides derived from the sequence of the HIV-1 gp41 CHR region could potentially inhibit HIV-induced cell-cell fusion and HIV-cell fusion (Jiang et al. 1993; Wild et al. 1994). One of the peptides, T20, was approved by the Food and Drug Administration (FDA) of the United States as the first HIV fusion inhibitor (brand name, Fuzeon; generic name, enfuvirtide) for treatment of HIV-infected patients who fail to respond to the current anti-HIV drugs.

CHR peptide blocks the six-helix bundle formation between the NHR and CHR regions of the viral gp41, a critical step in HIV-cell and cell-cell fusion events (Liu et al. 2007). Structurally, the synthetic peptide with the CHR sequence can compete with the viral gp41 CHR region to bind with the viral gp41 NHR region. Thus, the viral gp41 CHR is unable to form a six-helix bundle with the viral gp41 NHR, and viral fusion with the target cell is effectively blocked. Similarly, HIV-infected cells cannot fuse with uninfected target cells in the presence of the CHR peptide because this peptide blocks the six-bundle formation between the NHR and CHR regions of gp41 on the surface of the HIV-infected cells.

Compared to other anti-HIV drugs that target the HIV replication steps inside the host cell, enfuvirtide has more advantages because it targets the HIV fusion step and inhibits HIV infection before the virus can enter the host cell. This “keep the enemy outside the gates” strategy minimizes the harm of the virus to the body. Otherwise, once the virus enters the host cell, it inserts its genetic material into the human genome to establish a latent reservoir and produces viral proteins that affect the functions of the infected immune cell.

Because of the different mechanism of action, enfuvirtide can be used in combination with other anti-HIV drugs in highly active antiretroviral therapy (HAART) for treatment of HIV patients with multidrug resistance. However, the clinical application of enfuvirtide is limited because of its weaknesses: (i) lack of high potency, requiring it to be used at a dosage of 180 mg per day, resulting in high cost; (ii) short half-life, necessitating injection twice a day; (iii) tendency to quickly induce

drug-resistant HIV-1 strains (Cai and Jiang 2010). These problems have promoted the development of next-generation HIV fusion inhibitors. Currently, several peptides with improved efficacy, half-lives, and resistance profiles are under clinical development. Since enfuvirtide cannot be taken orally, several groups have been developing small-molecule HIV fusion inhibitors that also target the HIV-1 gp41 (Jiang et al. 2011). The advantages of the small-molecule compound-based HIV fusion inhibitors include their oral availability and relatively low cost of production.

Besides the pathways of HIV-cell fusion and HIV-induced cell-cell fusion, HIV can also be transmitted through endocytosis and virological synapses, as described above. However, it is questionable whether enfuvirtide can effectively block the transmission of HIV via endocytosis or virological synapse. Since this peptide drug cannot enter cells, it cannot block intracellular viral fusion. This calls for more extensive research on these alternative pathways of HIV fusion.

Conclusion

To infect a host cell, HIV must enter the target cell through virus-cell fusion, cell-cell fusion, or an alternative pathway. A cell-free virion first attaches to the target cell via the interaction of its envelope glycoprotein gp120 with its primary receptor (CD4) and Co-receptors (CCR5 or ► [CXCR4, Co-receptors](#)) on the surface of the target cell. Virus-cell fusion is subsequently initiated through direct membrane fusion or endocytosis. A virion in an infected cell can also enter the target cell through cell-cell fusion. The HIV-infected cell first attaches to the target cell via the interaction between the viral envelope glycoprotein gp120 on the surface of the HIV-infected cell and the receptor/Co-receptors on the surface of the target cell. Cell-cell fusion is then completed, resulting in syncytium formation. A virion in an infected cell can also enter the target cell through a virological synapse formed at the junction between HIV-infected and uninfected cells. The viral genetic material is then released to the target cell to initiate a new replication cycle.

Fusion between viral and target cell membranes (direct membrane fusion) has long been recognized as the primary means of HIV entry into the target cell. However, recent studies have challenged this hypothesis, suggesting that endocytosis may be the predominant pathway of HIV entry. However, more investigation is needed to gain better insight into the fusogenic mechanisms of HIV entry by endocytosis.

In contrast to cell-free HIV transmission via virus-cell fusion, it is widely agreed that cell-associated HIV transmission is much more effective via cell-cell fusion. On the other hand, recent studies indicate that spread of cell-associated HIV through the virological synapse is even more efficient than transmission via cell-cell fusion. Currently, most researchers believe that both pathways are important for HIV cell-to-cell transmission.

The discovery of CHR peptides as HIV fusion inhibitors, such as enfuvirtide, has opened a new avenue for developing anti-HIV drugs. However, the peptide drug is too expensive for production and lacks oral availability. Therefore, the development of small-molecule HIV fusion inhibitors with oral availability and low production cost is urgently needed. Research and development of new drugs targeting the alternative pathways of HIV fusion (e.g., endocytosis) are also needed.

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Gamma Delta T Cells

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Definition

Human T cells recognize infectious agents or cancer through two main types of cell surface antigen receptors. CD4+ or CD8+ cells express alpha and beta chains to form the $\alpha\beta$ T cell receptor (TCR). An alternate T cell receptor is made from gamma and delta chains ($\gamma\delta$ TCR). The $\alpha\beta$ TCR recognizes peptides presented by major histocompatibility complex (MHC) molecules. Molecular targets for $\gamma\delta$ TCR include non-peptidic antigens that are recognized without MHC presentation. During early phases of HIV disease, the major subset of $\gamma\delta$ T cells in blood is extensively depleted or inactivated. Consequently, HIV patients have profound $\gamma\delta$ T cell deficiencies and fail to generate normal $\gamma\delta$ T cell responses to phosphoantigens (five-carbon pyrophosphates made by the host or selected microbes). The phosphoantigen response is important for natural tumor immunity, for tuberculosis resistance, for

immune regulation and probably for HIV control. Thus, $\gamma\delta$ T cell depletion occurring in every infected patient is an important part of HIV-associated disease. After prolonged treatment, HIV patients begin to reconstitute the $\gamma\delta$ T cell subset but immunotherapy will be needed to reconstitute normal levels of cell function. The goals for HIV-related $\gamma\delta$ T cell studies are to define the impact of depletion on disease, including HIV-associated cancer or opportunistic infections, and test therapeutic interventions capable of activating critical effector functions in $\gamma\delta$ T cells once they have been reconstituted during prolonged antiretroviral therapy.

$\gamma\delta$ T Cell Functions in the Healthy Immune System

$\gamma\delta$ T cells make up 3–10% of T cells in circulation. They are distinguished from conventional CD4+ or CD8+ T cells because they express a unique T cell receptor (TCR) and recognize molecular targets other than processed peptides. $\gamma\delta$ T cells are present in mammals but their composition and functions in primates and human beings are distinct from all other species (Kalyan and Kabelitz 2013). The uniqueness of primates and man is due to a special γ -chain gene designated V γ 2 (V γ 9 in an alternate nomenclature). The V γ 2 chain is usually combined with V δ 2 chain to form the V γ 2V δ 2 T cell receptor. The unique V γ 2 T cell receptor γ -chain gene endows cells with

a capacity to recognize and proliferate in response to stimulation by five-carbon pyrophosphate molecules collectively termed phosphoantigens (PAg). The most common PAg are isopentenyl pyrophosphate (IPP), an intermediate of isoprenoid biosynthesis in man, and hydroxymethyl-but-2-enyl pyrophosphate (HMBPP) produced by bacteria, protists, fungi, and plants (Riganti et al. 2012). IPP concentrations increase to stimulatory levels when cells are stressed by infection or undergo transformation to malignant states. Drugs used commonly for treating bone disorders (bisphosphonates) block the conversion of IPP, and it then accumulates to levels sufficient for activating V γ 2 cells. Bisphosphonates also cause activation and proliferation of $\gamma\delta$ T cells in patients, but require simultaneous administration of interleukin-2 (IL-2). Animal studies and limited human clinical trials confirmed the ability of HMBPP to activate and expand V γ 2V δ 2 T cell populations in vivo. On a molar basis, HMBPP is much more potent than the mammalian analog IPP. Cells capable of responding to PAg or aminobisphosphonate stimulation represent approximately 4% of total lymphocytes among HIV-negative healthy adults. $\gamma\delta$ T cell subsets are usually named for the γ -chain and PAg-responsive cells are known as the V δ 2 T cell population.

In addition to the major V δ 2 T cell population in blood, a minor group of $\gamma\delta$ T cells expresses the V δ 1 TCR chain (along with any of several V γ chains) and comprises around 30% of $\gamma\delta$ T cells in blood. The V δ 1 cells are more common among intraepithelial lymphocytes in mucosal epithelial tissues than in blood. The roles for tissue-resident V δ 1 cells are not completely understood but they seem to regulate stress responses, cytokine expression, and recruitment of leukocytes or inflammatory cells to sites of damage and infection. The V δ 1 T cells respond to bacterial lipids or related molecules and participate in the complex regulation of mucosal immunity. These two subpopulations V δ 2 and V δ 1 T cells are more numerous than all others. Less is known about the functions of minor $\gamma\delta$ T cell subsets in man.

While V δ 2 cells and their responses to PAg are nearly ubiquitous among HIV-negative individuals,

their circulating levels are affected by age, gender, and race. V δ 2 cell levels peak at around 20 years of age and then decline steadily throughout life. During the decline, cell levels in men and women begin to diverge around the sixth decade, when women can have nearly three times higher levels of circulating V δ 2 T cells (Caccamo et al. 2013). In addition, V δ 2 levels in blood are affected by race with European-origin individuals in North America having up to fourfold higher baseline levels of V δ 2 T cells compared to matched populations of African-origin individuals (Cairo et al. 2010), and Asians being more similar to European-origin donors. However, strong responses to PAg stimulation were seen among all healthy donors and are sufficiently vigorous to assure a high degree of function. These numerical differences are mainly important for natural history studies of HIV disease where loss and reconstitution of V δ 2 T cells are valuable markers for treatment success or failure.

The Impacts of HIV Disease on $\gamma\delta$ T Cells

Our interest in $\gamma\delta$ T cells is driven by a need to explain the causes and consequences of dramatic changes to V δ 2 T cells during HIV infection (Li et al. 2013). The vast majority of V δ 2 cells do not express the CD4 receptor for HIV and are not susceptible to HIV infection either in patients or in the laboratory. Despite this property, V δ 2 T cells are one of the first T cell subsets impacted by HIV, being depleted and made less functional during early stages of disease. The V δ 2 T cell counts continue to decline or remain low as long as there is active viremia. Among advanced HIV patients with <200 CD4 T cells/mm³ of blood, the important PAg-reactive V γ 2V δ 2 T cells are nearly extinguished. Prolonged, successful antiretroviral therapy leads to slow reconstitution of V γ 2V δ 2 T cells in a process that depends on new T cell synthesis, but normal cell counts, robust effector-memory subsets, and strong responses to PAg are not recovered completely even after more than 10 years of treatment-associated virus suppression.

The loss of V δ 2 T cells seems greatest during acute or early stages of HIV disease when viremia

is high before starting antiretroviral therapy. One mechanism for deleting V δ 2 T cells depends on the unusually high levels of chemokine receptor CCR5. The CCR5 is so dense on V δ 2 cells that envelope glycoprotein of HIV (gp120) binds tightly even in the absence of cell surface CD4. The complex of HIV envelope glycoprotein and CCR5 signals cells through p38 MAP kinase and activates caspases that are eventually responsible for the death of V δ 2 cells (Li and Pauza 2011). This mechanism promotes depletion of a CD4-negative CCR5-positive cell without direct infection and likely explains why V δ 2 T cell depletion is greatest during high viremia when viral proteins, including envelope glycoprotein, are at peak levels. Other mechanisms may also promote V δ 2 cell depletion including apoptotic cell death resulting from chronic activation or cytokine exposure during HIV disease.

V γ 2V δ 2 T cells are thought to have direct roles in controlling HIV through cytotoxic destruction of infected cells, producing β chemokines that block HIV entry or promoting Th1 antiviral immunity (Agrati et al. 2011). Losing normal $\gamma\delta$ T cell functions of tumor surveillance, broad pathogen resistance, and immune regulation likely impact the comorbidities of HIV/AIDS. Accordingly, HIV-mediated depletion of V δ 2 T cells is a mechanism for viral immune evasion and an important part of the explanation for chronic/persistent HIV disease. It was often seen that the proportion or absolute count of V δ 1 T cells was increased in blood when V δ 2 T cell levels declined (Wesch et al. 1998). Whether this is a compensatory or homeostatic mechanism is not known. The increase in V δ 1 cells may be related to bacterial translocation with direct activation and proliferation of the responding subset.

Relationships Between Clinical Status of HIV Disease and $\gamma\delta$ T Cells

Relationships between $\gamma\delta$ T cell levels, functional responses to PAg, and disease progression were studied in many clinical settings including former plasma donors from southern China who became infected with HIV due to contaminated blood-

drawing equipment (Li et al. 2013). Because these individuals were infected at roughly the same time and with similar strains of HIV, there was a unique opportunity to understand relationships between HIV progression and $\gamma\delta$ T cells without the complicating factors of multiple virus strains and different routes for virus exposure. In these patients, V δ 2 T cell levels were highly sensitive to viral RNA burden but less affected by changing CD4 T cell count even though cell levels and function were lowest in patients with CD4 counts <200 cells/mm³ of blood. $\gamma\delta$ T cell levels and function were also evaluated in patients who control HIV without antiretroviral therapy (elite controllers), patients where virus replication is suppressed for up to 10 years by effective treatment, and patients who retain CD4 T cells despite chronic low-level viremia in the absence of therapy.

Approximately 1 in 1,000 HIV-infected individuals in the United States have the capacity to suppress virus replication to extremely low levels without the need for antiretroviral therapy. Termed natural virus suppressors or elite controllers, these individuals provide important models for understanding normal host immune mechanisms related to disease resistance. Compared to matched controls where virus was suppressed by antiretroviral therapy, elite patients had V δ 2 T cells equal to or above the levels found in HIV-uninfected controls indicating an association between higher V δ 2 T cell function and favorable clinical status (Pauza et al. 2011). Patients receiving antiretroviral therapy showed slow reconstitution of the V δ 2 T cell population but did not regain normal levels or functional responses after 7–10 years of treatment. V δ 2 T cell levels were lowest in patients with chronic viremia irrespective of CD4 count, reinforcing the relationship between viral RNA burden and V δ 2 T cell depletion.

Overall, quantitative depletion of V δ 2 T cells and reduced PAg responses characterize the normal course of HIV infection and disease. In advanced HIV disease where CD4 T cell counts are below 200 cells/mm³, the PAg-reactive V δ 2 T cell subset was extinguished in nearly all patients. Without these important cells, it is not

surprising that patients experienced increased risk for a variety of opportunistic infections and malignancies – both are conditions that are confronted in healthy individuals by the presence of functional $\gamma\delta$ T cell populations (Li et al. 2013; Chen and Letvin 2003).

$\gamma\delta$ T Cells and Immunity to Pathogens or Cancer

Much of our understanding of immunity is based on defining T cell or antibody responses against specific proteins derived from pathogens or tumor cells. Understanding PAg-reactive $\gamma\delta$ T cells is uniquely challenging because they do not respond to peptide antigens and do not require polymorphic histocompatibility molecules for antigen presentation. Significant efforts are underway to understand how $\gamma\delta$ T cells contribute to the control of pathogens including HIV or cancer. These studies will show whether therapeutic intervention aimed at $\gamma\delta$ T cell recovery is warranted as a treatment for HIV disease. First, we need to understand how $\gamma\delta$ T cells participate in the control of other diseases that are important for HIV+ patients.

A key function of $\gamma\delta$ T cells is the ability to recognize and kill certain types of tumor cells and cancer is emerging as a major cause of death among HIV patients. PAg- or bisphosphonate-stimulated V δ 2 T cells express a variety of cell surface recognition molecules capable of distinguishing tumor cells from normal cells. In vitro, V δ 2 T cells are potent killers of B cell lymphoma but not T cell leukemias (targeted by V δ 1 cells) and have varying capacities to kill cell lines representing many types of solid tumors. This capacity for tumor-specific cell killing gives $\gamma\delta$ T cells an important role in our understanding of natural mechanisms for tumor surveillance and immunity (Fournie et al. 2013). Since the main tumor-killing subset of $\gamma\delta$ T cells (V δ 2) is amplified in healthy individuals due to chronic PAg exposure, they serve as a constant guardian against malignant disease. Further, $\gamma\delta$ T cells have the ability to activate tumor killing by natural killer cells which lack a TCR but are also potent in recognizing and destroying tumor cells. Depleting

$\gamma\delta$ T cells during HIV disease eliminates their direct contribution to tumor immunity and degrades the functional capacity of NK cells. As might be expected, a reduced capacity for tumor surveillance leads to increased risk for many cancer types. The broad impact of HIV disease on cancer, where many types of cancer are at elevated rates among HIV patients, is distinct from other viruses that promote specific cancers such as human papillomavirus that promotes anogenital and oral epithelial cancer. Consequently, the elevated cancer risk in HIV patients is likely related to fundamental defects in tumor immunity including loss of V δ 2 T cells.

The V δ 2 T cells also comprise a natural barrier and important mechanism of resistance against many infectious diseases (Chen and Letvin 2003). The response to infection also shows the need for both PAg and cytokines to activate V δ 2 T cells. In vitro studies showed that IL-2, IL-15, or IL-21 is needed for PAg-mediated proliferation of V γ 2V δ 2 T cells. In vivo studies demonstrated that PAg plus IL-2 co-treatment, but not IL-2 or PAg alone, induced proliferation of V δ 2 T cells. Primary infections in macaques and humans with viruses or pathogens that are incapable of generating PAg failed to induce V δ 2 T cell proliferation despite high levels of cytokines produced during the infection. Mutant strains of *Listeria monocytogenes* that no longer produce the PAg HMBPP failed to elicit V δ 2 T cell proliferation during infection and proved that cytokine alone did not cause V δ 2 T cell proliferation. Other cytokines may also contribute to PAg-stimulated expansion of V δ 2 T cells during microbial infections. Mycobacterial infections of macaques induced major expansion of V δ 2 T cells and coincident expression of variant IL-4 (VIL-4) mRNA encoding a protein with the same N-terminus 97 amino acids found in IL-4, but a unique C-terminus 96 amino acids that included a signaling-related proline-rich motif. The expressed and purified VIL-4 protein acted as new cytokine to expand PAg-stimulated V γ 2V δ 2 T cells and promote heterologous effector cells capable of producing IL-4, IFN- γ , and TNF- α .

Plasmodium falciparum, a cause of malaria, also produces high levels of PAg and affects

V δ 2 T cell levels. First-time exposure to *Plasmodium falciparum* as an adult (traveler's malaria) caused severe, acute-onset disease with sharp depletion of V δ 2 T cells. In endemic malaria where disease is less severe, V δ 2 T cells are not depleted, and indeed, rising V δ 2 cell counts are associated with the convalescent phase of infection.

Strong V δ 2 T cell responses were also seen in nonhuman primates (Ali et al. 2007) infected with *Yersinia pestis* or *Listeria monocytogenes*, as well as in humans infected with pathogens of salmonellosis, brucellosis, legionellosis, tularemia, toxoplasmosis, leishmaniasis, and others. SIVmac- or SHIV-infected macaques were not able to sustain clonal expansion of V δ 2 T cells after infection with *M. bovis* BCG that is normally a strong stimulator of $\gamma\delta$ T cells. When retrovirus infection of macaques was controlled by antiretroviral therapy, V δ 2 T cells regained the response to mycobacterial coinfection. If V δ 2 T cells were activated early after SIV or SHIV infection, animals did not develop tuberculosis-like disease (Shen et al. 2004). The V δ 2 T cells are part of host defense against mycobacteria and loss of V δ 2 T cell function is an important factor in the increased susceptibility to mycobacterial tuberculosis.

V δ 2 T cells appear to be depressed in terms of frequency or effector function, both during chronically active tuberculosis and chronic HIV. The loss of V δ 2 T cell functions could be a cause or consequence of active TB. It is also possible that prolonged exposure of V δ 2 T cells to significant levels of mycobacterial PAg plus cytokines leads to phenotypic and functional changes that depress immune capacity. Studies of V δ 2 T cells in the setting of HIV-1/Mtb coinfection in humans demonstrated that HIV+ patients with active TB showed defects in total or cytokine (interferon- γ , IFN- γ)-producing V δ 2 T cells when compared to healthy individuals. Interestingly, the frequencies of PAg-stimulated IFN γ + V γ 2V δ 2 T cells were significantly higher in latent Mtb coinfection of HIV-1+ persons than in HIV-1+ TB patients or healthy individuals, suggesting a positive role for $\gamma\delta$ T cells in TB control. Potent V δ 2 T cell responses coincided with maintaining Mtb

latency in HIV-1+ persons with CD4+ T cell counts $>200/\text{mm}^3$ of blood (Shao et al. 2008).

These examples, both of pathogen resistance and tumor cell killing, describe a direct role for V δ 2 T cells in reducing the incidence and severity of infectious diseases. In addition, V δ 2 T cells are known to secrete high levels of cytokines capable of promoting viral or bacterial immunity. In HIV disease, loss of V δ 2 T cells reduces the capacity to resist both opportunistic infections and cancer, with the additional consequences of lowering thresholds for immune responses and contributing to the broad immunodeficiency seen in HIV/AIDS.

$\gamma\delta$ T Cells as Targets for Immunotherapy

Key observations that V δ 2 T cell levels and function are highest among elite controller patients and V δ 2 responses are related to Mtb control encourage efforts to develop immunotherapies aimed at increasing the levels and function of V δ 2 T cells in patients with HIV. The goal would be to stimulate and proliferate V δ 2 T cells in patients receiving antiretroviral therapy in order to match the levels of function found in elite HIV controllers or patients who suppress Mtb. In order to approach this goal, it is necessary to understand the unique status of V δ 2 T cells in patients receiving antiretroviral therapy.

Successful antiretroviral therapy achieves at least three outcomes: (1) changes in clinical status including increased lifespan, improved responses to opportunistic infections, decreased requirement for bacterial prophylaxis, and reacquisition of normal responses to conventional vaccines; (2) plasma viral RNA suppressed and maintained at undetectable levels; and (3) reconstituted CD4 T cell counts approaching the normal ranges for healthy adults.

Among treated HIV patients who successfully reconstituted CD4 T cells, neither V δ 2 T cell levels nor functional responses recovered to the same extent. In general, V δ 2 T cells remained below 0.5% of lymphocytes and manifested poor in vitro responses to PAg stimulation. When tested for tumor cell killing, V δ 2 T cells from treated

patients remained ineffective as tumor cell killers. A more detailed analysis of circulating V δ 2 T cells, based on DNA sequencing of individual V γ 2 chains present in blood of treated HIV patients, showed that CD4 reconstitution was accompanied by increased complexity in the V δ 2 T cell population. Unlike HIV-negative controls, these new cells retained a naïve phenotype indicating they had not responded to PAg or converted to memory cells as would be expected for cells from HIV-negative individuals. Without responding to PAg, the cells cannot switch to memory phenotype and do not proliferate or restore normal cell counts. These findings show that new V δ 2 T cells were produced and entered the circulating pool during the years of antiretroviral therapy, but functional responses were not recovered to the same extent.

V δ 2 T cell populations reconstituted during prolonged HIV therapy included V γ 2 gene sequences capable of responding to PAg stimulation, even if the observed response was low (Chaudhry et al. 2013). This is an encouraging sign for the possible development of immunotherapy aimed at activating V δ 2 T cells. Since V δ 2 T cell responses depend less on the genetic background of the host and can be triggered to proliferate and acquire cytotoxic killer cell function simply by adding PAg plus cytokine, one therapeutic approach can be tested in many individuals. Consequently, we now recognize opportunities for clinical trials using bisphosphonates plus IL-2 or PAg plus IL-2, to activate and proliferate V δ 2.

It remains unsolved why newly synthesized V δ 2 T cells appear during prolonged therapeutic suppression of HIV but fail to proliferate back to levels seen for matched, HIV-negative controls. Even though new cells were born into an environment essentially sterile of HIV due to effective treatment, the cells failed to acquire full functionality on par with uninfected individuals. If immunotherapy targeting V δ 2 T cells is to be successful in the future, appropriate stimulation conditions must be developed. Recent studies showed that the bisphosphonate zoledronate (Zometa) plus IL-15 stimulated V δ 2 T cells from treated HIV patients. Treated cells had moderate to strong

proliferation responses during in vitro culture and this approach might be suitable for in vivo therapy. In a pilot study treating HIV patients with zoledronate plus IL-2, there were few adverse events. With this as a starting point and strong arguments supporting the development of immunotherapies targeting V δ 2 T cells, we anticipate progress in this field to help reconstitute immunity in patients treated for HIV (Pauza et al. 2011).

Conclusions

Extensive destruction of V δ 2 T cells with loss of phosphoantigen responses is an important feature of HIV disease. Depletion and inactivation of this T cell subset reduces the capacity for natural tumor immunity and increases susceptibility to opportunistic pathogens including *M. tuberculosis* that can be devastating in HIV patients. V δ 2 T cell levels declined when vRNA was highest and only elite controller patients had normal levels and function of these cells, arguing that V δ 2 T cells are important for controlling viral disease. The V δ 2 subset of $\gamma\delta$ T cells can be stimulated in patients by clinically approved drugs (bisphosphonates or phosphoantigens plus IL-2) that are already showing promise in cancer treatment studies. Among treated HIV patients with prolonged virus suppression and CD4 T cell reconstitution, V δ 2 T cells were replenished, but not back to normal levels. This means that treatments to activate V δ 2 T cells, using potent, exogenous stimulation, are needed to activate their full immune function. Future immunotherapy studies in HIV patients will test the benefits of targeted V δ 2 T cell immunotherapy for reducing cancer risk, combating opportunistic infections including *M. tuberculosis*, and restoring normal immune capacity with the goal of slowing HIV disease.

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Gay men and other Men who have sex with men (MSM), Epidemiology of HIV/AIDS Introduction

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Definition

Men who have sex with men (MSM) include gay and bisexual men, MSM who do not identify as gay or bisexual despite their behaviors, male sex workers, and transgender men who have sex with men who identify as gay or have sex with other men. The term “MSM” was originally intended to describe same-sex behaviors between men rather than identities, orientations, or cultural categories. It was designed to be less stigmatizing than culturally bound terms such as gay, bisexual, or homosexual and has been useful as an epidemiologic tool to help characterize the HIV pandemic in challenging contexts. However, in the era of HIV, there are growing contingents who now identify as MSM and feel a connection with the MSM community challenging the initial intention

of the term. Separately, the term in 2016 is often used interchangeably with the term gay which can be dehumanizing to those who identify as members of the LGBT Community (Trapence et al. 2012). And given the dynamic nature of people, these definitions may further evolve in the years to come. However, for this paper, we will use the term MSM as a broad epidemiologic characterization in the context of the HIV pandemic.

Introduction

Globally, MSM are among the highest-risk people to acquire and transmit HIV infection (Fig. 1). The reasons for this are multifaceted and may be manifested in diverse ways for different individuals. Traditionally, epidemiologic studies have focused on measuring individual-level risk factors for HIV acquisition such as condom use and number of sexual partnerships. However, there is increasing recognition of the importance of social, network-level, and structural determinants for HIV infection risks among MSM. In addition to individual biological factors (e.g., the increased susceptibility of anal mucosa to HIV infection) or behavioral characteristics (e.g., frequent partner exchange among some MSM), social and sexual networks, community environments, law and policies, and the stage of HIV epidemic in the area in which the individual resides may also play a role in potentiating HIV spread (Baral et al. 2013a; Beyrer et al. 2012a; Sullivan et al. 2012). This chapter will begin with an overview of the drivers of HIV risk and then focus on the epidemic of HIV among MSM in various regions of the world, including concentrated and generalized epidemics in high- and low-income settings.

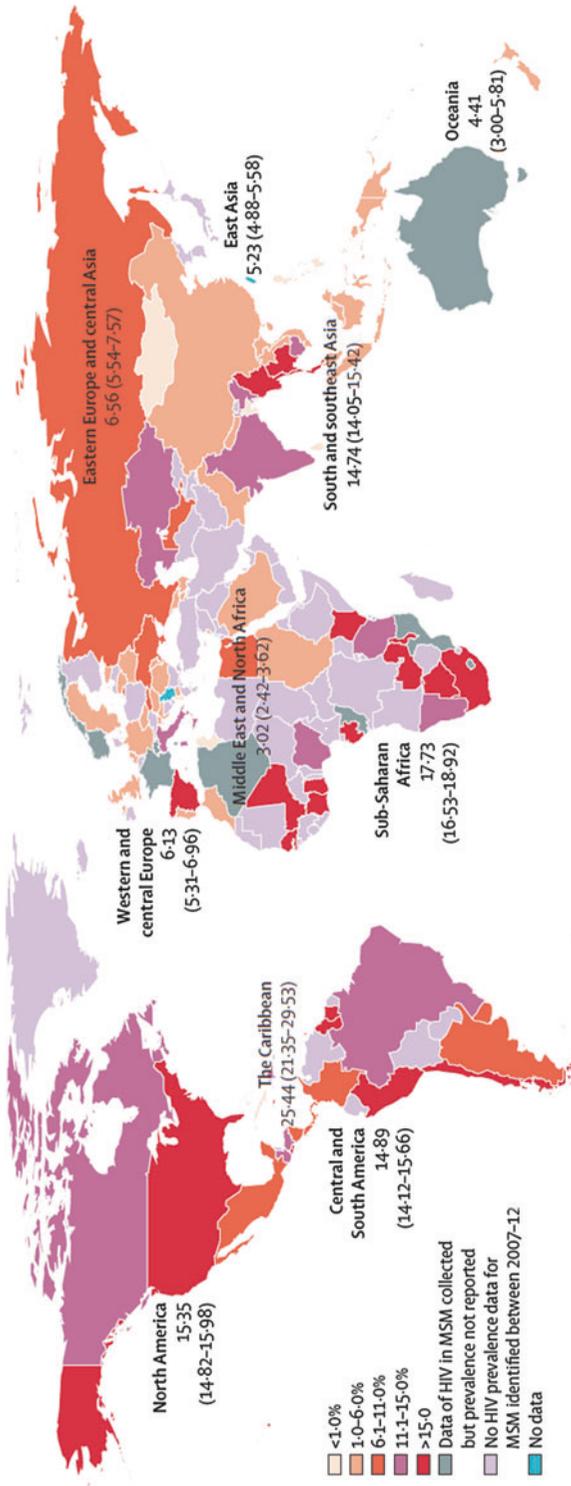
Individual-Level Risks

Individual-level risks for HIV acquisition in MSM have been well documented and include condomless insertive and receptive anal intercourse with serodiscordant and viremic sexual partners, high frequency of casual male partners, high number of lifetime male partners, injecting drug use, high viral burden in the index partner, and non-injection drug use, including use of

amphetamine. In addition, MSM may be at risk for a wide variety of other sexually transmitted infections, which can facilitate HIV transmission and acquisition through genital tract inflammation involving the local recruitment of more HIV target cells, ulcerations, and abrasions (Mayer et al. 2012; Mayer and Venkatesh 2011). Moreover, many of these infections are asymptomatic resulting in limited diagnosis and therapy in the context of syndromic STI surveillance. However, one of the primary driving forces behind the disproportionate HIV disease burden among MSM globally is the high per-act and per-partner transmission probability of HIV transmission from receptive anal sex. Rectal exposure to HIV infection is biologically different from vaginal exposure: the gut has a huge potential transmission space and is an immune organ with high density of HIV target cells and contains a friable and easily disrupted epithelium. These factors result in a high per-act HIV transmission probability during anal sex – more than a log higher than during vaginal sex (1.4% vs. 0.08%) (Baggaley et al. 2010).

Network-Level Risks

Among MSM, sexual-network-level risks can also facilitate HIV spread and key entry points for the delivery of interventions. Although individual-level risks are similar for men and women for receptive anal sex with a serodiscordant and viremic sexual partner, male-male sexual partnerships differ since each partner can engage in either insertive or receptive sexual positioning. This sex role versatility, coupled with the high per-act risk of HIV transmission during rectal exposure, leads to high individual, couple-based, and network-level HIV transmission risks. Social and peer-group norms, availability of condoms in networks, and high HIV/STI prevalence in networks can also affect the spread of HIV. Molecular epidemiologic studies of HIV conducted among MSM demonstrate substantial clustering of HIV infection within MSM networks, a high frequency of multiple transmitted viral variants, and more rapid HIV spread through sexual networks (Beyrer et al. 2012a). In addition, larger sexual networks provide increased



Gay men and other Men who have sex with men (MSM), Epidemiology of HIV/AIDS Introduction, Fig. 1 Global HIV prevalence in MSM, from studies published 2007–2011. Data are prevalence (95% CIs) (Reprinted from The Lancet, volume 380, Beyrer C, Baral SD, van Griensven F, Goodreau SM, Chariyaletsak S, Wirtz AL, and Brookmeyer R. Global epidemiology of HIV infection in men who have sex with men, pp 367–77, Copyright (2012), with permission from Elsevier)



opportunities for exposure to HIV-infected partners and consequently HIV acquisition among MSM. In the era of increasing use of digital spaces to find sexual partners, sexual networks may be increasingly large where people are less likely to know the status of their partner.

However, social network characteristics such as the provision of social support, reinforcement of protective social norms, and social capital, including social cohesion, participation, and inclusion, can help to reduce the spread of HIV. Other social associations with beneficial HIV-related outcomes include having a confidante, believing in collective efficacy, participating in same-sex-oriented public events, being “out” as an MSM (i.e., self-acceptance), and knowing other MSM in one’s city. Social factors can encourage consistent condom use and participation in HIV prevention programs, and they have been associated with decreased HIV infection (Baral et al. 2014a).

Community-Level Risks

Community environments, including network ties, relationships between organizations and groups, and geographical/political regions, can either promote health and well-being or serve as a source of stigma and HIV risk enhancement. Stigma toward MSM in communities can limit the provision and uptake of HIV prevention, treatment, and care services. For example, culturally insensitive health workers may result in MSM avoiding HIV prevention services; or even more problematically, MSM living with HIV may avoid HIV treatment services. Reduced utilization of health and HIV services by MSM, due to enacted or perceived discrimination, may limit knowledge of the risks of unprotected anal intercourse and opportunities for access to prevention services. Stigma and discrimination, such as exposure to homophobic abuse, homophobia, or homonegativity; a lack of social support; shame, blame, and social isolation; and victimization at school or work, have all been associated with negative HIV-related outcomes. These adverse outcomes include reduced rates of HIV testing, increased risk for

HIV infection, lower likelihood of discussing or disclosing HIV/AIDS status with male partners, increased condomless anal sex, and engagement in HIV treatment for those living with HIV. However, MSM community support such as MSM-specific health promotion can have positive impacts such as encouraging condom use through education and sex-positive messaging (Baral et al. 2014a).

Structural-Level Risk

Administrative policies such as the criminalization of same-sex practices in many countries and exclusion of MSM from national surveillance programs have contributed to the limited provision of focused, accessible HIV prevention and treatment strategies for MSM. These punitive policies present significant barriers to HIV prevention and meaningful engagement in treatment. Moreover, they have been associated with higher levels of violence and stigma against MSM, decreased funding for programs for MSM, increased fear of seeking healthcare leading to decreased clinical engagement, and ultimately higher HIV incidence and prevalence. Criminalization promotes multiple forms of stigma as well as structural and cultural violence, which in turn worsen health conditions for MSM and entire communities (Baral et al. 2014a).

Epidemic Stage

The individual-, network-, community-, and law- and policy-level risks discussed previously cannot create HIV infections, and moreover, there is nothing inherently harmful about condomless anal sex. These factors can only increase or decrease the probability of transmission of an infection that is currently prevalent. Consequently, it is the stage of the epidemic within the social and sexual network, community, and country that primarily determines the risk for HIV acquisition in the individual. For example, an individual living in an area of high HIV prevalence will have a greater chance of a shared sexual network with someone who is living with HIV and not virally suppressed than someone living in an area of low prevalence.

MSM in Concentrated Epidemics

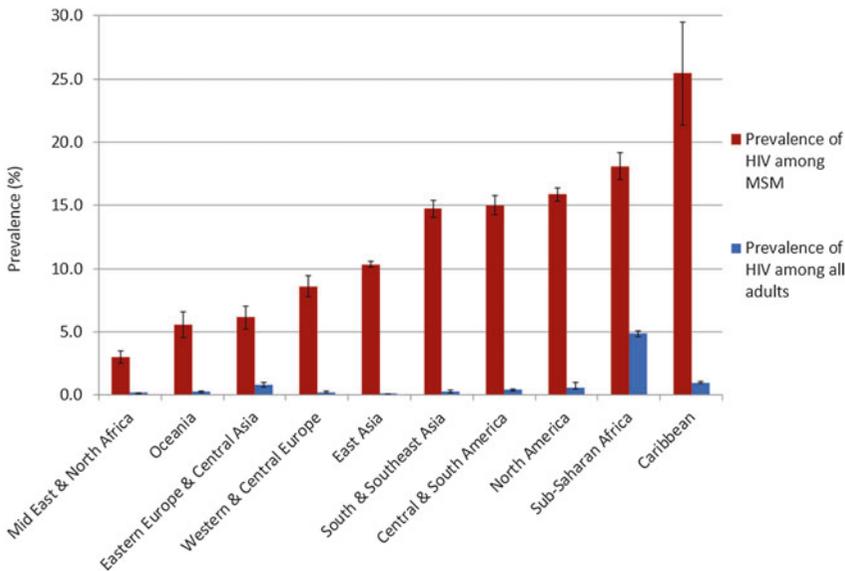
A concentrated epidemic has been traditionally defined as an HIV prevalence of less than 1% among all reproductive-age adults but more than 5% in any key population including MSM in a given country (WHO and UNAIDS 2000). Outside Eastern and Southern Africa, the epidemiology of HIV is primarily represented by concentrated epidemics among key populations including MSM, sex workers, people who inject drugs, and transgender women. In many regions, the HIV prevalence among MSM is over 10%, and the ratio of HIV prevalence in MSM to that of the others in the population is disproportionately high (Beyrer et al. 2013a) (Fig. 2).

High-Income Settings

The burden of HIV among MSM in many high-income settings can be characterized as a predominantly male-concentrated epidemic, with a median male/female case ratio of 2.5:1 and men making up more than 75% of the prevalent HIV

infections (Sullivan et al. 2014). Even as the HIV epidemic among adults is primarily stable or decreasing in most settings, the HIV epidemic among MSM continues to increase in many high-income countries, including the USA (Centers for Disease Control and Prevention 2010). The USA accounts for over 30% of total infections in high-income countries with significant disparities across income levels of MSM (Sullivan et al. 2014), with ethnic and racial minority MSM carrying a disproportionate local HIV burden (Millett et al. 2012).

High-income countries exhibit greater coverage of antiretroviral therapy (ART), larger extent of HIV diagnosis among people living with HIV, and greater access to healthcare services and social structures that facilitate accurate reporting of male-to-male sex risks. However, there have been reemergent epidemics in MSM in many high-income settings where the overall HIV epidemic is otherwise in decline – including Australia, France, the UK, and the USA (Sullivan et al. 2014). The disparities in the burden of HIV



Gay men and other Men who have sex with men (MSM), Epidemiology of HIV/AIDS Introduction, Fig. 2 Pooled HIV prevalence among MSM and among all men of reproductive age, by region, 2012. HIV prevalence among adults (Data from *UNAIDS report on the global AIDS Epidemic: 2012*. Joint United Nations

Programme on HIV/AIDS (UNAIDS). Geneva: 2012. Adapted from The Lancet, volume 380, Beyrer C, Baral SD, van Griensven F, Goodreau SM, Chariyalertsak S, Wirtz AL, and Brookmeyer R. Global epidemiology of HIV infection in men who have sex with men, pp 367–77, Copyright (2012), with permission from Elsevier)

observed within the USA among different populations of MSM suggest that the early initiation of appropriate ART does result in fewer HIV infections and reduced likelihood of HIV transmission. In the USA, HIV infections among MSM overall remained relatively stable between 2002 and 2011 though this average omits the increasing disparities in the burden of HIV observed in the US context among MSM generally attributed to socioeconomic determinants. HIV infections in MSM aged 13–24 years are estimated to be increasing at roughly 10% per year since 2002 (Johnson et al. 2014). Similar trends have been reported among gay and other MSM in the UK, where treatment coverage is higher than in the USA (Yin et al. 2014). Biological, couple, network-level, and community-level influences are likely crucial to understanding why HIV transmission rates remain so high in these populations of MSM. Given the rapid transmission that appears to take place within sexual networks of MSM when someone is acutely HIV infected, the bar for preventing HIV infection within these sexual networks is very high. In addition, breaking chains of HIV transmission necessitates not just coverage of HIV testing but the diagnosis of people during the acute infection stage followed by an effective intervention. Thus, we do not know yet whether universal treatment approaches will work for MSM since so many of these men are diagnosed often too late to prevent HIV transmission events (Sullivan et al. 2014).

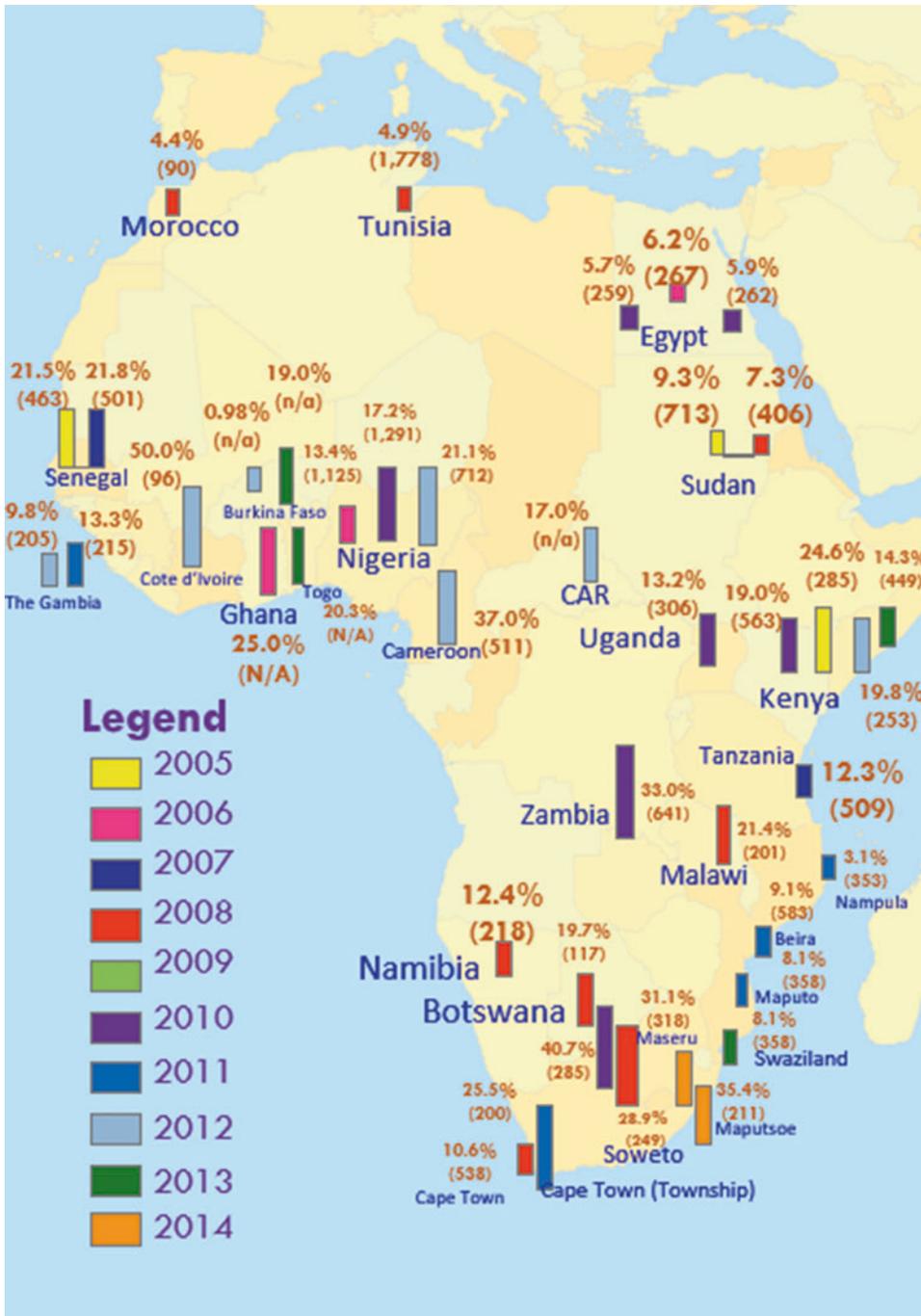
Low- and Middle-Income Settings

The concentrated HIV epidemic among MSM transcends country income level. In low- and middle-income countries, MSM are estimated to have 19 times the odds of living with HIV compared with other people (Baral et al. 2007; the Foundation for AIDS Research and Johns Hopkins Bloomberg School of Public Health 2012). According to a meta-analysis of 15 South American countries, the HIV prevalence among MSM is generally greater than 10%, and the odds of having HIV in MSM is almost 34 times than in the general population (Baral et al. 2007). In the Middle East and North African countries, HIV prevalence among MSM appears to be low, but emerging HIV epidemics have been documented

among MSM in Egypt, Jordan, Lebanon, Oman, and Syria (Mumtaz et al. 2010). In Eastern Europe and Central Asia, HIV incidence is highest among dual risk people who inject drugs (PWID) and are MSM, expanding the established local HIV epidemics driven by PWID combined with limited coverage of effective tools to decrease parenteral transmission of HIV. In South, Southeast, and Northeast Asia, MSM transmission, PWID transmission, and heterosexual transmission all contribute significantly to the HIV epidemic (Beyrer et al. 2010). In sub-Saharan Africa, the prevalence of HIV among MSM is higher than that of age-matched men in all settings (Fig. 3).

For several years the HIV epidemic among MSM in low- and middle-income countries was ignored by many governments, donors, and societies. Although attitudes have steadily shifted to address the needs of MSM, in many parts of the world, a hidden epidemic remains that is exacerbated by stigma, discrimination, and violence. Same-sex sexual practices are punished as crimes in roughly 75 countries, with penalties ranging from fines to imprisonment and in some cases even death. In much of the world, national HIV epidemiological surveys do not assess the impact of HIV on MSM. Unfortunately, this willful ignorance results in a lack of acceptable data regarding HIV risk in MSM, which is then used by governments to justify the limited investment in the HIV prevention and treatment needs of this population (the Foundation for AIDS Research and Johns Hopkins Bloomberg School of Public Health 2012).

To improve low- and middle-income countries' responses to HIV/AIDS, it is essential that services expand to include MSM as a key population in line with the attributable fraction of HIV infections. A more distinctive understanding of the diversity of epidemics among MSM in low- and middle-income countries is needed. Because these men are hidden from the broader community, there is notable underreporting of MSM behavior in population-based surveys, and there has been limited work on the type of surveillance necessary to characterize these men and the epidemics affecting them. Correlates of prevalent HIV infection in low- and middle-income countries include the individual-level HIV risk factors similar to those found in concentrated HIV epidemics, as



Gay men and other Men who have sex with men (MSM), Epidemiology of HIV/AIDS Introduction, Fig. 3 HIV prevalence among MSM in Africa (Modified from van Griensven et al. 2009)

well as community-level structural factors, such as stigma, being blackmailed, and history of homophobic abuse (Baral et al. 2014b). Additional risk factors for HIV in low- and middle-income settings

include social isolation, limited healthcare access, and social stigma that restrict HIV surveillance and the coverage of comprehensive HIV prevention programs.

Overall, the odds of having HIV infection are markedly and consistently higher among MSM in each geographic locale than among the adult populations across Asia, Africa, and the Americas. Indeed, MSM from low- and middle-income countries appear to be understudied and underserved and are in urgent need of prevention and care (Beyrer et al. 2012b). Factors contributing to the lack of sufficient knowledge include limited data regarding adolescent MSM and MSM from specific regions such as North Africa and the Middle East. Prevention strategies that lower biological transmission and acquisition risks, such as early treatment and PrEP, offer promise for controlling the expanding epidemic in MSM in these settings; however, their potential effectiveness may be limited by structural factors that contribute to suboptimal health-seeking behaviors and by the refusal of governments to address regulatory and other structural barriers and make PrEP available. More intensive efforts will be required to reach younger MSM, expand testing and treatment, and implement effective prevention tools such as PrEP in diverse geographic settings. In addition, these efforts will likely have to be coupled with community efforts around safe sex practices and condom use. Ultimately, the challenge will be not just linking people to these programs but will be about securing long-term retention – and this will require significant study leveraging the best implementation research approaches.

MSM in Generalized Epidemics

Generalized HIV epidemics are generally categorized as regions with HIV prevalence rates above 1% in adults or in antenatal clinics. With few exceptions, generalized HIV epidemics are limited to Southern and Eastern sub-Saharan Africa. In the context of generalized epidemics, it is often proposed that MSM do not constitute a significant component of the epidemic and that resources should not be diverted from addressing other populations deemed to have higher priority. As a result, in countries categorized as having concentrated epidemics, only a small proportion of HIV

prevention expenditures target the preventive needs of MSM, and an even smaller fraction of funding for HIV prevention supports prevention for MSM. However, recent data highlight the disproportionate burden of HIV among MSM that exists when compared with other men of reproductive age across countries with generalized epidemics.

Fortunately, there has been a recent notable decrease in HIV incidence among several countries with a generalized epidemic, including Malawi, Namibia, Botswana, and Zambia (UNAIDS 2013). However, in a review of 51 countries with generalized epidemics, the burden of HIV was consistently higher among MSM. Data were primarily available for Kenya, South Africa, and Thailand (Baral et al. 2014b). In Kenya, the HIV prevalence among MSM ranges 12.3–43.0% compared with 6.1% among the reproductive-age population. Similarly in South Africa, HIV prevalence among MSM ranges 10.0–40.7% compared with 17.9% among reproductive-age adults. In Thailand, one of the few countries outside of Eastern and Southern sub-Saharan Africa which has had a generalized epidemic, HIV prevalence among MSM ranges 8.2–68.2% compared with 1.1% among adults of reproductive age.

The HIV prevention needs of MSM in generalized epidemics may be different from those in concentrated epidemics. In generalized epidemics, HIV prevalence is higher in the population as a whole and usually disproportionately affects women. Thus, in generalized epidemics, MSM may have higher HIV acquisition risks secondary to their heterosexual partnerships, but some of the relatively higher risks among MSM may reflect their ability to acquire or transmit HIV with both female and male partners. Decriminalization of same-sex sexual practices is important in terms of establishing a facilitating environment for effective HIV programs focusing on MSM in these settings. However, stigma and discrimination, as well as access to effective interventions, will also need to be addressed to reduce impediments for MSM involvement in HIV prevention, treatment, and care. And decriminalization cannot achieve this on its own (Zahn et al. 2016). In

addition, because HIV prevention, treatment, and care services have been designed in a way to not specifically address any at-risk population, there is a common misconception among MSM that HIV transmission is more likely through vaginal intercourse than through anal intercourse (Baral et al. 2014b). A comprehensive agenda to understand who the “general population” is in terms of specific HIV acquisition and transmission risks will be needed in these contexts to inform HIV prevention and treatment response.

West Africa

The dynamics of the HIV epidemic in West Africa are significantly different from what has been observed in neighboring Southern and Eastern Africa. The HIV prevalence among reproductive-age adults is not as high as that in Southern or Eastern sub-Saharan Africa; however, the burden of HIV among key populations including MSM and female sex workers has been significant. West Africa includes both a generalized and concentrated epidemic, with significant transmission in both the general population and in most at-risk groups. In Senegal, a country known for an early and effective response to HIV with scaled-up ART, it is estimated that MSM have 50 times the risk of HIV infection than other reproductive-age men. Similar to Eastern and Southern Africa, homosexual activities are criminalized in a number of West African countries. Decreasing HIV incidence rates among these men will likely necessitate the integration of biomedical interventions such as treatment as prevention, oral PrEP, and eventually rectal microbicides. However, these approaches will have limited effectiveness if these men continue to have limited access to healthcare services as a result of stigma and discrimination (Drame et al. 2013).

Other Key Issues

Role of Community Organizations

Gay men and other MSM have historically led and participated in HIV intervention efforts through advocacy, education, research, and design and delivery of prevention, treatment, and care

programs that have benefited all affected by HIV. In stigmatizing environments, MSM and lesbian, gay, bisexual, and transgender (LGBT) community groups are often the only groups willing to advocate for recognition and rights or to provide HIV-related services to gay men and other MSM. Indeed, gay and MSM advocates have achieved important successes in the response to HIV, but they have faced challenges, including stigma and threats of violence, limited funding, and the need to represent highly diverse populations. Tailored community-based programs led by MSM have resulted in greater feelings of connection, social support, and self-esteem among community members. For example, men living with HIV in Cameroon have been able to obtain greater health services as the result of efforts by a dynamic community-based organization that provided HIV prevention, care, and treatment specifically for the LGBT population (Holland et al. 2015). In addition, MSM community groups have traditionally supported the collection of data that challenge the assumption that gay men and other MSM do not play substantial parts in the HIV epidemic in low-income and middle-income countries.

Protecting and improving personal health require community-level action, and this will continue to be essential in the response to AIDS worldwide. Communities will require increased resources, developmental support, and expanded opportunities to serve and lead the fight against the growing HIV epidemic in MSM (Trapence et al. 2012).

Transgender Women

Transgender women represent a separate population from MSM; they were assigned the male sex at birth but identify as women and are well known to be at exceptionally high risk for HIV acquisition and transmission. Worldwide, the odds for transgender women being infected with HIV is almost 50 times that of adults of reproductive age, with a pooled HIV prevalence of around 19% (Baral et al. 2013b). There are some shared determinants of risk between transgender women and MSM including the high transmission probability of unprotected anal sex. In addition, network-level

(e.g., HIV prevalence in subgroups), community-level (e.g., stigma, social exclusion) and structural-level (e.g., discriminatory laws, economic marginalization) risks contribute to the high burden of HIV among transgender women.

In contrast, transgender men, who were assigned the male sex at birth, have traditionally been considered low-risk for HIV. However, some of these men identify as gay and have sex with other men, sometimes termed trans MSM. Trans men and trans MSM report a range of sexual practices including vaginal or receptive anal sex with other men, which could put them at substantial risk in the context of HIV serodiscordant and viremic sexual partners. HIV prevalence in transgender men ranges from 0% to 2.9% based on two studies conducted in the USA (Reisner et al. 2013). However, there has been such limited programmatic investment and study, ultimately greatly limiting our knowledge of HIV-related risk behaviors, social and structural factors, and sexual health needs of transgender men.

Male Sex Workers

The global burden of HIV among male sex workers is high and in some cases increasing. Data on male sex workers are limited, with most research conducted as part of studies of MSM, female sex workers, or transgender women (Baral et al. 2015). However, available data indicate that male sex workers mostly offer sex to men and rarely identify as sex workers. Male clients sometimes do not identify as gay or bisexual, and many have regular female partners. Because of social exclusion, expulsion from higher education, and limited opportunities for other employment, sexual and gender minorities (including transgender women) are more likely to engage in survival or commercial sex work. In addition, male sex workers may have high numbers and frequencies of male partnerships (many of whom are older than they are), resulting in large sexual networks, which has been established as a risk factor for HIV among MSM. Individual-level behavioral HIV risks are compounded by criminalization and stigma of same-sex practices and commercial sex, leading to deferral of seeking testing, treatment, and prevention services. Other

risks include economic disparities, sexual and physical abuse, drug misuse, and low socioeconomic status. Dedicated advocacy, funding, surveillance, research initiatives, and diverse preventive options for male sex workers are needed to reduce the burden of HIV among male sex workers (Baral et al. 2015).

Internet Use for Finding Sex Partners

Among MSM, the Internet is a popular platform for meeting new sexual partners. Recent data suggest that MSM in low- and middle-income settings utilize the Internet for sex-seeking at rates equivalent to MSM in high-income settings such as North America or Europe. In response to the HIV epidemics among MSM, there has been some pushback against Internet access and other forms of social media – particularly in China but also in several other countries. This is a worrisome trend and is not evidence based. It is currently unclear whether online sex-seeking facilitates HIV transmission. MSM who meet partners online are more likely to report unprotected anal sex and higher numbers of sex partners, which would facilitate HIV transmission, but also with HIV status disclosure and seroadaptive sexual practices, which could protect against HIV transmission (Lewnard and Berrang-Ford 2014). Regardless, increasing evidence is pointing to the Internet as both an acceptable and feasible HIV intervention mechanism to reach MSM worldwide.

Biomedical Improvements in HIV Prevention

Daily oral use of PrEP is an effective HIV prevention intervention for MSM. In 2010, the iPrEx (Preexposure prophylaxis initiative) trial demonstrated a 44% reduced HIV infection rate among MSM in the PrEP arm compared to the placebo arm (Grant et al. 2010), but on average medication adherence was around 50%. In 2015, an open-label randomized trial (the PROUD study) conducted among gay men and other MSM in England ($N = 544$) successfully addressed concerns about real-world effectiveness and risk compensation for PrEP by demonstrating 86% efficacy among MSM who accessed STD clinics who initiated PrEP right away (McCormack et al. 2015). Further, the French and Canadian

Ipergay study found that peri-coital dosing before and after sex was found to be similarly effective (86%) (Molina et al. 2015). Overall, the findings of these studies strongly support the addition of PrEP into combination prevention packages that include behavioral, structural, and biological intervention components. At the time of writing, PrEP is being implemented in the USA, South Africa, France, and Kenya and including MSM in the populations recommended – a remarkably slow rollout for an intervention with 6 years of efficacy data. And indeed, this slow rollout may be further indicative of the limited support for interventions where the primary efficacy data for HIV prevention is among MSM. The coming years will likely introduce new formulations of oral PrEP and even long-acting injectable PrEP which may ultimately represent important components of the package of services for MSM.

Conclusion

There is an HIV pandemic among MSM – it is a global epidemic with more similarities than differences across income levels, HIV epidemic types, and geography. Improved HIV surveillance mechanisms leveraging mathematical modeling, phylodynamics, and meaningful indicators can inform the population attributable fraction of HIV among MSM across countries and will be needed to passively evaluate the success of intervention efforts. Among MSM, individual- and network-level HIV risks are compounded by structural-level risks such as community environments, laws, policies, and program failures. Universal HIV treatment, fourth-generation HIV tests, HIV self-testing, and daily oral PrEP using tenofovir-emtricitabine have emerged as integral to the prevention of HIV transmission, and such efforts should be immediately expanded for MSM and other populations disproportionately affected by HIV. Indeed, the bar for HIV prevention among MSM is extremely high. Only with true respect for human rights and efforts to combat stigma, discrimination, and social exclusion can the levels of coverage needed to change the trajectory of the HIV pandemic among MSM.

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Global NeuroAIDS

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Definition

The term NeuroAIDS encompasses all the neurological complications associated with HIV. This includes diseases that primarily result from direct infection of the CNS with HIV (HIV-associated neurocognitive disorders [HAND]), secondary conditions resulting either from the effects of associated immunocompromise (neurologic opportunistic infections or tumors) or the effects of treatment (such as the immune reconstitution

syndrome [IRIS]) as well as other diseases that could be influenced but not caused by HIV (e.g., stroke).

Types of HIV-Associated Neurological Syndromes

- Primary HIV neurological diseases
 - HIV-associated neurocognitive disorders (HAND)
 - Asymptomatic neurocognitive impairment (ANI)
 - Mild neurocognitive disorder (MND)
 - HIV-associated dementia (HAD)
 - Aseptic meningitis
 - HIV-associated myelopathy
 - HIV-associated peripheral neuropathies
 - Distal symmetric polyneuropathy (DSP)
 - Inflammatory demyelinating polyneuropathy (IDPN)
- Secondary or opportunistic neurological diseases
 - Opportunistic neurological infections
 - Viral: cytomegalovirus, progressive multifocal leukoencephalopathy, and herpes simplex encephalopathy
 - Bacterial: CNS tuberculosis
 - Fungal: Cryptococcal meningitis
 - Parasitic: Toxoplasmic encephalitis
 - Opportunistic CNS tumor
 - Primary HIV-associated CNS lymphoma
- Treatment-related neurological disease
 - Immune reconstitution inflammatory syndrome (IRIS)
 - Neurotoxic neuropathy
- Non-AIDS-related diseases
 - Stroke

Epidemiology of NeuroAIDS

HIV/AIDS remains a global public health problem with 35.3 million people infected and 2.3 million new infections worldwide in 2012 (UNAIDS 2013). The introduction of antiretroviral therapy (ART) from the mid-1990s led to a gradual reduction in the rates of new infections and increased the life expectancy of people living

with HIV; it also altered the manifestations of HIV disease. The global incidence of new HIV infections has declined by 19% since the peak of the epidemic in 1999. HIV has a wide range of effects on the nervous system, directly on the brain and spinal cord, which create cognitive, central motor, and behavioral abnormalities. In the pre-ART era, neurological disorders were a common presenting feature of HIV infection, with 7–20% of patients having neurological complications at first presentation. Severe cognitive impairment was commonly seen in patients with advanced immunosuppression; HIV-associated dementia was reported in 20–30%, and 39–70% developed opportunistic neurologic infections (Spudich and González-Scarano 2012; Tan et al. 2012).

Currently, in developed countries where there is widespread availability and access to combination antiretroviral therapy (cART), there has been a decline in the incidence of severe HIV-associated neurologic disorders and opportunistic infections by up to 80–90%. For example, between 1996 and 1997, the incidence of opportunistic infections in high-income countries was 13.1 per thousand patient-years; by 2006–2007, this had reduced to 1 per thousand patient-years (Tan et al. 2012). However, the spectrum of disease-causing agents has remained largely unchanged. Despite this reduction in incidence rates, neurologic complications still remain a significant cause of excess morbidity and mortality in people living with HIV. The overall prevalence of HIV-associated neurocognitive disorders (HAND) is on the rise despite satisfactory virologic control; the current prevalence of HAND ranges from 20% to 50% (Brew and Chan 2014).

The picture is somewhat different in low- and middle-income countries where only an estimated 34% of the 28.6 million eligible people in 2012 are receiving ART (UNAIDS 2013). The current picture of HIV neurological disorders appears similar to that of the pre-ART era. The problem in low-resourced areas of the world is further compounded by limited diagnostic capabilities for HIV-associated neurologic disorders and hence a paucity of reliable data on the actual rates of CNS complications in HIV populations in low- and middle-income countries (LMIC)

(Robertson et al. 2010). The little data available suggests that opportunistic infections are still the commonest neurologic complications of HIV.

The type and severity of the neurologic dysfunction related to HIV are influenced by the properties of the HIV virus, disease stage, genetic characteristics of the host, and interactions with the environment. Neurologic manifestations of HIV might be linked to HIV virus subtype. For example, HIV type 2 has less propensity to cause neurologic disease compared to type 1. Even within HIV-1 infection, different subtypes or clades have been associated with different rates of occurrence of neurologic dysfunction in preliminary studies (Sacktor et al. 2007).

Acute HIV infection is usually asymptomatic, but a few patients develop a neurologic illness. Common presentations include aseptic meningitis, facial palsy, Guillain-Barré (GB) syndrome, and transverse myelitis. As the disease progresses, with reduction in the CD4-positive cells, the risk for other conditions such as opportunistic infections, HIV-associated cognitive disorders, and peripheral neuropathies increases (Hogan and Wilkins 2011).

Pathogenesis of NeuroAIDS

HIV is able to infect the nervous system and cause central nervous system (CNS) and peripheral nervous system (PNS) disorders at any stage during disease evolution from seroconversion to late stage HIV disease. The neuropathology of HIV infection is very complex and as yet not fully understood; however, HIV is known to invade the central and peripheral nervous system soon after initial infection. The virus is believed to enter the CNS through HIV-infected macrophages and CD4+ T cells that are able to penetrate the blood-brain barrier. In the CNS, the virus commonly infects perivascular macrophages and the microglia. CNS infection is mediated by CD4 and the chemokine receptor (CCR5) serving as viral receptors (Spudich and González-Scarano 2012).

The mechanism for HIV neurotoxicity is not yet fully understood, but it is thought to result from either direct neurotoxicity from the virus

and its proteins or through sustained chronic immune activation and inflammation. Viral proteins such as glycoprotein 120 (gp-120) and transactivator of transcription (tat) have been shown to be neurotoxic. The chronic immune activation stimulates the microglia and macrophages to secrete neurotoxic products such as pro-inflammatory cytokines (tumor necrosis factor- α , interleukin-1, and interferon- α), quinolinic acid, and arachidonic acid metabolites. This results in disturbance of the blood-brain barrier and further entry of infected cells into the nervous system and eventually to decreased synaptic-dendritic density (Mirza and Rathore 2012). The common end pathway for neurotoxicity is through the excitation of *N*-methyl-D-aspartate (NMDA) glutamate receptors with potential for mediating apoptosis (Spudich and González-Scarano 2012).

HIV-Associated Neurocognitive Disorders (HAND)

HIV-associated neurocognitive disorder (HAND) is a chronic neurodegenerative condition characterized by cognitive, motor, and behavioral abnormalities. HAND is classified into three groups based on the severity of neurocognitive impairment that range from mild to severe: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) (Antinori et al. 2007). ANI is a subclinical decline in cognition, MND is a mild decline in cognition along with mild interference in everyday functioning, and HAD is significant decline in cognition along with significant functional impairment affecting routine activities. These disorders were previously known as AIDS dementia complex (ADC) and minor cognitive and motor disorder (MCMD).

With the introduction of antiretroviral therapy, there has been a substantial change in the epidemiology of HAND with significant decline in the incidence of severe neurocognitive impairment. For example, in the USA, current incidence of HIV-associated dementia is less than 1% compared to 7% in 1989 (Kranick and Nath 2012).

HIV-associated dementia (HAD) is associated with profound immunosuppression with low CD4 counts and the presence of other symptoms of AIDS. In low-resourced countries, where treatment is often delayed, HAD is still quite prevalent. The milder degrees of HAND (ANI and MND), on the other hand, remain common even in ART-treated patients and are no longer associated with the typical HIV disease biomarkers (viral load, CD4, and inflammatory response markers). Compared to patients without any impairment, patients with these milder forms of neurocognitive impairment have worse quality of life, reduced ability to perform everyday tasks, poor medication adherence, and reduced survival time.

The reasons for this persistence of cognitive dysfunction despite good viral control are unknown, but several explanations have been proposed. The host and viral factors that predispose to the development of HAND are also poorly understood. Recent evidence suggests that the subtype of HIV might have effect on disease progression and differ in neuropathogenicity. For example, a study from Uganda, where HIV clades A and D predominate, reported that adults with clade D infection had a more rapid progression and increased risk for dementia compared to clade A (Sacktor et al. 2007). Host factors that affect the occurrence of HAND include aging, substance abuse, and the presence of comorbid diseases such as hepatitis C virus infection (Shapshak et al. 2011).

HAND affects several areas or domains of neurocognitive functioning. The pattern of cognitive impairment observed in classic HAD is a subcortical dementia, more similar to deficits associated with disorders such as Parkinson's disease and Huntington's disease, rather than the pattern for cortical dementia associated with Alzheimer's disease. Cortical deficits were not observed until very late in the disease as the brain became globally affected. There is emerging evidence that HAND has some additional cortical features that are mostly observed in the neuropsychological profile. The most commonly affected domains are attention/working memory, executive functioning, psychomotor speed, memory

and learning, speed of information processing, and motor skills. Other domains less commonly or severely affected by HAND include language fluency and visuospatial domains. Furthermore, there is evidence that HAND is beginning to have more extrapyramidal features, sometimes overlapping with Parkinson's disease. These changes suggest that HAND is probably associated with the accelerated development of some neurodegenerative diseases and accelerated aging (Brew and Chan 2014).

Current "Frascati" criteria for the diagnosis of the milder forms of HAND require formal neurological assessment and neuropsychological testing (Antinori et al. 2007). Neuropsychological impairment is defined by performance below the appropriate normalized group mean on standardized tests. The formal criteria specify that at least five domains of functioning must be included to cover a range of cognitive abilities. ANI and MND impairment is defined as performance one standard deviation or more below the mean on at least two neuropsychological tests of different domains. HAD, however, must have impairment in performance two standard deviations below on two neuropsychological tests in two different domains.

In most clinical settings and in low-resourced settings where facilities for neuropsychological assessments are not readily available, a presumptive clinical diagnosis and use of brief screening tools are advocated. Examples of such brief screening tools include NEU screen, HIV Dementia Scale (HDS) and International HIV Dementia Scale (IHDS), and the ACTG ALLRT Neuroscreen, and even computerized screens such as CogState and CANTAB have also been utilized. However, no single tool is suitable across all practice settings, and these tests are generally not sensitive or specific for HIV-associated neurocognitive dysfunction.

Clinically, HAND presents with difficulties in learning new information, disturbances in attention, slowing of information processing, and disturbances in executive functioning (e.g., planning, abstraction). Motor symptoms include motor slowing, ataxia, incoordination, and tremors and may later progress to weakness, spasticity, and

paraparesis. Behavioral manifestations of HAND include apathy, psychomotor retardation, and irritability. Neuroradiological studies show generalized cerebral atrophy especially in the later stages of the disease; the degree of atrophy on MRI has been correlated with the degree of neurocognitive dysfunction. There is no specific laboratory diagnostic biomarker for HAND; HIV+ patients are diagnosed after ruling out confounding conditions such as CNS opportunistic infections, psychiatric disease, and age-related dementia. ART remains the most important treatment for HAND.

Peripheral Neuropathies

Neuropathy is one of the most common manifestations of HIV disease. Many forms of peripheral neuropathies have been associated with HIV including distal symmetric polyneuropathy (also called distal sensory peripheral neuropathy (DSPN)), mononeuritis multiplex, inflammatory demyelinating polyneuropathy, polyradiculopathy, and autonomic dysregulation. The commonest are distal symmetric polyneuropathy (DSP), acute inflammatory demyelinating polyneuropathy, and neurotoxic neuropathy.

Distal Symmetric Polyneuropathy: HIV-associated DSP is the commonest neurological problem of HIV in developed countries in this era of effective antiretroviral treatment. The rates have not been well studied in developing countries but appear similar to that from developed countries. The current prevalence estimates range from 19% to 66%. This wide variation results from widely varying definitions of HIV DSP used in studies and differences in the cohort studied. Some studies defined DSP as one clinical sign (i.e., reduced ankle reflexes or reduced pinprick sensation or reduced vibration sensation in the feet), others required two clinical signs, and some used validated screening or diagnostic instruments (Schütz and Robinson-Papp 2013). Prior to the introduction of ART, DSP prevalence was 55%, and almost all patients had evidence of DSP on autopsy even those without symptoms during their lifetime. Pre-cART, DSP was related to profound immunosuppression and high HIV

viral load, but recent studies report that the degree of immunosuppression or viral load does not predict the development of DSP. Recent studies report that age is the most important risk factor for DSP; rates are higher in older patients.

DSP is an ascending, symmetric predominantly sensory distal neuropathy. Symptoms typically begin in the distal lower extremities – toes and feet – and may gradually ascend up to the thighs and could similarly involve the hand: glove and stocking distribution of symptoms. Patients present with pain, paresthesia, and numbness. The signs seen on examination include decreased or absent ankle jerks and decreased pinprick and vibration sensation involving the distal lower extremities. Pathological changes may be absent on electromyogram and nerve conduction studies; definitive diagnosis is made on skin biopsy which typically shows a reduction in epidermal nerve fiber density in DSP patients. It is important to rule out other potentially reversible causes of sensory neuropathy such as diabetes mellitus, renal disease, liver disease, infections (syphilis, hepatitis C), and micronutrient deficiency (vitamin B12, folate, and vitamin D). There is presently no effective treatment for DSP.

Inflammatory Demyelinating Polyneuropathy: The true prevalence of this complication is not known. Two forms of inflammatory polyneuropathy have been described in HIV: acute inflammatory polyneuropathy (AIDP) and chronic inflammatory polyneuropathy (CIDP). AIDP typically occurs at seroconversion and may be the presenting symptom of underlying infection with HIV. It is indistinguishable clinically from Guillain-Barré syndrome (GBS); up to 10% of GBS cases are related to HIV. AIDP presents as a rapidly progressive primarily motor neuropathy, most commonly starting in the lower limbs and like GBS can lead to quadriplegia with respiratory failure over days or even hours. The treatment is the same as for non-HIV-associated AIDP. Treatment consists of supportive care, intravenous immunoglobulin, or electrophoresis and possibly starting cART.

CIDP on the other hand occurs in late infection and is associated with low CD4 count. CIDP resembles AIDP, but it is more slowly progressive

and may have a relapsing and remitting course. Electrophysiologic studies show slow or absent nerve conduction. The treatment for HIV-associated CIDPs is identical to that for non-HIV-related disease in addition to antiretroviral medications.

HIV Myelopathy

HIV is associated with a non-inflammatory, subacute vacuolar myelopathy (Previti and Marra 2012). Much of what is known about HIV-associated myelopathy is from the pre-cART era where it was found in up to 30% of autopsies and more commonly in patient with other AIDS-defining conditions. It is likely that the incidence is dramatically reduced in the current treatment era. HIV vacuolar myelopathy (HIV VM) is a diagnosis of exclusion; it has to be differentiated from other causes of spinal cord disease such as direct infections (CMV, HTVL I and II, herpes simplex virus, enteroviruses, varicella zoster, syphilis and mycobacterium tuberculosis), tumors, and nutritional deficiencies (vitamin B12). While vacuolar myelopathy can develop at any stage in the progression of HIV disease, it is more common at lower CD4 levels.

Clinically it presents as a subacute or chronic weakness in the lower limbs, spasticity, hyperreflexia, sensory loss (especially loss of vibration and joint position sense), sexual dysfunction, and sphincter dysfunction. Laboratory and imaging studies are needed to exclude other spinal cord diseases. Histopathological features are prominent vacuolation in the white matter of the dorsal and lateral columns that is most prominent in the thoracic spinal cord. There is no proven treatment for HIV myelopathy, and the condition is irreversible. The treatment is usually symptomatic; however, some patients may improve with ART.

Opportunistic Infections

Most CNS opportunistic infections (OIs) seen in people with HIV are due to reactivation of latent pathogens rather than new infections. The

strongest predictor for the development of opportunistic infections is decreased CD4 count; susceptibility is increased when the CD4 count falls below 200 cell/ μ l. These infections are usually comorbid, with up to 15% of cases having more than one infection (Tan et al. 2011). In the era of cART, opportunistic infections are often unmasked at the initiation of antiretroviral therapy. Many of the OIs that commonly infect the CNS are AIDS-defining conditions; they include toxoplasmosis, cryptococcal meningitis, progressive multifocal leukoencephalopathy (PML), CNS tuberculosis, and CNS cytomegalovirus. CNS OIs are associated with excess mortality. There is wide geographical variation in the occurrence of these infections. For example, the commonest CNS OIs in Asia and Pacific Regions are cryptococcal meningitis, cerebral toxoplasmosis, and tuberculous meningitis, while in Europe and North America, the commonest infections are PML, toxoplasmic encephalitis, and cryptococcal meningitis (Tan et al. 2011).

The diagnosis of CNS OIs is based on the clinical presentation, radiographic features, CSF findings, and isolation of the organism or its antibodies. In resource-rich settings, a battery of investigations which include neuroimaging studies (CT /MRI scans), CSF antibody testing or polymerase chain reactions to identify the organism, and image-guided stereotactic brain biopsy are used to confirm the diagnosis of CNS OIs. In places with limited resources, where diagnoses often depend on clinical presentation and minimal rudimentary investigations, the diagnosis of OIs is often missed or delayed contributing further to increased morbidity and mortality. In such resource poor settings where cART and the resources to manage OIs are not yet widely available, the current medical strategy is the use of prophylactic antimicrobial regimens to reduce the occurrence of OIs.

Primary CNS Lymphoma

Primary CNS lymphoma (PCNSL) is the second most common mass lesion in AIDS; it usually arises in and is confined to the CNS. It is therefore

important to rule out systemic lymphoma with secondary CNS lesions in making the diagnosis. PCNSL affected up to 5% of patients with HIV before the advent of cART, but rates have declined substantially since, and in most developed countries, it is now a rare disease. The major risk factor for PCNSL is a low CD4 count (less than 100cells/ μ l).

Primary CNS lymphoma is a high-grade B-cell non-Hodgkin's lymphoma that is associated with multiple genetic alterations and monoclonal Epstein-Barr virus infection. Factors that contribute to lymphoma development include HIV-induced immunosuppression, impaired immune surveillance, cytokine release and deregulation, and chronic antigenic stimulation. The clinical features of the tumor are usually non-specific and may include headache, lethargy, cognitive changes, seizures, and focal signs which depend on the anatomic site of the tumor. The tumor shows up on neuroimaging (CT or MRI) as contrast-enhancing lesions surrounded by edema and a mass effect. In making the diagnosis, CSF PCR to detect the presence of Epstein-Barr virus is helpful; diagnosis confirmed with brain biopsy. The clinical outcome with current treatments for primary CNS lymphoma in HIV-infected persons remains relatively discouraging; median survival time is about 2 months.

CNS IRIS

Immune reconstitution syndrome (IRIS) is a serious complication related to immune recovery on initiating effective antiretroviral therapy. IRIS can affect any organ system, and it occurs in 15–25% of patients starting ART, but the rates increase to 20–45% in patients with OIs. CNS IRIS is less common, found in only about 1% of patients initiating ART (Kranick and Nath 2012). Even though the rates have not been well documented in low-resourced settings, it is likely to be much higher as patients in such setting usually start ART at lower nadir CD4 levels when many already have an AIDS-defining illness. For example, in a prospective study of neurological disorders

occurring within the first year of starting ART in South Africa, 28% of the sample developed CNS IRIS (Asselman et al. 2010). CNS IRIS is associated with poor outcomes and high mortality rates ranging from 20% to 30% depending on the underlying infection and the immune status of the individual. This excess mortality is related to raised intracranial pressure and risk of brain herniation resulting from the excessive inflammation and swelling in the brain tissue.

The important infections predisposing to CNS IRIS include cryptococcal meningitis, CNS tuberculosis, and progressive multifocal leukoencephalopathy. However, CNS IRIS has been described occasionally with other opportunistic infections and more rarely without an identifiable pathogen. In addition to the underlying OI, other risk factors for developing CNS IRIS are degree of immune suppression prior to initiating ART (indicated by a low nadir CD4 count), rate of immune recovery, and possibly some host genetic factors.

Two forms of IRS are traditionally described: paradoxical and unmasking IRIS. In “paradoxical IRIS,” the individual is already known to have an opportunistic infection but is unable to mount an appropriate immune response against it; on initiating ART, the recovering immune system generates a heightened immune response that results in a worsening of the patients' clinical condition. In “unmasking IRIS,” the presence of a subclinical or occult infection is revealed when the individual commences ART. The exaggerated immune response and clinical deterioration seen in CNS IRIS are mediated by the infiltration of activated CD4+ and CD8+ T cells into the CNS.

The clinical features and time of onset for CNS IRIS vary significantly depending on the underlying OI; this suggests that pathogen-specific disease mechanisms may be responsible for IRIS seen in the context of each OI (Kranick and Nath 2012). CNS IRIS presents as paradoxical worsening of neurologic symptoms within the first 4–8 weeks of starting cART. Neuroimaging shows contrast-enhancing lesions on the MRI scan, indicating a breakdown of the blood-brain barrier. The management of patients who develop CNS IRIS is

guided by the nature of the underlying OI and the severity of presenting symptoms. Since the most important risk for developing IRIS is a low CD4 cell count prior to starting of ART, early initiation of ART will be important in reducing the incidence of CNS IRIS.

Neurotoxic Neuropathy

Some ARTs are known to cause a toxic neuropathy with features similar to and difficult to distinguish from distal symmetric polyneuropathy. The antiretroviral agents classically associated with peripheral neuropathy are the dideoxynucleoside antiretroviral drugs (d-drugs) including didanosine, zalcitabine, and stavudine. These drugs have largely been removed from antiretroviral medication regimens in resource-rich countries but due to their effectiveness and lower cost are still commonly used in resource-limited settings. Toxicity related to the d-drugs is additive, and combination therapy with these drugs should be avoided. Symptoms of ART neurotoxicity usually arise within 2–3 months of initiation of treatment. These drugs are thought to produce nerve damage through their competition with thymidine triphosphate and subsequent mitochondrial dysfunction. Withdrawal of the offending ART and substitution with an alternative that does not include a d-drug is the first step in patient management. Neuropathic symptoms may improve in 1–3 months following the discontinuation of the drug.

Non-AIDS-Related Diseases

The incidence of stroke is higher in HIV populations compared to the general population (Ovbiagele and Nath 2011). This excess risk has been attributed to factors that result directly from HIV infection and others related to complications or treatment of HIV. HIV has been implicated in causing small- and medium-vessel vasculopathy, with dyslipidemia and increased inflammation.

Future Directions

Cure, Eradication, and CNS Latency: Despite the successes achieved in HIV treatment with the introduction of ART, a major limitation of the currently available medications is their inability to eradicate the HIV virus from quiescent reservoirs. Known sanctuaries for latent HIV infection include the central nervous system, the lymphoid tissue, and the testes. Strategies targeted at eliminating persistent HIV from these reservoirs and thereby achieving a cure in patients who are receiving suppressive cART are being evaluated.

Treatment of HAND in Those on ART Who Are Suppressed: Antiretroviral therapy currently is the mainstay of treatment for HAND. However, the use of cART only partially reverses the symptoms or persistence of HAND despite adequate viral suppression. Trials of several adjuvant therapies aimed at attenuating CNS inflammation or acting as putative neuroprotective agents have not yielded much success in reversing neurocognitive impairments till date. Further research is needed to provide more evidence to guide the treatment of HAND in patients whose HIV infection is suppressed on ART.

CNS Penetration: Antiretroviral agents vary in their ability to penetrate the CNS in therapeutic concentrations; the effects of the CNS penetration of cART regimens are an ongoing area of research. While studies have shown that regimens with higher rankings on CNS penetration effectiveness (CPE) are associated with lower CSF viral loads, the effects on neuropsychological performance is as yet inconclusive (Brew and Chan 2014).

Conclusion

Despite advancements in the treatment of HIV, neurologic conditions remain an important cause of morbidity. In developed countries where there is widespread availability and access to combination antiretroviral therapy, there has been a significant decline in the incidence of more severe neurocognitive disorders and opportunistic infections (Sacktor and Robertson 2014); however,

milder forms of HAND are present in up to 50% of HIV-positive adults (Heaton et al. 2010). In low- and middle-income countries especially sub-Saharan Africa where the highest burden of HIV resides, limited resources hinder the diagnosis and treatment of neurological disorders in HIV (Joseph et al. 2013).

Cross-References

► HIV Neurocognitive Diagnosis, Natural History, and Treatment

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HAND Adjunctive Therapies: Reversing Neuronal Injury

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Definition

This entry focuses on the therapeutic potential of small molecule inhibitors of two kinases, leucine-rich repeat kinase 2 (LRRK2) and mixed-lineage kinase 3 (MLK3), for the treatment of HIV-1-associated neurocognitive disorders (HAND). In particular, this entry is focused on these small molecule inhibitors as a way of restoring normal synaptic architecture, the key substrate that is damaged by the neuroinflammatory milieu of HAND.

From a signaling perspective, MLK3 is a canonical MAP kinase kinase kinase (MAPKKK) that controls the phosphorylation state of the MAPKs JNK and possibly p38 (Gallo and Johnson 2002). While only relatively recently implicated in the neuropathogenesis of Parkinson's disease (PD), LRRK2 activation

has also been strongly associated with neurodegeneration and neuroinflammation (Kim et al. 2012; Moehle et al. 2012), and studies of its mechanism of action demonstrate that it may also contribute to the phosphorylation of p38 and JNK (Hsu et al. 2010). Further linking the structural and functional relationship of MLK3 and LRRK2, the kinase domain of LRRK2 exhibits a significant amount of homology with the kinase domain of MLK3 (Zimprich et al. 2004). Excessive p38 and JNK activity has been linked to many aspects of neurodegenerative disease, including loss of neuronal processes, increased neuronal apoptosis, and increased inflammatory cytokine production in brain-resident immune cells (Borsello and Forloni 2007; Dhanasekaran and Reddy 2008). These kinases are therefore considered prime targets with the potential for disease modification by small molecule inhibition in chronic neuroinflammatory disease.

Recent work using a commercially available kinase inhibitor relatively specific to LRRK2 demonstrated that inhibition of LRRK2 decreased inflammatory cytokine production by microglia in response to HIV Tat exposure in vitro (Marker et al. 2012). Additional studies using a novel microfluidic-based coculture system in which cultured neurons propagate along microgrooves in contact with cocultured microglial cells further demonstrated that inhibition of LRRK2 protected neuronal axons from destruction and phagocytosis by HIV Tat-exposed microglia (Marker et al. 2012). Using the same in vitro methods

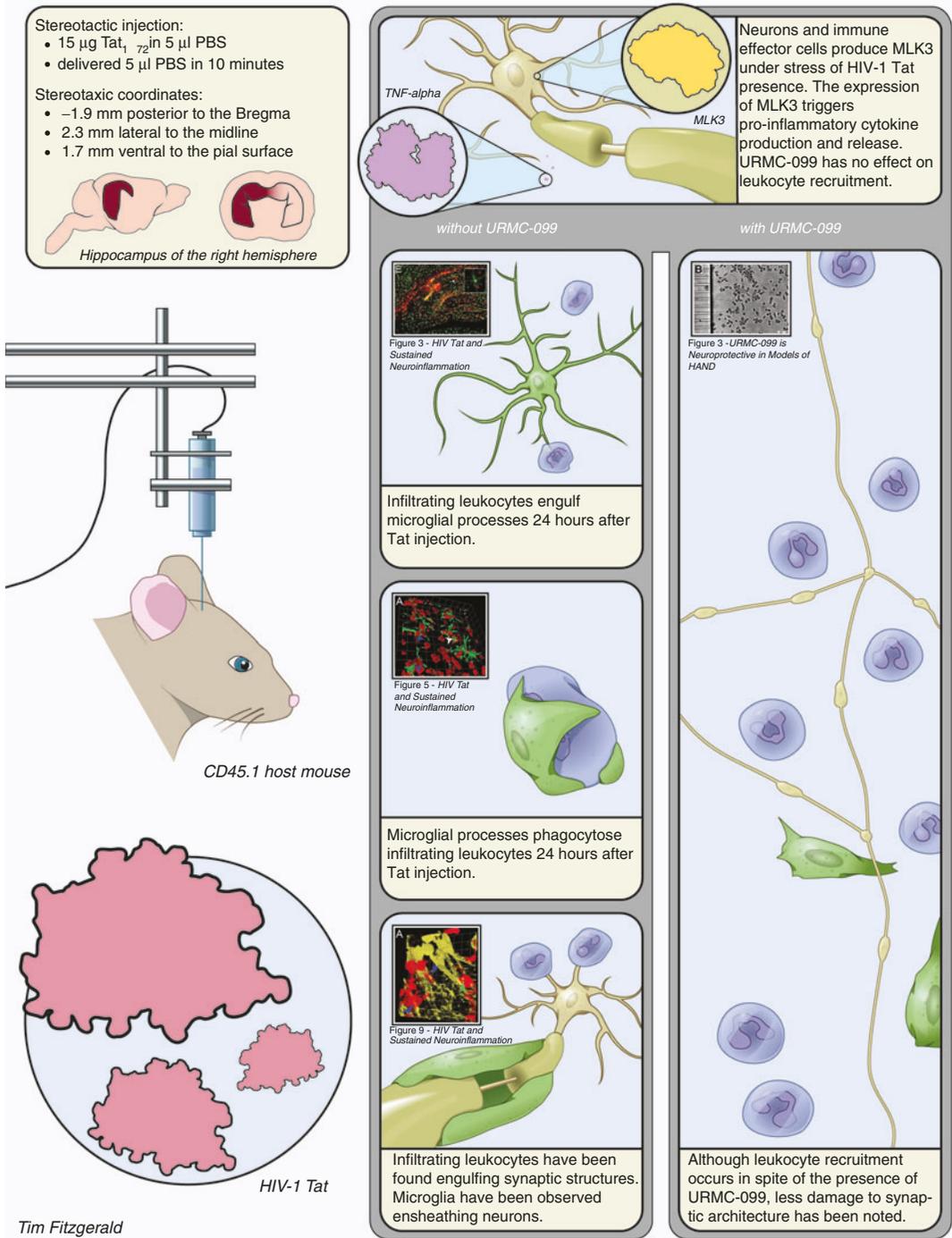
developed for these LRRK2 studies, the efficacy of a novel MLK3 inhibitor named URM-099 was evaluated for *in vitro* and *in vivo* neuroprotection against HIV Tat-induced neurotoxicity (see cartoon, Fig. 1). URM-099 is a kinase inhibitor that has low nanomolar affinity against MLK3 that was designed to be brain penetrant and orally bioavailable (Goodfellow et al. 2013). URM-099 treatment significantly reduced inflammatory cytokine production from cultured microglia in response to HIV Tat (Marker et al. 2013). It also significantly protected primary axons from destruction and phagocytosis by Tat-activated microglia (Marker et al. 2013). Using novel *in vivo* multiphoton imaging techniques in mice with genetically marked neurons and/or microglia, *in vivo* treatment with URM-099 significantly protected neuronal structures in murine cortex from the effects of a single exposure to HIV Tat. In this model, stereotactic injection in the cortex leads to prolonged neuroinflammatory changes that persist for at least 28 days without neuroprotective intervention. URM-099 treatment also altered the morphologic response of microglia exposed to Tat *in vivo* and normalized their interactions with synaptic elements at an ultrastructural level by greatly reducing phagocytosis of a subset of synaptic elements (Marker et al. 2013). These data provide compelling evidence for further study of URM-099 in more comprehensive models of HIV infection, such as the CD34-humanized severe combined immunodeficiency (SCID) mouse model of HAND (Dash et al. 2012), for the purpose of developing it into an adjunctive therapeutic treatment for HAND patients.

Small molecule kinase inhibitors like URM-099 can competitively prevent kinase activity through one of two characterized mechanisms of action: type 1 and type 2 inhibition. Both of these mechanisms prevent the binding of adenosine triphosphate (ATP) in the ATP binding domain of the kinase, and nearly all known kinase inhibitors target this ATP binding site (Liu and Gray 2006). The ATP binding site consists of a number of highly conserved domains: (1) the adenine-binding region, (2) the sugar region, and (3) the phosphate-binding region. Directly adjacent to the

ATP binding site is the “hinge” region, which contains a number of hydrophobic regions as well as “gatekeeper” amino acid residues that prevent ATP binding when the kinase is in an inactive form. Further, all known human kinases contain an aspartate-phenylalanine-glycine (DFG) amino acid sequence that is important for ATP binding in a distant area of the protein known as the activation loop. Type 1 kinase inhibitors form hydrogen bonds with amino acids in the hinge region of the kinase and competitively prevent ATP binding and kinase activity through steric hindrance of the ATP binding domain. In general, type 1 kinase inhibitors bind the active (i.e., phosphorylated) form of the kinase, as the ATP binding site is most accessible in the active conformation, but certain inhibitors can bind the inactive state as well (Liu and Gray 2006).

Type 2 inhibitors work through a different mechanism. They also form hydrogen bonds with the hinge region, but additionally they form hydrogen bonds with the DFG sequence of the activation loop, as well as other allosteric sites on the kinase. All known type 2 inhibitors bind with the DFG sequence in the “out” conformation, meaning these inhibitors bind the inactive form of the kinase (Liu and Gray 2006). These inhibitors may also bind the active form of the kinase in a different conformation or bind to the active form of the kinase and force it into an inactive state, but this has yet to be shown (Liu and Gray 2006). The additional allosteric and DFG binding sites make these inhibitors much more specific than type 1 inhibitors but also make them much more difficult to design.

Quantitative structure activity relationship (QSAR) analysis coupled with kinase activity data of URM-099 performed in cell-free assays has tentatively identified the compound as a type 1 kinase inhibitor that can bind the inactive form of MLK3 (Goodfellow et al. 2013). The data that demonstrate decreased phospho-JNK with URM-099 treatment in cultured BV-2 microglial cells exposed to Tat (Marker et al. 2013) are not inconsistent with this hypothesis. In that experiment, cells with URM-099 showed a reduction in phospho-JNK in response to Tat treatment. This result is consistent with a scenario in which



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HAND Adjunctive Therapies: Reversing Neuronal Injury, Fig. 1 A cartoon summary of data from Lu et al. (2011) and Marker et al. (2013) depicts in the left-hand panels the in vivo administration of HIV Tat into mice chimeric for either thy1-GFP or CX3CR1-GFP to identify neurons or myeloid cells including microglia, respectively.

The middle and right panels depict the sequence of events that occur after intracerebral inoculation of HIV Tat, including infiltrating inflammatory leukocytes from the peripheral circulation, sustained microgliosis, and amelioration of damage to synaptic structures after treatment with URM-099

URMC-099 binds to the inactive form of MLK3 and prevents activity, although it does not rule out the ability of URMC-099 to inhibit the active form of MLK3, as well.

While URMC-099 was initially designed based on its ability to inhibit MLK3 as the primary target, it was later found to significantly inhibit the LRRK2 in the results of a large-scale kinase screen, the Ambit KINOMEScan (Goodfellow et al. 2013). KINOMEScan measures the activity of potential drug-like small molecules against 456 human kinases, using proprietary methods to determine IC_{50} in a non-ATP-dependent fashion. The off-target effects of URMC-099 against LRRK2 are most likely due to the significant homology between the MLK3 and LRRK2 domains. These results suggest that URMC-099 may have multiple mechanisms for neuroprotection by inhibiting pathologic activation of both MLK3 and LRRK2, but, of course, does not detract from its promise as a therapeutic agent. Thus far, attempts to determine URMC-099's mechanism of action by genetically silencing MLK3 and/or LRRK2 in the microglia *in vitro* have utilized siRNA species using lipofectamine-based methods, but have been hampered by non-specific inflammation. Recent publications have demonstrated significant activation of the JNK pathway in control-transfected microglia (Kim et al. 2012), indicating that lipofectamine-based transfections may also activate cultured microglia. As microglia are a monocyte lineage immune cell that becomes activated in all *in vitro* cultures, it is possible that siRNA treatment further activates innate defense mechanisms normally triggered by infectious agents as well. These confounds make siRNA expression and lipofectamine-based transfection ill-suited for *in vitro* studies directed at elucidating potential signaling pathways relevant to URMC-099's mechanism(s) of action. These results further suggest the need for *in vivo* approaches to address pathologic outcomes of LRRK2 and MLK3 signaling using conditional genetic deletion of one or both of these control points for neuroinflammatory signaling.

Another interesting question raised by studies of HIV Tat-induced neurotoxicity is whether

URMC-099's neuroprotective effects are exerted through kinase inhibition in the microglia, neurons, or both. To answer this question, it will be important to first examine the extent of JNK activation and the effect of URMC-099 treatment in Tat-treated neuronal monocultures. While significant inhibition of the JNK pathway in cultured microglia exposed to HIV Tat occurs (Marker et al. 2013), it remains technically impossible to demonstrate this in neurons exposed to HIV Tat because of the extremely high baseline phosphorylation of JNK in cultured neurons (Barnat et al. 2010). In order to examine Tat and URMC-099's effect on the JNK pathway in cultured neurons, pull-down assays of the individual JNK isoforms may be the most technically feasible way of determining phosphorylation status in neurons exposed to HIV Tat with or without URMC-099. This will demonstrate whether Tat is activating MLK3-controlled pathways in neurons on its own and whether URMC-099 has any effect on these pathways. Efforts to better understand proximate downstream signaling events relevant to MLK3 activation have been hampered by the unavailability of either monoclonal or polyclonal antibodies to phospho-MLK3 since loss of the single commercial source in 2010; thus the effects of URMC-099 treatment on MLK3 phosphorylation using either immunoblotting or immunocytochemistry remain unknown due to the lack of suitable reagents. This also leaves the question as to whether URMC-099 inhibits the active phosphorylated form of MLK3 and/or the inactive form of MLK3 unanswered. However, if Tat alone does not induce MLK3 activity in neurons, it will be necessary to determine if soluble factors produced by Tat-exposed microglia upregulate MLK3 activity. This can be simply tested by exposing neurons to supernatant from Tat-exposed microglia cultures. Finally, it is possible that direct contact between microglia and neuronal elements (such as synapses or axons) is necessary to induce MLK3 activation in neurons. This can only be tested using high-magnification, high-localization ICC, by examining levels and translocation to the nucleus of phospho-JNK (since pMLK3 antibodies are unavailable) in fixed cultures, or by dissociating and sorting the

cultured cells using fluorescence-assisted cell sorting and blotting against the individual isoforms of phospho-JNK in the neuronal fraction.

A previous study demonstrated that the number of synapsin-1-positive puncta at the Tat injection site appeared to decrease between the 7 and 28-day time points (Marker et al. 2013). Because of this progressive loss of synaptic elements, mice were treated with URM-099 at twice daily doses to insure brain levels above the K_i for MLK3 (Goodfellow et al. 2013) and subsequently evaluated by immunocytochemistry for the ability of URM-099 to confer neuroprotection in a chronic model of neural injury like HAND (Marker et al. 2013). This data supports the hypothesis that Tat exposure creates a self-perpetuating inflammatory environment that leads to progressive neurodegeneration (see cartoon, Fig. 1), even after the initial insult is no longer present. It also opens the possibility that delayed URM-099 treatment could still be neuroprotective. Because published data involved pretreatment with URM-099 prior to administration of intracerebral Tat to obtain the maximal degree of neuroprotection, future experiments will investigate the effects of withholding treatment for various periods of time after Tat exposure, to determine the length of the treatment window. If URM-099 is working by breaking an inflammatory feedback cycle, it is also possible that continuous treatment is not needed in the mice for neuroprotection.

Conclusion

Despite initial hopes that the advent of cART would eliminate HIV neurologic disease, over the last decade, it has become clear that this is not the case. In its current form, cART alone simply cannot eradicate the HIV virus from the host, and the low-level production of viral particles and proteins even with efficacious central nervous system-targeted antiretroviral therapy (Ellis et al. 2014) is sufficient to drive HAND in its current manifestations. For the foreseeable future, HIV will remain an incurable but treatable condition, with patients approaching normal life spans. For this reason, the development of an

adjunctive therapy to protect neurologic function in these patients is paramount for their quality of life. Published work using preclinical in vitro and in vivo models of HAND lays a strong foundation for the use of URM-099 in the treatment of HAND. Future studies of this drug concerning safety and efficacy are the obvious next step, but the safety and tolerance of MLK3 inhibition in phase II PRECEPT trials for PD (Parkinson Study Group PRECEPT Investigators 2007) bode well for URM-099's transition to human trials. While CEP-1347, the nonselective MLK3 inhibitor failed to demonstrate efficacy in the PRECEPT trial (Parkinson Study Group PRECEPT Investigators 2007), previously published studies demonstrated that CEP-1347 has a very poor profile of brain penetration and metabolism (Goodfellow et al. 2013) compared to URM-099. This further buttresses the need to address safety, tolerability, and ultimately efficacy of URM-099 for the treatment of HAND.

Cross-References

- ▶ [Long-Acting Nanoformulated Antiretroviral Therapy](#)
- ▶ [Neuro-AIDS, Immunopathogenesis of](#)
- ▶ [Neuroinflammation and HAND: Therapeutic Targeting](#)
- ▶ [Prevention Clinical Trials: Highlights of Evidence and Research](#)

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Harm Reduction for Injection Drug Users

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Definition

Harm reduction for injection drug users (IDUs) refers to programs designed to reduce or minimize negative health consequences related to substance use among IDUs without the expectation of substance use abstinence. Harm reduction arose as a response to emerging HIV epidemics driven by injection drug use in the mid- to late 1980s. The goal of harm reduction is to reduce individual- and societal-level harms that can result from drug use rather than focusing on stopping drug use altogether. Some harm reduction programs focus on reducing the amount and frequency of drugs used with the eventual goal of abstinence. However, the vast majority of harm reduction programming focuses on the reduction of physical harm to the individual injector, their immediate social network, and the public as a whole, particularly in reducing the transmission of HIV and other infectious diseases. Because the harm reduction approach in dealing with substance use is nontraditional, implementation of harm reduction programs can be controversial and politicized. Despite this, harm reduction strategies have been implemented widely both globally and in the United States. Common harm reduction programs include needle or syringe exchange programs (NEPs or SEPs), supervised injection facilities (SIFs), drug treatment, and provision of condoms and education about sexual transmission of HIV.

Needle and Syringe Exchange Programs (NEPs and SEPs)

History and NEP Coverage

Needle exchange programs (NEPs), also referred to as syringe exchange programs (SEPs) and needle syringe programs (NSPs), provide new sterile syringes to IDUs in exchange for used needles. Use of these programs has been the preeminent form of harm reduction among IDUs both in the United States and internationally and has led to decreases in HIV and hepatitis B and C virus (HBV and HCV) incidence among IDUs.

In 1983, the first NEP was opened in Amsterdam, Netherlands, in order to stem an outbreak of HBV among IDUs. A few years later, driven by the HIV/AIDS epidemic, many new NEPs were established to halt the spread of HIV among IDUs. The first NEPs in the United States were established in the late 1980s. At least 82 countries are known to have one or more operational NEPs (Mathers et al. 2010).

Through 2010, there were 203 known NEPs operating in the United States (Green et al. 2012) – more than triple the number operating in 1995 (Des Jarlais et al. 2009a). American NEPs reported exchanging more than 36 million syringes in 2010 (Green et al. 2012). Along with distributing new sterile needles, the majority of NEPs also provide male and female condoms, have on-site screening for HIV and hepatitis, and offer education on safer sex and injections. Despite these successes, many NEPs cite operational issues in implementation of their programs; foremost among these problems is lack of resources and funding, followed by shortage of staff, and staff burnout (Des Jarlais et al. 2009a).

Evidence for HIV Transmission/Acquisition Reduction

In 2004, the World Health Organization (WHO) released a report stating that there was compelling evidence that NEPs helped to significantly reduce the spread of HIV among IDUs and cited NEPs as a cost-effective method of harm reduction. The WHO report also said, “there is still no persuasive evidence that needle syringe programmes increase the initiation, duration or

frequency of illicit drug use or drug injecting” (Wodak and Cooney 2004).

More recently, researchers have shown that while NEPs are effective at reducing HIV transmission, there is insufficient evidence to conclude that they are associated with reductions in HCV transmission (Palmateer et al. 2010). Reductions in HIV prevalence and incidence among IDUs have been predominantly driven by the use of new sterile needles at each injection, behaviors promoted by NEPs. Use of NEPs has also been shown to be associated with decreases in the frequency of injection and providing used syringes to friends. IDUs who accessed services at NEPs were also more likely to be linked into drug treatment programs.

NEP Policy Issues

In 1988, the US Congress enacted a federal funding ban on NEPs. Ten years later, Lurie and Drucker reported that legalization of NEPs in the United States could have prevented 10,000–20,000 new HIV infections (1997). The 2004 WHO report stated that this legislation on the distribution of injecting paraphernalia was a serious blockade to controlling HIV among IDUs (Wodak and Cooney 2004). The ability to access syringes was hindered by a number of laws, including drug paraphernalia laws in 47 states, syringe prescription laws in eight states, and regulation of pharmacy dispensation of syringes in 23 states. In order to facilitate access to clean syringes, many states enacted legislation to exempt NEPs from these laws. However, the existence of these laws still deterred many IDUs from seeking clean needles due to fear of arrest and incarceration.

In 2009, the Congress voted to overturn the federal funds ban on NEPs in the United States through the Consolidated Appropriation Act. Limited funding was the main concern for most NEPs in the United States (Des Jarlais et al. 2009a), and nearly 80% developed plans to apply for federal funding in 2011/2012 (Green et al. 2012). More than two-thirds of American NEPs received either state or local funding, and increased access to funds would allow them to expand their distribution ability. However, only

2 years after the federal ban was overturned, it was reinstated in December 2011 by the US Congress.

Types of NEPs

There are several different types of NEPs, including those operated through community-based organizations, hospitals, and pharmacies, mobile vans, and vending machines for distribution. In the United States, the majority of NEPs are stationary distribution sites, although more than one-third of all NEPs offers mobile distribution services. While harm reduction is associated with accessing NEPs regardless of type, there are significant differences in those who access specific types. In the United States, mobile-based NEPs attract twice as many high-frequency injectors as a NEP based within a pharmacy. In addition, mobile-based NEPs and syringes dispensed via vending machine were more likely to be accessed by younger IDUs. New and innovative forms of needle exchange, such as backpack exchanges employed in Rhode Island, where individuals roamed high-risk neighborhoods on foot to distribute needles, have been shown to be effective at reaching hard-to-reach injectors.

Supervised Injection Facilities (SIFs)

Supervised injection facilities (SIFs), also called safe injection facilities (SIFs), safe injection sites, drug consumption facilities (DCFs), drug consumption rooms (DCRs), and medically supervised injection center (MSIC), are legally sanctioned sites at which IDUs can inject pre-procured drugs under safe and hygienic conditions under the supervision of medical personnel. SIFs also provide sterile injection equipment, basic wound care, referrals to drug treatment, safer injection education, and access to medical personnel. The availability of a low-stress, safe, and sterile environment for IDUs to inject helps to prevent the transmission of blood-borne infectious diseases, such as HIV, HBV, and HCV, and reduces the risk of development of other negative health consequences, such as endocarditis and abscesses. Because these facilities are medically supervised, overdose deaths can also be averted.

There are more than 60 SIFs globally found in more than two dozen European cities, Sydney, Australia, and Vancouver, British Columbia, Canada (Des Jarlais et al. 2009b). Currently, there are no existing SIFs in the United States, although the acceptability and feasibility of a SIF has been explored in San Francisco, CA (Kral et al. 2010). Among IDUs surveyed, 85% reported they would use an SIF to comply with potential regulations if one opened. Community perceptions in San Francisco were mixed; community stakeholders voiced concerns about whether the burdens from operating a SIF would outweigh the benefits.

SIFs are believed to appeal to more socially vulnerable IDUs, such as those who are homeless or obtained their income from illegal or marginal sources. Previous research in Vancouver revealed that the SIF attracted higher-risk and higher-need IDUs who would benefit the most from a safe, sterile injecting environment (Wood et al. 2006). Extensive evaluation of SIFs has provided evidence of the reduction of overdose mortality as well as the reduction of borrowing used syringes and indirect sharing of injection equipment, increased linkage to drug treatment and detoxification, and the reduction of other medical consequences of non-sterile injection, as well as reductions in public drug use and publicly discarded syringes. In addition, the presence of the SIF has not had an effect on the amount of drug dealing and drug-related crime near the SIF, the rate of transition to injection drug use, or the rate of injection relapse among former IDUs (Wood et al. 2006).

Another feature of SIFs includes the provision of education to help reduce the negative consequences from injecting; in Vancouver, approximately one-third of participants had received safe injecting education from the SIF (Des Jarlais et al. 2009b). SIFs may also act as a gateway to the health-care system which ordinarily may not be as accessible to this highly marginalized population. Furthermore, SIF attendance was found to be positively associated with drug treatment and detoxification entry and a reduction in violence against the women who attended the SIF by allowing the women to avoid gender-related violence

surrounding injection practices norms (Des Jarlais et al. 2009b). Benefits to society have also been examined; one Australian ecological study conducted in New South Wales found that ambulance calls in the vicinity surrounding a SIF had decreased significantly after the SIF opened compared to other areas of New South Wales (Salmon et al. 2010).

The Vancouver SIF was created in response to a sharp increase in overdose mortality in the early 1990s as a result of increasing purity of imported heroin. This SIF has received much local community support and a positive reception from the IDU community. Despite this and extensive evaluation highlighting many individual- and societal-level benefits of the SIF, the SIF in Vancouver has faced many federal legal challenges to close it down (Small 2010). The Vancouver SIF won its latest legislative battle against the Canadian Government on September 29, 2011, when the Supreme Court of Canada ruled unanimously to uphold the SIF's exemption from the Controlled Drugs and Substances Act, allowing it to remain in operation.

Drug Treatment

Drug treatment, in particular opiate substitution treatment, is another component of harm reduction. The use of drug treatment such as methadone maintenance, buprenorphine, and naloxone and naltrexone reduces individual- and societal-level harms resulting from procurement and use of illegal injection drugs by facilitating the engagement of IDUs into a structured treatment program. Being enrolled in a drug treatment program has been shown to effectively reduce the risk of HIV transmission (Metzger et al. 1993). Drug treatment for opiate injectors can be categorized into three main groups: opiate agonist treatment (including use of methadone and buprenorphine), opiate antagonist treatment (including naloxone and extended-release naltrexone), and the use of opiates as drug treatment.

Opiate Agonist Treatment

Methadone maintenance therapy. Methadone hydrochloride is an opiate agonist that has been

utilized as replacement therapy for opiate addiction since the mid-1960s. It has been shown to effectively reduce heroin use and injection behaviors, improve treatment retention, and prevent transmission of HIV and other infectious diseases compared to non-opioid agonist treatments (Metzger et al. 1993). Methadone is dosed on an individualized basis, with an average dose of 80–120 mg used to establish narcotic blockade. It is long acting, with a half-life of 24–36 h, and therefore must be taken on a daily basis. Experimental studies have suggested that higher dose levels result in greater reductions in illicit opioid use.

In the United States, the provision of methadone as opiate substitution therapy is limited to federally approved treatment facilities, which require specific provisions to be met including the use of pre-specified induction dosing schedules, on-site counseling services, and regular urine toxicology. Methadone can either be prescribed as being received on a daily basis at a methadone clinic or, as individuals stabilize on treatment and have sustained drug-free urine toxicologies, as take-home doses. Different approaches toward the use of methadone treatment exist among treatment providers and substance use counselors, with one faction supporting abstinence-based treatment and the other supporting continued substitution treatment.

Globally, methadone maintenance is widely available in Europe and Australia, in which general medicine practitioners are allowed to prescribe methadone to patients, which is then subsequently dispensed through pharmacies (Tetrault and Fiellin 2012). Other regions with large IDU populations, including China, Malaysia, Vietnam, and Ukraine, have recently implemented and expanded the availability of methadone in response to HIV epidemics driven by injection drug use (Mathers et al. 2010). Russia, which has one of the largest HIV epidemics driven by injection drug use, does not allow for any opioid agonist therapy (Mathers et al. 2010).

Other models of methadone maintenance exist, such as low-threshold methadone and interim methadone. Unlike conventional methadone maintenance, in which the ultimate goal is abstinence

from illicit drug use, the goal of low-threshold methadone maintenance is to engage actively using IDUs who may be unwilling or unable to adhere to all necessary requirements of higher-threshold methadone maintenance, such as participation in counseling sessions and abstinence from illicit drug use. These low-threshold programs are designed to engage the highest-risk IDUs to begin the relationship between the consumer and the health-care provider in order to facilitate future engagement in a higher-threshold program.

Several different models of low-threshold methadone exist, including the provision of high-dose methadone without time limitations or requiring abstinence (Torrens et al. 1996). A program in Spain resulted in a 2-year retention rate that was higher than that reported with conventional methadone maintenance treatment. In the Netherlands, low-threshold methadone consists of low doses of methadone dispensed in a variety of settings (including a mobile methadone unit) without the requirement of enrollment into a higher-threshold drug treatment program; other harm reduction paraphernalia are available in these settings including clean injection equipment and condoms (Buning et al. 1990). The philosophy behind the Dutch model of low-threshold methadone is to engage refractory or treatment-resistant drug users who are not ready or willing to enter drug treatment by reducing the harms experienced by continued injection, with the eventual goal of engaging them into a higher-threshold treatment program.

Interim methadone has been used in the United States as a treatment bridge for IDUs who want to enter drug treatment but are on a waitlist for a treatment slot, in which IDUs are given an adequate dose of methadone and emergency counseling as long as they are on a waitlist for drug treatment. A randomized trial found that IDUs engaged in interim methadone maintenance were significantly more likely to enter comprehensive methadone maintenance and reported significantly fewer days of heroin use, reductions in heroin-positive drug tests, and reductions in the amount of money spent on drugs, and self-reported illegal income, compared to those who were on a wait list for treatment but did not receive

interim methadone maintenance (Schwartz et al. 2006).

Buprenorphine and buprenorphine/naloxone. Buprenorphine is a partial opioid agonist that was approved for use as opiate addiction pharmacotherapy in 1996 in France and 2002 in the United States. Because it is a partial agonist, its ability to provide euphoric effects and respiratory depression is dampened compared to that of other pharmacotherapies. Buprenorphine is longer acting and administered every 2–3 days versus methadone which is taken daily. Single formulation of buprenorphine as drug treatment resulted in considerable injection misuse globally, which led to the introduction of a buprenorphine/naloxone combination therapy. Buprenorphine and buprenorphine/naloxone are formulated as either tablets or films for sublingual administration. The buprenorphine/naloxone formulation results in little to no euphoric effects from sublingual administration yet can result in antagonistic effects if injected (Veilleux et al. 2010).

In the United States, as a result of the Drug Addiction Treatment Act of 2000, buprenorphine can be prescribed in primary care office-based settings by certified physicians, which allows for increased access for IDUs who might not otherwise seek treatment at a drug treatment facility. Current research is ongoing to explore the integration of HIV medical care with buprenorphine treatment.

Globally, buprenorphine and buprenorphine/naloxone in combination are widely available in Australia, New Zealand, Western Europe, and most parts of Eastern Europe, with the main exception being Russia. It has some availability as a treatment option in most East, Southeast, and South Asian countries that allow opioid substitution treatment, and there is limited to no availability of buprenorphine in all other parts of the world (Mathers et al. 2010).

Opiate Antagonist Therapy

Naloxone and extended-release naltrexone. Naloxone and naltrexone, which is a longer-acting derivative of naloxone, are opiate antagonist therapies which bind to the μ -opioid receptors in the brain, displacing any exogenously administered

opioids. The successful use of naltrexone as a treatment modality has been met with limited success; because its affinity to opioid receptors is so strong, external sources of opioids are displaced and blocked from the receptors, resulting in the precipitation withdrawal for dependent individuals. The successful initiation of naloxone and naltrexone first requires prolonged abstinence and therefore a long induction period, often resulting in withdrawal, relapse, and early dropout (Veilleux et al. 2010). A systematic review of the literature revealed that oral naltrexone with or without psychotherapy was not more effective in terms of treatment retention or reduction in the use of substance of abuse compared with placebo or no pharmacological treatment (Minozzi et al. 2011). However, some studies have demonstrated beneficial effects of using naltrexone in conjunction with contingency management and among those who are employed and highly motivated (Veilleux et al. 2010).

A recent randomized controlled trial conducted among opiate injectors in Russia comparing injectable, extended-release naltrexone to placebo demonstrated positive reductions in opiate use and craving, longer treatment retention, and a larger proportion of time opiate-free in the extended-release naltrexone group (Krupitsky et al. 2011). Because of the prohibition of the use of methadone and buprenorphine as drug treatment, positive results from this trial may signal a new and viable treatment option for opiate injectors in Russia.

Opiate-Assisted Treatment

Opiate-assisted treatment uses injectable heroin (diacetylmorphine) or other opiates such as morphine or hydromorphone (Dilaudid) as treatment for heroin addiction. In this harm reduction model, heroin maintenance is used as a last-resort treatment among long-term, treatment-refractory IDUs who have failed other conventional forms of drug treatment. The prescription of injectable heroin is used as a means to engage these individuals into a comprehensive treatment program and reduce their need for street-procured drugs.

A review of opiate-assisted treatment studies from six different countries (Switzerland,

Netherlands, Germany, Spain, United Kingdom, and Canada) found that among treatment-resistant individuals with long-term opiate dependence, heroin-assisted treatment compared to methadone resulted in longer treatment retention, better health outcomes, reduced illicit drug use and criminality, and increased social functioning (Ferri et al. 2011). In addition, a cost utility analysis found heroin maintenance to be cost effective. However, adverse events such as respiratory depression and seizure were more frequently reported among the heroin maintenance groups, although no treatment-related deaths were reported. Because of these reasons, heroin-assisted maintenance has been recommended as a last-line treatment option for individuals who have repeatedly failed conventional forms of drug treatment. Availability of heroin-assisted treatment is limited and is currently found in only seven European countries but not on other continents (Mathers et al. 2010).

HIV Testing and Education

Reduction of Sexual Risk

Many programs that target IDUs incorporate HIV testing and/or education about sexual and injection transmission risks into their harm reduction plans. Although injection drug use is the primary mode of HIV transmission for IDUs, IDUs are also still at risk for HIV infection through sexual transmission. HIV prevalence among IDUs who are men who have sex with men (MSM) is more than five times greater than among male IDUs reporting no sex with men, indicating a potential link with sexual behavior. Additionally, among female IDUs, it has been shown that sexual risk, rather than drug use risk, is significantly associated with HIV incidence. Research has also shown that many HIV-positive female IDUs report inconsistent condom use with serodiscordant male partners, providing a risk of sexual transmission to these partners (Latka et al. 2006).

Since risk of HIV acquisition via sexual behaviors is of concern among IDUs, many harm reduction programs seek to target both sexual and drug

use practices. In the United States and Canada, the majority of NEPs provide both male and female condoms to IDUs, along with education on how to prevent transmission and acquisition of HIV through sexual contact (Des Jarlais et al. 2009a). In Vancouver, Canada, an SIF provides safer sex materials to all IDUs who use the facility in order to encourage consistent condom use. Researchers found that HIV-positive IDUs who had been enrolled in the facility's program longer were significantly more likely to consistently use condoms with regular and casual sex partners (Marshall et al. 2009).

Conclusion

Implementation of IDU-focused harm reduction programs has been vital to decreasing the incidence and prevalence of HIV and other blood-borne diseases among IDUs, both in the United States and globally. The foremost method of harm reduction has been the development of needle/syringe exchange programs – providing IDUs with clean, sterile needles and injection equipment significantly decreased the prevalence of sharing syringes and other paraphernalia. Although conventional drug treatment plays a significant role in reducing drug-related harms by focusing on substitution or abstinence, the development of other harm reduction programs such as safe injection facilities and opiate-assisted treatment has helped to expand harm reduction possibilities to injection drug users who may not be ready to cease their drug use. Although NEPs, SIFs, and other more controversial harm reduction programs face a myriad of legal issues and community resistance, especially in the United States, they continue to provide an invaluable service to IDUs.

Cross-References

- ▶ [HIV Testing and Counseling](#)
- ▶ [Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#)

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Health Care Workers, Epidemiology of HIV/AIDS

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Definition

It is well documented and understood that healthcare personnel are at times at risk for occupational exposure to blood-borne pathogens including human immunodeficiency virus (HIV).

While accurate, and an appropriate concern, this topic deserves to be unpacked and better examined to fully understand the implications of this risk among healthcare workers.

To start, a healthcare worker (HCW) is any healthcare personnel working in the medical or dental fields, or providing related services in an inpatient, outpatient, or community setting. Approximately 7% of the US labor force are health services employees (Bell 1997). These HCWs are employed in a great variety of workplaces which can include emergency departments, inpatient units, urgent care clinics, primary care settings, healthcare facilities in correctional settings, tuberculosis, STD, or substance abuse clinics, outpatient facilities, public health department clinics, skilled nursing facilities, community clinics, etc. There are also over 20,000 provider agencies that deliver home health care in the USA, with thousands of HCWs providing services to patients/clients in these home settings (Siegel et al. 2007).

Perhaps most important to consider is the distinction of which cases of HIV transmission constitute an “occupationally acquired infection?” The Centers for Disease Control and Prevention (CDC) defines an occupationally acquired HIV infection as a case in which “HIV seroconversion [is] temporally associated with an occupational exposure” (Bell 1997).

Possible routes for HIV transmission include blood, semen, pre-seminal fluid, vaginal secretions, rectal fluids (secretions, not feces), and breast milk. Notably, transmission of HIV is not possible via saliva, vomit, sweat, urine, or feces, though especially in healthcare settings, there exists the possibility that any of these materials could also contain HIV-infected blood. While most new HIV infections in the US occur via sexual contact or the sharing of injection needles, occupationally acquired HIV cases are those that occur through percutaneous or mucocutaneous (or both) routes. Percutaneous (through the skin) exposures most commonly occur via punctures from needlesticks or cuts from other sharp medical instruments (e.g., scalpel, glass, etc.). Mucocutaneous (pertaining to mucous membranes or skin tissue) exposures occur if blood or other transmissible bodily fluids come in contact

with the HCW's eyes, nose, mouth, or non-intact skin (CDC 2003).

Risk of HIV Transmission for HCWs

While HIV transmission is possible in healthcare settings, it's important to understand the reasons why occupational HIV transmission is very rare for HCWs.

There are several factors influencing a HCW's risk for occupational transmission, including: (1) the prevalence of HIV infection (in other words, the number of individuals with HIV) in the patient population, (2) the type of exposure(s), (3) the total number of exposures, (4) the amount of blood or other bodily fluid involved in the exposure, and (5) the amount of virus (i.e., viral load) in the infected individual's blood or other bodily fluid at the time of exposure (CDC 2003).

According to the CDC (2003), most occupational exposures (99.7%) do not lead to transmission of the virus. By 1997, US hospital-based HCWs experienced an estimated 500,000 percutaneous blood exposures each year, and of these exposures, about 5,000 involved HIV-infected blood (Bell 1997). Among HCWs with percutaneous exposure (i.e., needlestick or cut) to HIV-infected blood, the average risk of transmission has been estimated to be 0.3% (in other words, about 3 in 1,000), though there are higher risks with different types of percutaneous exposures (e.g., the deeper the needle enters, the more blood transferred, etc.) (CDC 2003, 2013). The average infection risk from mucocutaneous exposure (i.e., eye, nose, mouth, etc.) and exposure of non-intact skin (i.e., open wound, chapped skin, rash, lesions, etc.) to HIV-infected blood is estimated to be 0.09% (or about 1 in 1,000) (CDC 2003). Though it should still be prevented when possible, it's believed that the presence of a small amount of HIV-infected blood on intact skin poses no risk for infection (CDC 2003).

The reporting and documenting of occupationally acquired HIV cases began in 1985. To date, there have been 58 documented cases of US HCWs acquiring HIV through an occupational exposure, with only one confirmed case since

1999 (CDC 2011; CDC 2013; Joyce et al. 2015). There have also been 150 possible cases of occupationally acquired HIV infection in the USA (CDC 2011; CDC 2013; Joyce et al. 2015). These infections were possibly acquired via a workplace exposure, but due to lack of information/documentation regarding a specific exposure incident, these are not confirmed cases (CDC 2005).

Prevention Measures

Infection control experts strongly agree (and a preponderance of evidence strongly supports) that carefully adhering to recommended infection control procedures decreases infection transmission in healthcare occupational settings. As required by OSHA (2011) and recommended by the CDC, HCWs must follow the proper use of safety techniques to prevent exposure to HIV in health care settings (CDC 2003). This includes the proper use of protective practices, safety devices for sharp medical equipment, and personal protective equipment (PPE). PPE includes an array of barrier equipment to protect mucous membranes, skin, airways, and clothes from potential contact with any infectious agents (Siegel et al. 2007). HCWs are advised to act on the assumption that all blood and body fluids are potentially infectious and should follow the most up-to-date safety precautions at all times (CDC 2013).

Of HCWs with documented cases of occupationally acquired HIV, most transmission occurred via percutaneous exposures to HIV-infected blood, and a majority of those involved hollow-bore needles (Bell 1997; Joyce et al. 2015). Motivated in part by these findings, the prevention of percutaneous exposures "has always been an essential element of Universal and now Standard Precautions" (CDC 2003; Siegel et al. 2007). These precaution guidelines address measures that will prevent injury to the user (and others) when handling needles and other sharp objects. These include:

- The use of needles with hands-free recapping systems or other safety devices and injury

prevention equipment. The Needlestick Safety and Prevention Act became a federal law in 2000 and gave OSHA authorization to universally require the use of “safety-engineered sharp devices” in work settings (Siegel et al. 2007). This also requires that HCWs follow precautions by avoiding any recapping of used needles by hand.

- The use of sharp disposal containers for used needles or other sharp patient care equipment.
- The use of gloves. In the event of a needlestick or other puncture, the presence of a glove covering the skin may decrease the amount of blood on the outside (external) surface of the sharp object, though not the inside (lumen) of the needle; thus the amount of protection provided from gloves isn’t certain, but wearing gloves should still be advised when using/handling sharp devices (Siegel et al. 2007).

Guidelines for mucocutaneous protections have been mandated by the OSHA Blood-borne Pathogens Standard and require the use of proper protective barriers for PPE (such as gloves, goggles or other eye protection, face masks with a protective shield, and/or gowns) when anticipating potential contact with blood or other bodily fluids (OSHA 2011). Specifically:

- Gloves are to be used universally to protect the HCW’s hands from having direct contact with blood or bodily fluids, mucous membranes, non-intact skin, other potentially infectious materials (OPIM), or contaminated patient care equipment and surfaces; for nonsurgical patient care, one pair of gloves is adequate (Siegel et al. 2007).
- Isolation gowns and/or other protective apparel must be worn if there is any anticipation that the HCW’s arms and other exposed body parts could possibly have direct contact with blood or bodily fluids, mucous membranes, non-intact skin, OPIM, or contaminated patient care equipment and surfaces (Siegel et al. 2007; OSHA 2011). It should be noted that clinical/lab coats are not considered sufficient PPE protective apparel.

- Masks should be used to protect against sprays or smears of blood or other bodily fluids and may be used in conjunction with eye protection/goggles or a face shield for greater face protection. Personal eyeglasses, sunglasses, or contact lenses are not considered adequate eye protection (Siegel et al. 2007).

It’s also required that healthcare organizations “clean, repair, and replace” all necessary PPE as needed and keep these supplies up-to-date and available for HCWs at no cost to them (OSHA 2011). The CDC advises that all healthcare organizations and agencies create and sustain “an infrastructure to guide, support, and monitor adherence to Standard and Transmission-Based Precautions” (Siegel et al. 2007; CDC 2013). Incorporation of these precautions into their organization’s practice will not only help create an institutional safety culture aiming to keep HCWs and patients safer from the spread of infection, but will also ensure that each organization is working to achieve goals set by the Joint Commission on Accreditation of Healthcare Organization to decrease hospital-acquired infections (Siegel et al. 2007).

Indications for Exposure Occurrence

In the event of an exposure, or a suspected exposure, to a patient’s blood or other bodily fluid(s) capable of transmitting HIV, all HCWs are advised to immediately (1) perform first aid and cleaning to the exposure site, and (2) report the incident to appropriate management personnel. For the purposes of cleaning the exposure site:

- If percutaneous exposure:
 - Wash the exposure area (i.e., site of needlestick or cut) with soap and water.
 - Avoid using a caustic or corrosive agent (e.g., bleach) at exposure site.
- If mucocutaneous exposure:
 - Perform water flushes to the affected mucous membrane (i.e., nose, mouth).
 - Provide irrigation to eyes with saline, clean water, or other sterile irrigants.

-Wash any contaminated skin (intact or non-intact) with soap and water.

For the purposes of reporting and managing the exposure case, healthcare settings and employers are required to have a system in place for HCWs to immediately report possible exposures. Usually HCWs will be encouraged to first report the incident to their manager or supervisor, then to occupational/employee health or infection control personnel, who are then charged with managing the HCW's exposure case (CDC 2003). It is the responsibility of the employer to ensure that as soon after the exposure as possible the HCW (1) is evaluated; (2) receives education about and, if needed, immediate access to postexposure prophylaxis (PEP) medications; (3) is monitored for signs of illness and/or potential PEP side effects; (4) receives appropriate testing to determine if HIV transmission occurred; and (5) is provided ongoing education, support, and/or counseling regarding the situation (CDC 2003). If it is not possible to identify, locate, or provide testing to know the source patient's HIV status, the exposure risk should still be weighed carefully, and employers must immediately follow postexposure procedures and make suitable testing available for the HCW (CDC 2003; Kuhar et al. 2013).

At present day, there exists no vaccine or cure for infection with HIV. However, certain antiretroviral (ARV) drugs have proven effective in reducing the likelihood of HIV infection if taken within a specified time frame after exposure to the virus. The administration of these PEP medications has become the recommended emergency response to certain occupational (and other) exposures that are deemed a risk for HIV transmission. After assessing the exposure risk, if PEP is indicated the HCW must be educated regarding and prescribed the appropriate prophylaxis treatment regimen, ideally starting the medications as soon as possible (but no more than 72 h) after the exposure incident. As of 2013, the US Public Health Service (PHS) recommends a 4-week (28-day) course of three (or more) ARV drugs if the circumstances of the exposure were such that it poses a risk for HIV infection (Kuhar et al. 2013). The PHS no longer recommends choosing

PEP regimens based on severity of exposure, but rather routinely prescribing a 3-ARV drug course for all occupational HIV exposures (Kuhar et al. 2013). Notably, ARV medications are expensive, but if the HCW was exposed in the course of their work, their employer's health insurance or workers' compensation will typically pay for the medications and care management.

There exists several combination options for the three-drug PEP regimens, and these drugs come from six classes of ARVs: nucleoside and nucleotide reverse-transcriptase inhibitors (NRTIs), non-nucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), a fusion inhibitor (FI), an integrase strand transfer inhibitor (INSTI), and a chemokine receptor 5 (CCR5) antagonist (Bartlett et al. 2009; Kuhar et al. 2013). For most PEP regimens, the PHS currently recommends a fixed-dose combination pill of tenofovir (TDF) and emtricitabine (FTC) (both NRTIs, which when combined is known as Truvada), plus the INSTI raltegravir (RAL) (Bartlett et al. 2009; Kuhar et al. 2013). The PHS PEP recommendations may be modified on an as-needed basis, depending on the specifics of each exposure case, and/or the HCW's response to the medications prescribed (CDC 2003, 2013). Ideally, a medical provider with expertise in HIV and/or ARVs (e.g., an infectious disease specialist) should be consulted for proper and effective PEP regimen management (CDC 2003; Kuhar et al. 2013).

It's worth reiterating that occupational exposure to HIV should be considered a medical emergency, and if indicated, PEP ought to be started as soon as possible, ideally within only hours of the exposure. PEP is most effective when the recommended 28-day treatment course is adhered to, and the exposed HCW should notify their managing provider immediately if they need to stop/change their regimen for any reason (Roland 2005; CDC 2013). Though the optimal time interval in humans has not been well studied related to ethical concerns and small sample populations, animal studies have suggested that PEP treatment becomes less effective with the more time that elapses from the exposure (CDC 2003). Even when taken as advised, PEP is still not 100% effective (Gerberding 2003; Roland 2005).

Worldwide there are 21 known cases in which HCWs became HIV-infected despite timely PEP treatment (Gerberding 2003). Of the 58 confirmed US cases of occupationally acquired HIV infections, in six of these cases, the HCWs had started the recommended PEP regimen within 2 h of being exposed (Bartlett et al. 2009).

Unfortunately, all ARV medications used for HIV treatment and PEP are associated with some type of potential side effects, the most commonly experienced being nausea, vomiting, diarrhea, fatigue, and headache (CDC 2003; Gerberding 2003). The most serious side effects reported from PEP treatment include kidney stones, hepatitis (inflammation of the liver), and decreased blood cell production (CDC 2003). According to CDC surveillance data, almost 50% of exposed HCWs who received PEP treatment reported adverse side effects (Gerberding 2003). It's imperative that the managing physician discusses with the exposed HCW the risks, benefits, and potential side effects of PEP prior to starting the medications and, if needed, addresses management for any other medications being taken. The exposed HCW should receive lab testing (including complete blood count, renal, and hepatic function testing) at baseline and 2 weeks after starting PEP to monitor for drug toxicity and other possible adverse side effects (CDC 2003; Gerberding 2003). And perhaps most importantly, the HCW should also be closely monitored for any possible signs or symptoms of HIV infection, such as sudden or severe flu-like illness including fever, rash, aches, etc. The HCW should be encouraged to contact the managing provider immediately with any questions or concerns.

Baseline HIV antibody testing should occur as soon as possible after the occupational exposure, and then follow-up testing should occur periodically for a minimum of 6 months following the date of exposure to detect and ideally rule out HIV infection. As their status will be unknown during this follow-up period, to prevent possible further HIV transmission, the HCW should follow considerable precautions, including refraining from donating blood, semen, or organs, engaging in sexual intercourse, and if lactating, women should consider alternative infant feeding methods (CDC 2003).

It should be noted that PEP is not indicated if blood or bodily fluid contact was only to HCW's intact skin, or if the source patient is found to be HIV-negative (Gerberding 2003). If PEP was started in such instances, it may be safely discontinued after consulting with the managing provider. In all circumstances, if the occupational exposure is not deemed a risk for HIV infection, PEP should not be taken, as serious side effects can result, and risk for these should also be avoided when possible (CDC 2003).

Dispelling Myths

While there is some risk of infection from their work practices, the vast majority of HIV infections contracted by individuals who work as HCWs are in fact not related to their occupation. Most HCWs who contracted the HIV infection in the 1980s and 1990s did so via sexual intercourse, not from occupational exposures (CDC 2003). According to CDC data from 1995, of adults with AIDS who had worked as HCWs, 94% reported that they had acquired HIV via risks not related to their occupational setting, and the other 6% reported having "undetermined risk" (Bell 1997).

Though the majority of this chapter has been devoted to discussing the rarity of HIV transmission from patients to HCWs, it's worth noting that there are cases, however even more rare, in which HCWs with HIV have infected their patients. These more anecdotal cases of HIV transmission, whether intentional or inadvertent, deserve to be better understood as well.

The first documented case in the US in which a HCW spread the HIV infection to his patients was that of Dr. David J. Acer (Altman 1993). Dr. Acer was a Floridian dentist who had contracted HIV in 1986 (via sexual intercourse), and between then and his death from AIDS in 1990, he continued to practice dentistry. Though a great deal of unknowns surround the circumstances of the infections, it's been confirmed that within those 4 years Dr. Acer infected six of his patients with HIV, four women and two men, ranging in age from 15 to 65. Dr. Acer denied ever intending to

hurt his patients, but law enforcement and public health surveillance officials still remain uncertain if these were accidental or deliberate cases of transmission (Ciesielski et al. 1994).

A significant outbreak of HIV infections was discovered in 1989 among Romanian infants and young children who were cared for in various orphanages and pediatric hospitals. It's now believed that these infections occurred inadvertently as a result of policies and practices that encouraged injection transfusions of small volumes of unscreened blood to malnourished and anemic children, as well as inadequate sterilization procedures and reuse of injection syringes at these underfunded and understaffed facilities (Dente and Hess 2006). By 2002, there were 12,559 documented cases of HIV infection in Romania, of which 9,936 were among Romanian children, which at the time equated to 60% of Europe's pediatric HIV/AIDS cases (Dente and Hess 2006). Though initially slow to recognize and respond to the outbreak, the Romanian government eventually sprung to action and by 2003 was the first Eastern European country to provide free universal ARV coverage to all persons infected with HIV (Dente and Hess 2006).

From 1997 to 1998, more than 400 Libyan children were infected with HIV while being treated at Al-Fateh Hospital, Benghazi's public pediatric hospital. Community and international outrage fueled Libyan government and prosecutors to charge five Bulgarian nurses and one Palestinian doctor who worked at the hospital with intentionally infecting the children, over 60 of whom have died to date. However, experts who investigated the outbreak have reported the HIV strains involved in the outbreak were already present in the hospital prior to the arrival of the six foreign HCWs, and the likely cause of the massive spread of HIV infection was poor hygienic standards and inappropriate sterilization of medical equipment (Ahmad 2004; De Oliveira et al. 2006). Though initially convicted and sentenced to death, the six HCWs, themselves not HIV-positive, were extradited to Bulgaria in 2007 and released.

As recently as December 2014, a man named Yem Chrin, a local medical practitioner in Roka,

Cambodia, had been charged with causing an HIV outbreak among more than 100 community members, both adults and children. Chrin, who was charged by a provincial court with (1) spreading HIV, (2) cruel murder, and (3) violating medical ethics, admitted to reusing syringes between patients (Al Jazeera 2014).

Despite these extremely rare examples of transmission based on either substandard care or harmful practices, the majority of HIV-infected HCWs are eager and capable of continuing to provide safe and effective care to their patients. In the US, the 2010 SHEA guidelines recommend that HIV-positive HCWs should "not be prohibited from participating in patient-care activities solely on the basis of their HIV infection" (Henderson et al. 2010). The SHEA guidelines state that the HCW should be freely permitted to participate in/perform procedures in which HIV transmission is either extremely miniscule or "theoretically possible but unlikely" (Henderson et al. 2010). To perform procedures that do present a "definite risk" of transmission, the HCW should have their clinical status and lab data reviewed every 6 months by a managing physician, have a reduced viral load, and seek prior approval from an expert review panel (Henderson et al. 2010). The guidelines also state that if the HCW is compliant with all other SHEA guidelines, they should not be required to disclose their HIV-infection status to patients as part of the informed consent process prior to performing procedures, though there is nothing in the guidelines that prevents them from doing so (Henderson et al. 2010).

Exposure Management Resources

In the US, it's required that employers report cases of suspected (and confirmed) occupationally acquired HIV infection to the local and/or state health department, who in turn are required to report the cases to the CDC (Bell 1997). Still, it can be difficult to track all such cases since accurate surveillance relies on voluntary reporting to health department and CDC officials (Bell 1997; CDC 2013). As summarized by Ippolito et al., "surveillance and research programmes of

occupational exposures are needed to monitor the risk of exposure to and transmission of bloodborne pathogens, the efficacy and tolerability of post exposure treatments, and the effectiveness of safety devices and safety practices” (Ippolito et al. 1999). Though today there exists a far greater body of knowledge regarding occupational exposures, including best prevention and treatment practices, this statement rings as true as it did then, and active surveillance efforts must continue in earnest to continue monitoring HCWs’ risks.

For reporting suspected or confirmed cases of occupationally acquired HIV and/or possible PEP failure:

1. Report to state health department HIV surveillance personnel.
2. Report to CDC Coordinator for “Cases of Public Health Importance”: 404-639-0934 or 404-639-2050 (<http://www.cdc.gov/hiv/risk/other/occupational.html>).

For questions regarding possible or confirmed occupational exposure to HIV:

- Clinical Consultation Center (CCC) Post-Exposure (PEP) Consultation: 888-448-4911 (<http://nccc.ucsf.edu/clinician-consultation/post-exposure-prophylaxis-pep/>)
- Division of Healthcare Quality Promotion: 800-893-0485 (www.cdc.gov/ncidod/hip)
- National Institute of Occupational Safety & Health: 800-356-4674 (www.cdc.gov/niosh)
- National AIDS Hotline: 1-800-342-2437

Conclusion

Though this chapter has focused mostly on US HCWs and their risk, the international community is increasingly collaborating to better protect all HCWs against workplace HIV transmission. Addressing the issues of occupational safety at a global scale, the International Labor Organization (ILO) is a special division of the United Nations (UN) that focuses on HIV/AIDS in workplaces around the world (ILO 2015). The ILO acts to

leverage support and mobilize action by international governments, Ministries of Labor, employers, labor organizations, and employees in efforts to protect workers’ rights, combat discriminatory practices and/or policies against HIV-infected employees, and improve occupational safety and health (ILO 2015). Such collaborative efforts by the international community will ideally foster greater support for and commitment to evidence-based safety precautions in global healthcare occupational settings, which can ultimately reduce risk for HIV transmission in the workplace.

In conclusion, while risk of HIV infection from occupational exposure is low, and lowered further by proper adherence to an appropriate PEP regimen, it’s impossible to guarantee that an exposed HCW will not become infected with HIV, which only highlights the importance of ensuring that all HCWs follow the recommended safety precautions to prevent exposure (Siegel et al. 2007).

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Healthcare Workers, Shortage and Task Shifting of

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Definition

A robust health workforce, one that is capable of providing effective, efficient, and high-quality services, is a vital component of health system functioning and plays a critical role in addressing the global HIV epidemic. Extensive challenges persist in the recruitment, distribution, and retention of healthcare workers. Current figures estimate a worldwide shortage of nearly 7.2 million physicians, nurses, and midwives, a scarcity most acute in sub-Saharan Africa, which already has some of the world's weakest health systems. Brain drain, or the migration of healthcare workers away from low-resource settings, is one of the many complex contributing factors to healthcare worker shortages. Brain drain can be internal or external to national health systems; both types must be addressed in order to improve the availability, accessibility, acceptability, and quality of the health workforce.

Task shifting is an approach to rapidly scale-up the overall health workforce and mitigate the effects of brain drain by systematically delegating responsibilities from highly qualified health workers to those who are less qualified. There are two basic forms of task shifting – one that reassigns responsibilities from highly skilled to less skilled workers, such as the WHO-promoted method in which trained nonphysician clinicians, midwives, and nurses are responsible for conducting HIV testing, prescribing and/or dispensing ART, and clinical monitoring and side effects management for patients who are positive for HIV. The second form of task shifting moves tasks from generalized workers to those who have been narrowly trained to fulfill particular duties, as is done when responsibilities are standardized

and delegated from healthcare professionals (e.g., nurses) to a new cadre of lay workers (e.g., community health workers). The efforts of such community health workers, especially those living with HIV who are successfully managing their condition, have demonstrated an increased uptake of HIV services, more timely diagnoses, enhanced adherence to treatment, and reduced loss to follow-up. Task shifting is an essential component of the WHO's coordinated strategy, called "Treat, Train, Retain," for addressing specific challenges related to shortages in the health workforce.

A robust health workforce, one that is capable of providing effective, efficient, and high-quality services, is a vital component of health system functioning. Health workers, the individuals "whose primary intent is to enhance health," are responsible for protecting and improving the lives and well-being of members of their communities through preventive, promotional, or curative health services (World health report 2006b). Traditional examples of human resources for health include nurses, midwives, pharmacists, physicians, and dentists, but auxiliary staff, such as community health workers, practitioners of traditional medicine, technicians, and other paraprofessional personnel, are just as important in the achievement and sustainment of local, national, and global health-related goals. Global health differs from general community health in that it emphasizes difficult to reach populations over wider geographic areas and prioritizes health equity for people worldwide.

Health workers play a seminal role in addressing the healthcare needs of a population, yet extensive challenges persist in their recruitment, distribution, and retention. Recent modeling formulas suggest a higher deficit in the global health workforce than previously estimated. Worldwide, 100 countries fall below the minimum density of 34.5 skilled health professionals/10,000 persons, a threshold delineated in the context of establishing universal health access. Amounting to a current estimated shortage of nearly 7.2 million physicians, nurses, and midwives, this scarcity is expected to surge to 12.9 million health workers by 2035. In most nations, the rate at which new

workers are trained and added to the workforce is not commensurate with the accelerating demands of growing populations (A universal truth: no health without a workforce 2014). If not immediately addressed, this issue will lead to serious consequences for the health of billions of people across the globe.

These workforce shortages constitute a public health emergency, and the extent of the problem is grossly imbalanced across the globe; in many places, the dire situation demands immediate action. Countries with the lowest relative need and the strongest health systems have the highest numbers of health workers, while, conversely, those with the greatest burden of disease and the most fragile systems must cope with significantly fewer personnel. For example, the WHO Region of the Americas contains only 10% of the global burden of disease, yet employs approximately 37% of the world's health workforce; in contrast, the African Region bears more than 24% of the global burden of disease but has access to only 3% of health workers (World health report 2006b). Even within national borders, the availability of healthcare workers typically exhibits considerable geographic variation, with significantly better availability within cities and urban centers.

Although parts of Asia have the greatest projected deficits over the next two decades in absolute terms, worker shortages are expected to be most acute in sub-Saharan Africa, which already has some of the world's weakest health systems. Current training facilities are insufficient to meet the growing needs, as there are a mere 168 medical schools in the 47 countries that comprise sub-Saharan Africa. Of those countries, 11 have no medical school at all, and 24 have only one medical school (Mullan et al. 2011).

These disparities between the supply and demand of healthcare workers have led to crippling effects on poorer countries' prospects for development. Efforts to achieve the health objectives of the Sustainable Development Goals set by the United Nations in 2015 have been thwarted due to the lack of healthcare staff in many countries. As a result, human resources for health have received significant attention in the global health policy realm in recent years.

The causes of this health worker crisis are multidimensional. In addition to unprecedented world population growth, an ageing workforce, whereby staff retire without being replaced, is occurring alongside an inadequate number of young people newly entering health professions. The workforce shortage, particularly in low- and middle-income countries, is a major barrier to the prevention and treatment of HIV. These workers comprise the frontline efforts to combat this disease, and there is a direct correlation between a greater number of health providers and higher rates of access to antiretroviral therapy (ART) (World health report 2006b). At the end of 2015, 36.7 million people worldwide were living with HIV. Globally, only 18.2 million of these people were accessing in ART treatment, which is key to extending life expectancy, preventing and reducing morbidity, reducing transmission, and halting AIDS-related deaths. The WHO's recommendation in late 2015 was revised to a "test and treat" strategy, whereby everyone with HIV should be offered ART as soon as they are diagnosed. (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2013). This increased eligibility, coupled with the fact that people with the virus are living longer as the disease transitions to one that can be successfully managed on a long-term basis, makes the demand on healthcare workers even more prominent. A strong health workforce is critical to attaining, sustaining, and accelerating progress toward universal access to ART for the half of eligible persons, or roughly 18.5 million people, currently lacking access to this lifesaving treatment (UNAIDS 2016).

Brain Drain

While the causes of the ongoing health worker scarcity are multifaceted, the perpetual migration of trained health workers, colloquially known as "brain drain," is widely recognized as a primary contributory factor. Brain drain occurs both when members of the workforce in resource scarce countries migrate to wealthier nations, and when workers leave the public health sector for other

jobs within domestic borders. Heavy workloads stemming from an already limited workforce and high disease burdens mean losses due to brain drain are particularly costly to health systems. Not only does the continuous efflux of health workers negatively affect continuity of care, but it also increases the potential for further turnover of existing employees who suffer from stress and burnout as they take on additional workloads.

External Brain Drain

The migration of healthcare workers from low-resource settings with immense need ("source" countries) to resource-abundant locations with better living and working conditions ("destination" countries) is the consequence of a complex set of decisions and contextual factors. Since the era of modern globalization, many poor nations have been losing up to half of their newly trained health workforce to the rich world on an annual basis. Such external brain drain, or out-migration, has precarious consequences on the national health systems of poor nations. When developing nations pay to educate health workers who in turn decide to temporarily or permanently settle abroad, the poorer countries are, in essence, subsidizing the health systems of wealthier nations (Kuehn 2007). The International Organization for Migration (IOM) estimates that low-income countries spend US \$500 million per year to train healthcare workers who eventually migrate to high-income countries. Moreover, these poor countries' already limited supply of skilled labor, often considered the brightest of the bunch, is even further reduced (Lopes 2008).

Internal Brain Drain

Internal brain drain, or the migration of workers away from the public health sector and toward opportunities within private institutions or non-government organizations, further intensifies these workforce imbalances. Additionally, the maldistribution of the health workforce, characterized by urban concentrations and rural deficits, is an inherent challenge in many countries, irrespective of their level of economic development. In most nations, health workers move from lower resource, rural settings to higher resourced,

urban areas at a faster rate than the general population, leaving these locations understaffed in comparison to wealthier regions and cities. About half of the global population lives in rural and remote areas, yet they are served by less than one quarter of the world's doctors and fewer than one third of the world's nurses (A universal truth: no health without a workforce 2014).

Push and Pull Factors in Brain Drain

At face value, it may seem that workers are simply moving toward jobs with better pay. While issues surrounding employee remuneration and compensation are critical, many studies have shown that financial compensation alone does not explain the brain drain predicament. Both "external" and "internal" brain drain phenomena are the result of a multitude of complex, interrelated "push" factors encouraging workers to leave. Low-resource health systems frequently have poor working conditions, including unrealistic or unsafe workloads, deteriorating physical environments, and inadequate or faulty supplies and equipment. Issues surrounding human resource management, including insufficient opportunities for professional development or continuing education, limited prospects for career advancement, and weak performance monitoring are also important contributory factors. Many of the countries with the most fragile health systems and extensive brain drain are those with the highest levels of social tension, gender discrimination, and political instability, creating a climate which further pushes health workers away from employment within the public sector.

Simultaneously, numerous "pull" factors in wealthier nations entice workers to migrate there. Such factors in developed countries include growing ageing populations and an increasingly technology-driven healthcare system, which creates a high demand for employees. Poor planning and underinvestment in health worker education has left many developed nations with too few domestic workers to meet this demand, causing them to rely on immigrants, many of whom migrate from developing nations (Kuehn 2007).

The dynamic interplay of such "push" and "pull" forces that perpetuate the asymmetric shifts

in skilled human resources is a central part of the discourse surrounding health workforce development (Tankwanchi et al. 2013, 2014, 2015). The human resource crisis stemming from these forces must be approached holistically, in which political, technical, and economic aspects of the problem are taken into account. This process is political in that it requires deliberate coordination on the part of various sectors and constituencies in society at different levels of government; it is technical in that it necessitates expertise in human resource planning, education, and management; and it is economic in that it entails substantial investments in workers and institutions. Meanwhile, it is important that any strategies confronting these arenas maintain a delicate balancing act whereby healthcare workers maintain their individual rights to migrate. Plans to attract, retain, and motivate healthcare workers must address both financial and nonfinancial incentives, and governments must increase the number of healthcare training institutions (Sales et al. 2013).

The health worker shortage has too often been addressed through fragmented initiatives or temporary, simplistic solutions. As this problem is projected to grow significantly in the coming decades, it is imperative for governments to do away with piecemeal efforts and take immediate action toward long-term, comprehensive workforce planning. Such steps must align with overarching national development efforts. Consequently, it is essential to rethink traditional models of education, structure, deployment, and remuneration for the health workforce.

Task Shifting as a Response to the Global Shortage of Workers

The roots of the health workforce crisis are multifaceted; therefore, one solution alone cannot singlehandedly resolve this challenge in all of its complexities. Efforts to globally increase the number of physicians, nurses, midwives, pharmacists, and technicians are imperative, but such recruitment and training requires considerable time. Training takes at least 6 years for doctors, 3–4 years for nurses, and 4 years for midwives. Waiting for enough new workers to enter public

health systems through conventional education streams will result in lengthy delays in the provision of urgently needed services, a situation which can devastate the response to current and emerging crises such as the HIV epidemic. Instead, the roles of healthcare workers need to be reexamined, reassigned, and decentralized.

The conversation surrounding global human resources for health is shifting from a focus exclusively on the *number* of health workers to more explicitly considering their availability, accessibility, acceptability, and quality. *Availability* means having a sufficient supply of workers with skills to match a community's healthcare needs, whereas *accessibility* indicates members of a population, including those living in remote and underserved areas, have equitable access to workers. *Acceptability* is the ability of the workforce to create a sense of trust and treat everyone with dignity, while *quality* refers to the skills, knowledge, and behavior of health personnel, evaluated in the context of professional norms and perceptions from the recipients of health services (A universal truth: no health without a workforce 2014).

When implemented as a component of overall community development and poverty alleviation strategies, task shifting is a viable method capable of enhancing the availability, accessibility, acceptability, and quality of the healthcare workforce. Task shifting is an approach in which certain responsibilities are systematically delegated, where rational and appropriate, from highly qualified health workers to those who are less qualified. The task shifting strategy aims to redesign roles in a way that aligns to local health needs while creating a more streamlined chain of care, relieving pressure on individual workers, and rapidly scaling up the overall health workforce.

Historic Examples of Task Shifting

Though only a recently coined term, task shifting is not a new phenomenon but has been practiced for centuries with varying levels of formality. In France, the medical credentialing system was abolished in the late nineteenth century during

the French Revolution, and the absence of teaching and licensing institutions left the medical system in a state of chaos. Second-tier doctors known as *Officiers de Sante* served to increase the country's medical manpower, eventually becoming a formally recognized cadre of non-physician healthcare workers.

In mid-twentieth century China, peasants were selected for an intensive 3- to 6-month course in medical training to bring healthcare to rural areas of the country. Known as "barefoot doctors," these workers provided basic services to underserved populations, including immunizations, family planning, first aid, preventative care, and health education. The barefoot doctors were widely utilized across the country and eventually incorporated into the national health system.

Overview of Task Shifting

In healthcare, task shifting has similar principles to the lean model used in industry and production, where the goal of any process is to use less to do more. By minimizing waste (in this case, the time of highly skilled workers), lean thinking standardizes tasks and processes in ways that maximize the duties each worker performs at his or her highest skill level. The goal of this practice is to redistribute and decentralize the health workforce to more efficiently utilize available human capital while rapidly expanding the overall talent pool to cope with growing patient loads. There are two basic forms of task shifting – one that reassigns responsibilities from highly skilled to less skilled workers, and one that moves tasks from generalized workers to those with narrow but specialized training.

The first form of task shifting involves delegating responsibilities from workers with higher levels of education and skill to those in lower cadres within the existing health workforce, where workers have fewer qualifications. This model is predicated upon the notion that experts with highly specialized abilities are too scarce to waste on jobs others can do, including primary care, general management, and administrative duties. Instead, roles are redefined in such a way

that experts spend more time on tasks only they can perform. For example, since doctors are in such short supply, WHO promotes a method in which trained non-physician clinicians, midwives, and nurses are responsible for HIV testing, ART prescribing and/or dispensing, and clinical monitoring and side effects management (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2013). Only complicated cases are referred to specialists or senior clinicians, and, as a result, these physicians are able to dedicate more time to the management of a greater number of complex cases across a wider geographic region.

The second type of task shifting occurs when specific duties are transferred from workers with generalized medical training to those who have been narrowly trained for a particular task. This model most commonly occurs when explicit responsibilities are standardized and delegated from health professionals (e.g., nurses) to a new cadre of lay workers (e.g., community health workers). In rural areas where scarcities of doctors and nurses are particularly severe, many countries successfully utilize a lay health worker strategy. These lay health workers must perform their duties according to strict protocols, as they lack the broad education, experience, and professional judgment required to make competent decisions when complications arise or protocol deviations occur. Levels of community health worker responsibility vary considerably, but WHO guidelines support a model whereby trained and supervised community health workers care for patients already initiated in ART by preliminarily assessing for new signs or symptoms, providing adherence monitoring and support, and dispensing medication between regular clinical visits (Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection 2013). Depending on the competencies required, it takes between 1 week and 1 year to train a community health worker in contrast to the 3–4 years needed to obtain a nursing certification. These workers are typically recruited from local communities and are often HIV-positive themselves.

Community members, especially people living with HIV who are successfully managing their

condition, have the potential to be an important resource in the delivery of ART, yet are often underutilized. Community health workers are able to improve the accessibility of HIV services, particularly across remote and underserved regions. Numerous studies have confirmed that appropriately trained and supervised staff of lower-level cadres are not only capable of carrying out shifted tasks but may even perform certain duties better than physicians (Penazzato et al. 2014). In addition to providing more manpower for the workforce and creating employment opportunities, the community health worker model is advantageous in that it can build important bridges between health facilities and surrounding populations. These health workers can be especially supportive in reducing HIV-related stigma and providing support for ART adherence within defined locations. Care that is localized, decentralized, and delivered by community-based workers has been shown to bring positive health benefits, including an increased uptake of services, more timely detection, enhanced adherence to treatment, and reduced loss to follow-up. For example, community members called adherence counselors have helped many remote clinics outperform hospitals in the retention of patients in ART treatment, a result attributed to the close relationship between the adherence counselors and the patients at the village level (Kredo 2014). The acceptability of HIV services is improved when the profile of the health workforce – its age and sex composition, language, skill mix, and level of cultural awareness – meets the expectations of its recipients, which is more likely when workers and patients are from the same communities.

Current Status of Task Shifting

In May 2006, WHO launched a coordinated global plan addressing specific challenges related to shortages in the health workforce and its consequences in delivering HIV care and treatment. This strategy, called *treat, train, retain* (TTR), explores both the causes and effects of health worker shortages, and delineates a three part solution within the context of HIV – first, increase the

delivery of HIV treatment, prevention, care, and supportive services for health workers infected or affected by the epidemic (treat); second, empower workers to deliver comprehensive and universal access to HIV services (train); and third, retain clinical and public health employees through improved working conditions, financial compensation, and other incentives in effort to reduce migration and mitigate brain drain (retain) (Taking stock: health worker shortages and the response to AIDS 2006a).

Embedded within the “train” step of the TTR framework, task shifting is an essential component of the strategy to expand HIV services while providing an important new opportunity for strengthening human resources for health more generally. Under the umbrella of the TTR model, WHO, UNAIDS, and PEPFAR jointly released 22 recommendations for task shifting in HIV care in 2008 (Task shifting: global recommendations and guidelines 2008). These important guidelines promote a formalized framework that supports task shifting under the auspices of national priorities for holistically organizing and reinforcing the health workforce. As such, TTR is considered a strategic approach to health worker mobilization, rather than a program, meaning it requires substantial in-country leadership, ownership, and direction in order to avoid parallel systems and duplicated efforts (Task shifting to tackle health worker shortages 2007).

It is important for countries to professionalize the task shifting process and formally recognize new cadres of the health workforce. Task shifting is most successful when employees have clearly defined roles and responsibilities, which requires standardized training and examination criteria. Training culminating with formal credentialing further legitimizes this process. As such, the certification of previously nonprofessional cadres (for example, volunteer community health workers), should be prioritized, with salaries commensurate to their added responsibilities. As these employment levels are legitimized, opportunities for continuing education should be available to staff and occur at an appropriate frequency.

Additionally, as responsibilities are downshifted, employees must have clearly delineated

avenues for patient referral. It is essential to have adequate mentoring and supervision so employees do not step outside of their roles, which should involve implementing employee performance monitoring and evaluation systems.

Conclusion

Reaching 18.5 million people with universal access to HIV care and treatment depends on effective and efficient health systems capable of delivering services at a scale much larger than that at which they are currently operating. There is no denying that the absolute number of healthcare workers must grow, and coordinated efforts to increase the quantity and quality of health training institutions in the developing world, as well as the retention of workers who graduate from them, are imperative. Task shifting is a process which empowers those in the health workforce by utilizing them more efficiently, thereby promoting teamwork, improving worker morale, reducing burnout, and increasing employment retention. When coupled with national strategies for health system strengthening and poverty reduction, task shifting is a viable option for responding to crippling health worker shortages and ensuring there is more human capital available to address the health needs of people living with HIV.

Cross-References

- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Clinical Ethics in HIV/AIDS Prevention, Care, and Research](#)
- ▶ [HIV Counseling and Testing, Prevention of HIV](#)
- ▶ [HIV Testing and Counseling](#)

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Hepatitis C Virus Infection and HIV

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Definition

Hepatitis C virus (HCV) infection in HIV-infected patients is frequent since both viruses share common routes of transmission. HIV/HCV coinfection is associated with higher HCV viral loads and accelerated liver fibrosis progression particularly in those with low CD4 T cell counts, compared to HCV infection alone. While AIDS-related mortality dramatically decreased since the introduction of antiretroviral therapies (ART), there is an increasing impact of chronic viral hepatitis on hospital admissions and mortality among HIV-infected patients. Hepatic decompensations and HCCs are among the most common causes of death in HIV/HCV-coinfected patients. However, the recent major advances in HCV treatments have the potential to substantially reduce HCV-related morbidity and mortality among HIV-infected patients.

Hepatitis C in HIV-Infected Patients: A Frequent and Complex Coinfection

It is estimated that worldwide two to three million HIV-infected patients are coinfecting with HCV (Rockstroh 2015). The clinical manifestations of this coinfection are extremely diverse and range from asymptomatic HCV infection to life-threatening complications including liver cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) ► [Hepatocellular Carcinoma in HIV-Positive Patients](#). Moreover, HCV infections cause a wide range of extrahepatic complications which include highly unspecific

Hepatitis C Virus Infection and HIV, Table 1 Characteristics of three exemplary HIV/HCV-coinfected patients

Characteristic	Patient A	Patient B	Patient C
Sex	Male	Male	Female
Age	45	55	35
Mode of HCV transmission	IDU (currently in opioid substitution program)	MSM	IDU (short period 5 years ago)
HCV genotype	1	3	4
Time since HCV transmission (years)	15	2	5
Liver stage (Metavir scale)	F4 (child B)	F2	F0
Symptoms/complications	Jaundice, esophageal varices, thrombopenia	Severe fatigue	None
CD4 count (/μl)	150	550	450
Antiretroviral treatment	Tenofovir, zidovudine, lamivudine, darunavir/ritonavir	None	Tenofovir, emtricitabine, efavirenz
Further problems and comorbidities	Alcohol abuse	None	Planned pregnancy

Abbreviations: *IDU* intravenous drug use, *MSM* men who have sex with men

symptoms such as chronic fatigue or typical HCV-related diseases such as mixed cryoglobulinemia (MC) vasculitis. In this overview, key aspects of screening, diagnosis, and treatment of HIV/HCV-coinfected patients will be reviewed by discussing the clinical management of three exemplary patients (Table 1).

Epidemiology and Screening

The three patients described in Table 1 are very different with regard to their social situation, risk behavior, and mode of HCV transmission. For many decades, HCV infections occurred almost exclusively in people who inject drugs (PWID) or in hemophiliacs. Most PWID acquired HCV before HIV, and, accordingly, up to 90% of HIV-infected PWID are coinfecting with HCV (Rauch et al. 2005). In this context, it is not surprising to diagnose HCV coinfections in HIV-infected PWID (**patients A and C**). In countries with effective drug substitution and harm reduction programs, there was a marked decline in HCV incidence in recent years. However, in other areas including Eastern Europe and Southeast Asia, HIV/HCV coinfection incidence is increasing and affects up to 50% of PWID.

Screening for HCV is recommended in all HIV-infected patients at the time of HIV diagnosis. In case of HCV seropositivity, an HCV RNA has to be ordered and the genotype determined. The status of liver damage has to be assessed including staging of liver fibrosis. In recent years, most clinicians preferred noninvasive tests such as transient elastography (FibroScan) or serum biomarkers to assess the stage of liver fibrosis. The APRI and FIB-4 scores are commonly used noninvasive tests of liver fibrosis. These scores have been well validated including in HIV/HCV coinfection and can be calculated in almost all patients based on age, transaminases, and platelet levels. Liver biopsies are ordered if noninvasive assessments do not provide conclusive results or to exclude other liver pathologies. More detailed recommendations for the care of HIV/HCV-coinfected patients are available from the European AIDS Clinical Society (EACS) guidelines (www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html).

Patient A suffers from liver cirrhosis. His 5-year risk of hepatocellular carcinoma (HCC) is about 10% (Hill 2015). Because early detection of HCC improves survival, this patient should be screened 6-monthly with liver ultrasound for HCC ► [Hepatocellular Carcinoma in HIV-Positive](#)

Patients. In addition, the patient needs regular surveillance and treatment for esophageal varices, hepatorenal syndrome, and encephalopathy. Furthermore, the patient needs support to reduce his alcohol consumption. Before starting HCV treatment, zidovudine should be substituted with another antiretroviral drug due to the increased risk of severe anemia when combined with ribavirin (RBV) (see below for HCV treatment options).

Patient B is an MSM with a recent HCV transmission. Incident HCV infections were very rare until 12 years ago. However, in the last decade, there has been a new epidemic of incident HCV infections among men who have sex with men (MSM). Unprotected anal sex, use of sex toys, and recreational drugs have been associated with incident HCV infections among MSM. The “classic” HCV epidemic among PWID (**patients A and C**) differs in many ways from the “new” epidemic among MSM (**patient B**). In the PWID epidemic, nearly all HCV infections occurred before HIV infection. In contrast, in the MSM epidemic, HCV infections occur in a host with at least some degree of HIV-mediated immune deficiency. This might partially explain the relatively low (approximately 20%) spontaneous clearance rates after incident HCV infections in HIV-infected patients. Another marked difference between the two epidemics is in treatment uptake. HCV treatment uptake has been low in most cohorts of HIV-infected PWID. Typically, less than 20% of patients started therapy due to multiple barriers to treatment including contraindications to interferon, comorbidities, and uncontrolled addiction. In contrast, the majority of HCV-infected MSM start HCV therapy early after infection (Boesecke et al. 2012).

Worldwide, the large burden of HCV disease is still within the PWID population. However, in Western Europe, the majority of incident HCV infections among HIV-infected patients occur in MSM ► [Men who have sex with men \(MSM\)](#), [Epidemiology of HIV/AIDS](#). It is important to note that these epidemiological features differ substantially in other countries. As an example, in Eastern Europe and Southeast Asia, the large majority of incident HCV infections still occur in PWID.

Because of the frequency and the clinical relevance of HCV infections, the European guidelines

recommend yearly HCV screening in all HCV seronegative HIV-infected patients.

HIV Infection Adversely Affects the Natural Course of HCV Infection

Acute HCV infection induces a wide range of innate and adaptive immune responses. Despite these immune responses, the virus is able to establish a chronic infection in most infected individuals. HCV specific immune responses are severely impaired in HIV-infected patients. Accordingly, spontaneous clearance rates are lower and HCV RNA levels higher in HIV/HCV-coinfected compared to HCV-monoinfected patients (Fig. 1).

Coinfection with HIV accelerates liver fibrosis progression. A landmark study with repeated biopsies estimated that the median time from infection to cirrhosis was 12 years shorter (26 years vs. 38 years) in HIV/HCV-coinfected compared to HCV-monoinfected patients (Benhamou et al. 1999). HIV coinfection has also been associated with higher rates of hepatic decompensation and with shorter survival times in patients with HCC. Accordingly, HCV-related complications are among the leading causes of death in HIV/HCV-coinfected patients (Weber et al. 2006).

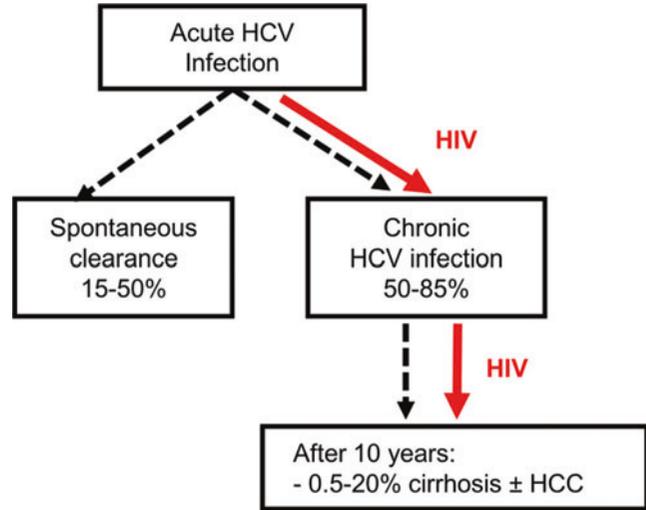
There are several reasons for the harmful effects of HIV on the natural history of HCV infection. First, cellular immune responses to HCV antigens are severely impaired in HIV-infected patients. Second, untreated HIV infection causes chronic immune activation with upregulation of pro-inflammatory cytokines which can accelerate fibrogenesis. Third, the HIV-associated damage of gut mucosa and bacterial translocation to the portal system increase hepatic inflammation ► [Microbial Translocation](#). And finally, HIV can infect and activate hepatic stellate cells promoting fibrogenesis in the liver (reviewed in Rockstroh et al. (2014)).

ART in HIV/HCV-Coinfected Patients

Given the detrimental effects of HIV on the natural course of HCV infection, it is logical to postulate

Hepatitis C Virus Infection and HIV,

Fig. 1 Natural course of HCV infection and impact of HIV



that an effective ART should partially revert the harmful effects of untreated HIV infection ► [Anti-retroviral Medications, Adult Care and Treatment](#). And indeed, several studies demonstrated that ART is beneficial with regard to viral control and liver damage (Rohrbach et al. 2010; Thein et al. 2008). However, even patients with successful ART remain at increased risk for liver-related complications, in particular those with ongoing HIV replication during ART or with CD4 T cell counts below 200/μl (Lo Re et al. 2014; Qurishi et al. 2003). The beneficial effects of ART on HCV related complications support recent recommendations to start ART in all patients irrespective of CD4 counts.

The choice of ART is mainly driven by HIV-related factors, further comorbidities, and comedications. Drugs with hepatotoxic potential should be avoided. In the era of direct-acting antiviral agents (DAAs), the choice of ART should also consider potential drug-drug interactions between HIV and HCV drugs (see below).

HCV Therapy in HIV-Infected Patients

Eradicating HCV infection dramatically reduces HCV-related complications. The benefits include improvements in extrahepatic manifestations such as chronic fatigue or MC vasculitis, as well as an approximately tenfold reduction in

the risk of hepatic decompensation and a three-fold reduction in the risk of HCC (Hill 2015). In coinfection studies SVR also reduced non-liver related deaths, suggesting that cure of HCV infection decreases systemic inflammation. Furthermore, eradicating HCV eliminates infectiousness. Modeling studies suggest that treating HCV could be a very effective measure to reduce HCV incidence and prevalence by preventing HCV transmissions through needle sharing or high-risk sexual behavior (Martin et al. 2013).

The recent improvements in HCV treatments are a major breakthrough in the care of HIV/HCV-coinfected patients. For many years, treatment uptake and outcomes were unsatisfactory. The previous standard of care treatments consisted of weekly injections with pegylated interferon alpha (IFN) combined with daily doses of ribavirin (RBV). The typical treatment duration for coinfecting patients was 48–72 weeks. These therapies were often poorly tolerated, and the cure rates between 30% and 70%, depending on the HCV genotype, were unsatisfactory. Multiple contraindications to treatment and the fear of side effects were important barriers to HCV therapy. Furthermore, IFN-based therapies in those with the most urgent need (such as **patient A**) could lead to life-threatening hepatic decompensations. Therefore, in most settings, only a minority of patients started therapy.

The new era of IFN-free DAA treatments has revolutionized the management of hepatitis C in HIV-infected patients. Compared to interferon-based therapies, these treatments are more effective and eradicate infection in the large majority of patients. Importantly, patients who would not have tolerated interferon have now excellent treatment options. Previously reported treatment barriers for IFN/RBV therapy do not apply anymore in the large majority of cases. Sustained virological response (SVR) rates were typically lower in HIV-infected patients with traditional IFN-based therapy compared to HCV-monoinfected patients. Fortunately, treatments including DAAs are equally effective in patients with and without HIV coinfection. This has led guidelines (EASL, EACS, AASLD) to no longer separate between HCV-mono- and HIV-coinfected subjects. Indication and choice of DAAs is now the same for all patients. The only remaining issue is the need to check for drug-drug interactions between HIV and HCV drugs.

Treating HCV Infection from 2015: The Future Is Bright

The hepatitis C treatment options and recommendations change rapidly. As an example, first-generation HCV protease inhibitors were approved 4 years ago but have already been replaced by better tolerated and more effective compounds. It is therefore crucial to consult regularly the updated treatment recommendations. The joint guidance by the Infectious Diseases Society of America (IDSA) and the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL) and the European AIDS Clinical Society (EACS) guidelines updates online detailed recommendations and includes a specific guidance for HIV/HCV-coinfected patients (www.hcvguidelines.org, www.eacsociety.org/guidelines/eacs-guidelines/eacs-guidelines.html and www.easl.eu/research/our-contributions/clinical-practice-guidelines).

The rapidly evolving HCV treatment options make it difficult to maintain a good overview.

However, some basic principles which are very familiar to HIV physicians facilitate the oversight in this changing field. JM Pawlotsky has proposed in a recent review three requirements for an HCV cure which can be applied to any DAA combination treatment (Pawlotsky 2014). To understand this concept it is important to remind the two main phases of HCV RNA decline during HCV therapy. The first-phase decline is the result of the direct antiviral effect of DAA treatments and characterized by a steep decline in HCV RNA within days of starting therapy. DAAs block almost immediately the production of new virions and reduce HCV RNA by at least three logs within 3–5 days of therapy. However, as in HIV, monotherapy leads to rapid selection of resistance variants causing a rapid rebound of HCV viremia. Therefore, DAA needs to be combined with other antiviral agents (IFN, ribavirin, or preferably a second DAA). Combination of two DAAs results in a significant increase in the barrier to resistance, and thus the decline in HCV RNA is sustained. The second-phase decline is characterized by the clearance of infected cells through the host immune response and the progressive degradation of HCV RNA. To achieve cure, HCV treatment has to be sustained until all infected cells are eliminated. The approved DAA combination treatments fulfill the requirements of potency and high barrier to resistance. Nevertheless, several factors need to be considered when discussing the optimal treatment choice. Key parameters are summarized in Table 2.

HCV DAAs in 2015

Effective DAA combinations inhibit different steps of the HCV life cycle. The three main DAA targets are the HCV protease, the NS5A protein, and the NS5B polymerase. Only approved compounds will be discussed here. Many other agents are in clinical development and will partially replace the currently available drugs.

HCV Protease Inhibitors

The first approved protease inhibitors (PIs) were boceprevir and telaprevir. When combined with

Hepatitis C Virus Infection and HIV, Table 2 Factors to be considered in DAA therapy

Parameter	Examples	Consequence
HCV genotype	The first-generation HCV protease inhibitors telaprevir and boceprevir are only active against HCV genotype 1; simeprevir is not active against HCV genotype 3 Some DAA combination treatments (e.g., ombitasvir-paritaprevir + dasabuvir) are not active against HCV genotype 3	DAA choice needs to consider HCV genotype
Liver fibrosis stage	Relapse rate is higher in cirrhotic patients	Prolong treatment duration and/or maximize potency (e.g., by adding a third antiviral agent)
Treatment experience	Nonresponse to previous IFN-based therapy is associated with higher relapse rates	Prolong treatment duration and/or maximize potency (e.g., by adding a third antiviral agent)
Comedication	Drug-drug interactions (DDIs) between HCV and HIV protease inhibitors Increased tenofovir exposure together with sofosbuvir	Choose DAA treatment without relevant DDIs or adapt ART before DAA therapy Adapt ART or frequent monitoring for renal toxicity

IFN and ribavirin, the addition of these first-generation PIs increased cure rates from about 40% to 70% in treatment-naïve HCV genotype 1-infected patients. However, IFN-related side effects, a high pill burden, and multiple side effects were major drawbacks of these first DAA-based therapies. Therefore, most guidelines do not recommend the use of first-generation PIs anymore.

In 2013, the second-wave PI simeprevir was approved in the USA and subsequently in Europe. This PI is active against HCV genotypes 1, 2, and 4 and is combined with either IFN/RBV or the NS5B inhibitor sofosbuvir (see below). The simeprevir/sofosbuvir combination has been widely used in clinical routine including in HIV-infected patients, and cure rates above 80% have been reported. One limitation is that due to drug-drug interactions, simeprevir cannot be combined with HIV protease inhibitors, etravirine or efavirenz.

NS5A Inhibitors

These drugs inhibit the HCV NS5A protein and block viral replication, assembly, and release. Daclatasvir has been approved in Europe in combination with either sofosbuvir or IFN/RBV. The combination of daclatasvir and sofosbuvir is an important treatment option for HCV genotype 3-infected patients with cirrhosis as other currently

available DAAs are not sufficiently active against this genotype. In this context it is interesting to note that HCV genotype 3 was considered as “easy to treat” in the IFN/RBV era but is now considered as “hard to treat,” as many currently approved DAAs have poor antiviral efficacy against this genotype.

NS5B Polymerase Inhibitors

The first approved NS5B inhibitor is sofosbuvir. This nucleotide analogue inhibitor has already been used extensively together with IFN/RBV or in combination with other DAAs. Sofosbuvir exhibits pangenotypic activity, has a high barrier to resistance, and has few drug-drug interactions. One exception is an increase in tenofovir exposure which warrants caution especially in those with impaired renal function.

Of note, there has been a recent report on significant arrhythmias during treatment with sofosbuvir and daclatasvir together with the anti-arrhythmic drug amiodarone. This unexpected side effect exemplifies the importance of continued monitoring for unexpected adverse events as the new DAAs are introduced into clinical routine.

Fixed Combinations

In 2015, two fixed-dose DAA combinations were approved. Sofosbuvir/ledipasvir has been approved for the treatment of HCV genotypes

1, 3, and 4. This combination results in cure rates above 90% in both HCV-monoinfected and HIV/HCV-coinfected patients. Ombitasvir-paritaprevir-ritonavir plus dasabuvir is another combination therapy (approved for HCV genotypes 1 and 4) which achieved cure rates above 90% in HIV/HCV-coinfected patients.

Drug-Drug Interactions

HIV-infected patients are at particular risk for significant drug-drug interactions, as nearly all patients start ART before commencing HCV therapy. In addition, many HIV-infected subjects are treated for comorbidities that increase the number of drugs and the potential for significant drug-drug interactions. Therefore, HIV-infected subjects are at risk for inadequate drug levels of both HCV and HIV drugs. Drug-drug interactions occur between many antiretroviral and HCV drugs that are substrates, inhibitors, or inducers of different cytochromes, UDP-glucuronyltransferase, or efflux pumps. It is therefore crucial to check for drug-drug interactions before starting DAA treatments. Regularly updated databases such as www.hepdruginteractions.org are of great help in this context. In some cases, therapeutic drug monitoring can be helpful to tailor ART and HCV treatments. In most patients, drug-drug interactions are manageable and should not be a barrier to starting HCV therapy.

Who and How to Treat HCV Infection

In light of the current knowledge, how should the three patients described above be treated?

Patient A suffers from decompensated liver cirrhosis and has an urgent indication for HCV treatment. Curing HCV would dramatically reduce his risk of liver-related complications. Several studies have demonstrated that HCV therapy can achieve high success rates in opioid substitution programs. A key advantage is that treatment can be supervised daily during the opioid substitution. Reinfection rates are low in this

population, and, therefore, a history of intravenous drug use must not be a reason for not starting therapy. As outlined above, zidovudine should be replaced in this patient because of increased mitochondrial toxicity when combined with ribavirin. Interferon-based treatments should be avoided because of the risk of hepatic decompensation. In this situation, the current guidelines recommend either fixed-dose combination ledipasvir/sofosbuvir and RBV for 12 weeks or in those with anemia or RBV intolerance ledipasvir/sofosbuvir without RBV for 24 weeks. Further combinations including ombitasvir-paritaprevir-ritonavir plus dasabuvir and ribavirin are currently tested in clinical trials but are not recommended yet in decompensated cirrhosis.

Patient B has a relatively recent HCV infection with significant liver fibrosis but no signs of cirrhosis. Even without therapy, the risk of a severe liver-related complication within the next 5 years is below 10% (Macias et al. 2015). However, he suffers from severe fatigue which is a very common complaint that can be relieved by eradicating HCV infection. Furthermore, recent modeling work suggests that deferring treatment until advanced fibrosis (Metavir stage F3) could increase the lifetime risk of liver-related deaths even after eradicating HCV infection. HCV clearance is often associated with fibrosis regression, but some individuals may also experience liver fibrosis progression after SVR. This is plausible considering that many risk factors associated with fibrogenesis including drug toxicity, alcohol use, coinfections, or metabolic liver disease persist. Furthermore, the time of infectiousness could be shortened substantially if treatment would start immediately instead of deferring therapy until F3. Taken together, there are several arguments to start therapy immediately in this patient. Unfortunately, the very high current treatment costs (above 50,000 USD/treatment course) led to reimbursement restrictions to patients with at least advanced fibrosis in many countries. If HCV treatment was reimbursed, DAA treatment would almost certainly permanently eradicate HCV infection in this patient. To avoid drug-drug interactions, the start of ART could be deferred until HCV treatment has terminated. Currently

available treatment options would include combinations of sofosbuvir/ribavirin, sofosbuvir/daclatasvir, or sofosbuvir/IFN/RBV in this HCV genotype 3-infected patient.

Patient C has no liver fibrosis and is asymptomatic. However, the treating physicians should discuss the options of eradicating HCV infection before pregnancy. In the IFN/RBV era, a planned pregnancy was a contraindication to HCV therapy because of the teratogenic effects of ribavirin during therapy and 6 months thereafter. However, with the new DAA therapies, HCV infection could be eradicated within 3 months and before conceiving. An HCV regimen without ribavirin should be chosen in this patient. Current recommendations for HCV genotype 4 infections in non-cirrhotic patients include combinations with sofosbuvir with either ledipasvir, simeprevir, or daclatasvir for 12 weeks. These interferon- and ribavirin-free treatments achieve cure rates above 90%. Curing HCV infection before pregnancy would eliminate the risk of a vertical transmission which is 10–20% in the setting of HIV/HCV coinfection.

Conclusions

The new advances in HCV treatments offer unprecedented opportunities to improve the health of HIV/HCV-coinfected patients and to reduce horizontal and vertical HCV transmissions. However, to achieve a major impact on the hepatitis C disease burden at the population level, treatment uptake has to be increased substantially. It is now possible to dramatically reduce the risk of HCV-related complications in the large majority of HIV/HCV-coinfected patients. However, to make this affordable for healthcare systems worldwide, the prices for DAA treatments need to be lowered substantially. Until recently, the most urgent problem in the field of HIV/HCV coinfection was to improve efficacy and tolerability of HCV therapies. To date, nearly all patients can be cured with very well-tolerated and safe treatments. The most urgent priority today is to massively increase treatment uptake. If this can be achieved, the future is bright, and

millions of HCV-infected patients will benefit from a true breakthrough in medicine.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Hepatocellular Carcinoma in HIV-Positive Patients](#)
- ▶ [Gay men and other Men who have sex with men \(MSM\), Epidemiology of HIV/AIDS Introduction](#)
- ▶ [Microbial Translocation](#)

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Hepatocellular Carcinoma in HIV-Positive Patients

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Definition

Hepatocellular carcinoma (HCC) is a primary tumor of the liver that arises from hepatocytes. The principal factors that increase the risk of HCC include infection with hepatitis B virus (HBV), infection with hepatitis C virus (HCV), alcoholism, and aflatoxin. Patients with HIV infection also have an increased risk of HCC, but the role of HIV and immunosuppression is unclear, and much of this risk appears to be because of coinfection with HBV and/or HCV. Whether HIV plays a direct role in HCC pathogenesis remains to be established, but it can increase the risk of HCC in individuals coinfecting with HBV and/or HCV. The clinical course of HCC depends on the stage of cancer disease, performance status, and comorbidities. Therapeutic options include liver transplantation, local antitumor chemotherapy,

and biological agents. In the HIV setting few data are available about treatment options. The increased longevity of patients with HIV appears to be contributing to an increased incidence of HCC in this population and imposes new strategies for prevention and therapeutic management of patients.

Introduction

HIV has been linked to malignancies since the beginning of the AIDS epidemic in 1981, when Kaposi's sarcoma was reported for the first in young never-married men. Soon thereafter, aggressive non-Hodgkin lymphoma (NHL) was found to be linked to HIV, and these two tumors were considered AIDS defining when they occurred in an HIV-infected patient. Subsequently, invasive cervical cancer was also noted to be more common in HIV-infected patients and became the third AIDS-defining tumor. Since that time, numerous studies have shown that HIV infection raises the risk of a number of other tumors that do not confer a diagnosis of AIDS; these are called non-AIDS-defining cancers (NADCs). NADC cancers include carcinoma of the anus, testis, lung, skin (basal cell skin carcinoma and melanoma), Hodgkin disease, and hepatocellular carcinoma (HCC) (Dal Maso et al. 2009; Polesel et al. 2010; Pantanowitz et al. 2006).

The advent of highly active antiretroviral therapy (HAART) has dramatically extended the survival rates of patients with human immunodeficiency virus (HIV), leading to effective suppression of HIV and prolongation of life (Nunnari et al. 2005; Polesel et al. 2010). Most patients receiving HAART have an increase in their CD4 counts and a decrease risk of AIDS-defining tumors associated with severe immunosuppression, such as Kaposi sarcoma or central nervous system lymphoma. However, as these patients live longer, we are seeing a rise in a wide range of other cancers, and cancer is an increasing cause of morbidity and mortality in HIV patients; in fact, in some recent studies, cancer is the most common cause of death in

HIV-infected individuals (Bonnet et al. 2004; Simard et al. 2010, 2011; Meijde et al. 2013; Centers for Disease Control and Prevention 1992).

Zucchetto et al. evaluated the mortality for NADCs among 10,392 Italian patients with AIDS, who were diagnosed between 1999 and 2006, compared with the general population of the same age and sex. NADCs were the underlying cause of death for 7.4% of HIV-infected patients. The authors found a 6.6-fold elevated risk of death for NADCs among persons with AIDS. Most of these cancers with significantly elevated standardized mortality rates (SMRs) have a viral etiology, including anal cancer, which is associated with a human papilloma virus (SMR 270); Hodgkin lymphoma, which is associated with Epstein-Barr virus (SMR 174); and HCC, which is associated with chronic hepatitis B and C virus infections (SMR 11.1). In absolute terms, the most common cause of death for NADCs was lung cancer (24.6%), followed by liver cancer and Hodgkin lymphoma (both with 11.9).

The particularly enhanced risk for infection-related cancers could be explained by the fact that the altered immune system in HIV-infected persons may reduce its ability to control and suppress the oncogenic viral process or tumors expressing foreign antigens (Dubrow et al. 2012). This mechanism is supported by Grulich et al. who compared, in a meta-analysis, the cancer risk for HIV-positive patients and organ transplant recipients. These populations had a common risk factor for cancer: immunosuppression. Indeed, most of the cancers seen with a higher frequency in both populations had a known infectious cause. The exact role of HIV-induced immunosuppression in the pathogenesis of NADCs remains controversial and may vary from tumor to tumor: certain tumors appear to be associated with low CD4 counts, while in other tumors this relationship is less clear or nonexistent. Silverberg et al. analyzed a cohort of 19,280 HIV patients, followed from 1996 to 2007 and matched for age and sex with 202,303 HIV-negative persons. The authors found that the risk of mouth-throat cancer, anal cancer, colorectal cancer, lung cancer, and Hodgkin

lymphoma rose as recent CD4+ cell count fell, even after adjusting for other cancer risk factors such as age, smoking status, substance use, and viral hepatitis. Longer duration of HIV infection and a history of repeated opportunistic or other infections are also considered as relevant risk factors for development of various NADCs. It has been suggested based on animal studies that some anti-HIV drugs may contribute to one or more NADC, but this has not been shown in humans.

Focusing on HCC, it is known that the major risk factor for HCC is liver cirrhosis, and in many countries, the most common cause of cirrhosis is infection with HCV or HBV. Other causes of cirrhosis and HCC include alcoholism and, especially in part of Asia, aflatoxin exposure. HCC usually develops only after several decades of HCC or HBV infection. The role of HIV in the development of HCC is not clear; most HIV-infected patients who develop HCC re-coinfected with HBV and/or HCV, and these are the major risk factors for the development of HCC. Evidence seems to suggest that HIV by itself is not a risk factor for HCC. It is not known whether HIV infection alone is a risk factor for HCC, and indeed, evidence from several large retrospective studies suggests it is not. There is some, although conflicting, evidence that among patients coinfecting with HIV and either HBV or HCV, HCC is more common in patients with low CD4 counts, suggesting that HIV or HIV-induced immunosuppression may be a contributory factor in the development of HCC. HIV coinfection also seems to accelerate disease progression of HCC. The ability of HAART to reduce HIV-associated immunosuppression may thus be beneficial in reducing the risk of HCC. At the same time, patients receiving HAART have a prolonged survival, and in countries where HAART is widely used, the HIV-infected population is increasing in number and becoming more aged. These latter trends are contributing to a substantially increased overall burden of HCC in HIV-infected or AIDS patients. In addition to potential indirect effects on HCC risk through improvements in immune reconstitution and survival, HAART is known to have some direct

hepatotoxic effects, which might be amplified among HIV-positive patients chronically infected with HBV or HCV. Whatever the contributory causes of the increased number of cases of HCC in the HIV-infected population, this is an increasingly important problem, and there is an urgent need for more effective preventive, diagnostic, and therapeutic approaches.

HCC: A Rising Problem Among Patients with HIV

Epidemiology and Risk Factors

HCC is the commonest primary cancer of the liver and, according to the WHO report, the fourth commonest cause of cancer-related death. The estimated incidence of new cases worldwide is about 500,000–1,000,000 per year, causing 600,000 deaths globally per year. Although there are large areas of the world where the incidence of HCC is still unknown, several regions including parts of East Asia and sub-Saharan Africa are affected by a very high incidence of HCC (over 20 cases/100,000 population). Areas with moderately high risk (11–20 cases/100,000 population) include Italy, Spain, and Latin America; France, Germany, and the United Kingdom have instead an intermediate risk (5–10 cases/100,000 population). A relatively low incidence (less than 5 cases/100,000 population) is found in the United States, Canada, and Scandinavia. The incidence of HCC has been rising in developed Western countries in the last two decades, driven in part by the increased prevalence of HCV infection and the rise of immigration rates from HBV-endemic countries. In addition, even though the incidence of HCC reaches its highest peak among persons over 65 years, an increased incidence among younger individuals has been noted in the last two decades both in the United States and Europe.

In HIV-positive patients, the incidence of HCC is substantially higher (82/10,000 according to the Data Collection on Adverse Events of Anti-HIV Drugs) than in the general population. Overall, HCV infection is the strongest predictor for liver-related death, followed by HBV; many studies have confirmed this datum. The role of HIV is

less clear. A large retrospective cohort study on US veterans demonstrated that HIV-positive persons had a higher risk to develop HCC than HIV-negative persons, but, after adjusting for HCV and alcohol abuse, HIV status was not independently associated with cancer. The 2001 French Mortavic study (Rosenthal et al. 2003), a prospective 1-year cohort study involving 25,178 HIV-positive patients, showed a significant increase in death from end-stage liver disease (ESLD) and HCC, as compared to similar cohorts in 1995 and 1997; death due to ESLD rose from 1.5% to 14.3% of all deaths, whereas deaths due to HCC rose fivefold, from 4.7% to 25% of all deaths; interestingly, essentially all deaths from HCC were in patients with HCV coinfection. Throughout the same period, deaths directly due to AIDS fell from 91.6% of all deaths (in 1995) to 48.7%, suggesting that the increased longevity in the HAART era could be a reason for the increased HCC rate in the 2001 cohort. In a prospectively followed cohort of HIV-infected individuals in France, HCC deaths related to HCV infection also increased during the period from 2000 to 2005 (Bonnet et al. 2004). By contrast, the incidence of HCC development and related deaths among HIV-HBV-coinfecting individuals seemed to be relatively stable (Bonnet et al. 2004). A retrospective study conducted on a cohort of US veterans with hepatitis C between 1991 and 2000 showed that the incidence of HCC did not differ between HIV-/HCV-coinfecting and HCV mono-infected patients in the HAART era, whereas it was significantly lower among HIV-/HCV-coinfecting individuals previously to HAART introduction. This datum supports the premise that, in the pre-HAART era, HIV patients did not survive enough to develop HCC. Other retrospective studies examining cohorts from countries where HAART is largely unavailable found the incidence of HCC to be lower or equal to average population rates, also supporting this conclusion.

In 2004, the Italian Cooperative Group on AIDS and Tumors (GICAT), while collecting data on malignancies occurring in HIV patients since 1986, identified a total of 41 consecutive patients with HCC (from a joint Italian and

Spanish database) and retrospectively investigated the main epidemiological characteristics of these patients as compared to a control group comprised of 384 HIV-negative patients diagnosed over the same period. The GICAT study emphasized the younger age of HIV-positive patients at the diagnosis of HCC (age 40–46 vs. 60–70 in HIV negatives). HCV infection was the main risk factor for HCC development in both HIV-positive and HIV-negative subjects. The median time to develop HCC after HCV infection was found to be around 22 years in HIV-positive patients: 10 years shorter than that reported among HIV-negative patients who acquired HCV infection with transfusion. Alcohol abuse (which is often associated with HIV risk behaviors) and insulin resistance (which causes non-alcoholic fatty liver disease and frequently occurs in HIV-infected individuals in part from certain antiretroviral drugs) are other potential risk factors for the earlier development of HCC among HIV-positive patients.

HIV-HBV and HIV-HCV Coinfection: Prevalence and Significance of a Complex Interaction

Coinfection with HCV and/or HBV is common among HIV-infected persons, because of shared routes of transmission, although the prevalence of coinfection varies markedly according to the geographic origin and demographic characteristics of infected patients. Approximately 25% of HIV-positive persons in the Western world have HCV coinfection. In a study of 3,048 patients in the European SIDA cohort, the prevalence of HIV/HCV coinfection was 33% overall but 75% in injection drug users (IVDUs). In the United States, the highest rates of HIV/HCV coinfection were also seen among IVDUs. As regards HBV, up to 9% of HIV-positive patients in Europe are coinfecting with HBsAg (Konopnicki et al. 2005). In Italy, between 3% and 4% of HIV-infected individuals are chronic carriers of HBsAg. The recorded prevalence is likely to be inaccurate, however, because of the large number of patients with occult HBV infection, associated with detectable HBV DNA on quantitative PCR.

HCV usually leads to the development of HCC through the stage of cirrhosis, which can

take 28–30 years to occur. Cirrhosis is almost a prerequisite for the development of HCV-related HCC: HCV is not able to integrate into the host genome, and the major hypothesis to explain hepatocarcinogenesis in patients with HCV is related to immune-mediated inflammation and hepatocellular injury. HBV chronic infection is another major cause of HCC, but, differently from HCV, HCC may occur in HBsAg carriers without cirrhosis, because of the direct involvement of a number of viral-related factors (viral proteins, BCP mutation in the viral genome, pre-S deletion mutants). Furthermore, HBV has a retroviral intermediate stage in its replication and can integrate its DNA into the host genome. This can lead to a variety of mutagenic consequences, including large inverted duplications, deletions, amplifications, and translocations, resulting in chromosomal instability. As expected, patients with HCV-HBV coinfection have yet a higher risk of developing HCC than those infected with just one or the other, and vaccination against HBV should be proposed to all patients with chronic hepatitis C who are not infected with HBV.

A number of studies have examined the role of HIV in the pathogenesis of HCC. In vivo studies in murine models have shown a potential role of the HIV *Tat* gene in liver tumorigenesis. In transgenic mice expressing this gene, a greater incidence of hepatocellular carcinoma and extrahepatic malignancies has been found, suggesting that the potential oncogenic effect of *Tat* gene is not liver specific. There is suggestive evidence that *Tat* may have oncogenic activity because of its anti-apoptotic activity, pro-angiogenic activity, and ability to induce expression of growth factors, cytokines, and transcription factors. Although murine experiments suggested a direct role of *Tat* in HCC, a number of epidemiological studies have not shown a clear role of HIV itself on HCC development; in a large retrospective study by Giordano et al., for instance, the rate of HCC was not higher in HIV mono-infected patients without HCV or HBV infection than in general population. At the same time, however, there is a clear evidence that HIV can accelerate the progression of HCV- and

HBV-liver disease to cirrhosis and HCC. In this regard, the presence of HIV alters the natural history of HCV infection, increasing the likelihood of chronicity (over 90%) due to the lack of CD4+ T-cell responses against HCV. Moreover, once chronic HCV infection is established, liver disease progression is much faster in HIV-infected patients, resulting in a higher frequency of cirrhosis and its complications compared to HCV mono-infected patients.

The molecular mechanisms of accelerated fibrosis in coinfecting patients are not fully understood. The studies of Galastri et al. suggest that HIV gp120 may play a role by exerting multiple effects on human hepatic stellate cells (HSCs), modulating their phenotype in a profibrogenic way. Incubation of HSCs with gp120 significantly increased the migration of HSCs and their expression of proinflammatory cytokines, including monocyte chemoattractant protein-1 (MCP-1) and type 1 procollagen. Recent data suggest that the binding of HIV gp-120 to the CXCR4 coreceptor, which is expressed on the surface of hepatocytes and HSCs, is able to upregulate tumor necrosis factor (TNF)-related apoptosis, inducing ligand (TRAIL) R2 expression. By this mechanism, HIV infection may make hepatocytes more susceptible to liver injury. During HIV coinfection, increased liver damage may also be mediated by the effects of antiretroviral drugs and indirectly by immune reconstitution syndrome (Bonnet et al. 2004; Puoti et al. 2012).

Further prospective studies are needed to better evaluate the role of HIV in subjects coinfecting with HCC. It will be important to control for potential confounding factors in epidemiologic studies. In some studies, for example, not all HCV-HBV patients had been tested for HIV, thus implying the possibility to underestimate the prevalence of coinfecting persons. Also, since time of infection with HBV or HCV is often missing, it is hard to calculate the effect of HIV coinfection on the development of HCC. For example, if patients with isolated HCV or HBV acquired this infection earlier than HIV-coinfecting patients in a study, this variation in the duration of infection could contribute to an apparent effect of HIV infection. Given these considerations, key points

of an ideal prospective study should include cross-testing for coinfections before individual allocation to groups, standardized screening for HCC, and regular evaluation of HIV viral load in the coinfecting cohort, in order to evaluate the potential effect of HAART-induced viral suppression on HCC pathogenesis.

Clinical Characteristics

During its initial stages, HCC is generally asymptomatic. In more advanced phases, hepatomegaly, jaundice, and abdominal pain may appear. Overall, the clinical presentation and prognosis considerably vary according to the number and size of tumor lesions. Liver cancer may appear either as a single nodular or infiltrating lesion with an eccentric growth or as a multinodular widespread tumor *ab initio*. In some patients HCC lesions have a slow growth rate, with a twofold increase in 20 months, while in others it can double in less than 1 month. Multinodular HCC is more often found in patients with more than one risk factor and needs to be classified as primitive multicentric HCC or metastatic cancer from a primitive HCC. This distinction has important clinical implications because primitive multicentric HCC are less aggressive and recur less frequently after ablation than metastatic cancers from a primitive HCC.

Among HIV-infected patients, cumulative clinical data suggests a more aggressive course of HCC. Patients with HIV from the HIV-HCC Italo-Spanish group showed a more advanced and infiltrating HCC (also with extranodal metastases), a more advanced stage of cirrhosis at presentation, and a reduced survival rate in comparison with HIV-negative patients. A 2007 US-Canadian multicenter retrospective study identified 63 HIV-infected patients with HCC from 1992 to 2005 and compared them to 226 HIV-negative HCC patients. Patients with HIV not only were younger and more frequently symptomatic than HIV-negative patients but also showed higher median α -fetoprotein levels. By contrast with other studies, tumor staging and survival were similar between cases and controls in this study. In untreated HCC cases, the presence of undetectable HIV-RNA was an independent

predictor of a better survival. In a recent, large, multicenter, observational study, Berretta et al. (2011) confirmed that HIV-positive HCC subjects tend to be younger and to have a shorter survival time after treatment than HIV-negative patients.

HCC: Treatment Options

HCC treatment is usually classified as curative or palliative. The curative treatments are surgical resection, orthotopic liver transplantation (OLT), and local ablative therapies, including percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA). Surgical resection is the treatment of choice in solitary tumors less than 5 cm in diameter that are without vascular invasion or extrahepatic spread, developing in patient with preserved hepatic function who does not have portal hypertension; in this setting, the 5-year survival rate is about 50%. OLT is the best option for cirrhotic patients with a solitary lesion less than 5 cm in diameter that is not a candidate for resection or with up to three lesions smaller than 3 cm and where there is no vascular invasion or metastasis according to the Milan criteria. In these cases the 5-year survival rate can be as high as 70%. In patients that are not eligible for resection or transplantation, owing to comorbidities, liver dysfunction, or limited surgical resources, PEI and RFA are a potential treatment for small tumors, usually less than 3 cm in size. For early stage HCC, RFA has been reported to induce a complete response in about 80% of patients, with a 5-year survival of 50% and recurrence rates comparable to surgical resection.

Unfortunately, however, most patients with HCC have advanced disease at diagnosis. They are candidates for palliative treatments that include transarterial chemoembolization (TACE), chemotherapy, hormonal compounds, and immunotherapy. TACE has been shown to improve survival when applied to carefully selected patients. It is indicated for unresectable multinodular HCC, without vascular invasion or extrahepatic spread. To date, durable remission has rarely been reported with chemotherapy, and no significant survival benefits have been conclusively demonstrated with this approach, probably

because of the chemoresistance of HCC cells. More recently sorafenib (an oral multikinase inhibitor of the vascular endothelial growth factor receptor) prolonged median survival time as compared to placebo as well as time to radiologic progression in patients with advanced HCC, and it has been approved for advanced disease.

Other antiangiogenic therapies that may have some utility in HCC are sunitinib and the combination of bevacizumab and erlotinib; unfortunately, no data are available concerning their use in HCC patients infected with HIV. Also, mammalian target of rapamycin (mTOR) inhibitors have shown a certain rate of efficacy in small cohorts of patients with HCC, but these data need to be validated in wider clinical settings. The presence of sexual hormone receptors on HCC cells raised the possibility of using antiestrogens like tamoxifen against those HCC cases not amenable to resection: unfortunately, several trials failed to demonstrate any benefit either in terms of response or in terms of overall survival in patients with advanced HCC treated with tamoxifen.

HCC in patients with HIV is often advanced at presentation, not allowing curative therapeutic strategies. Until a few years ago, HIV infection was an exclusion criterion for liver resection or transplantation. An important concern was the risk of HIV progression after OLT, a poor post-transplantation prognosis, and eventually the waste of graft (Di Benedetto et al. 2008; Terrault et al. 2012). Ettore et al. (2003) showed that almost half of HIV-positive patients affected with end-stage liver disease were not suitable for surgical treatment and comprehensively only 28% of them had the opportunity to receive a successful surgical treatment. An analysis of the GICAT cohort showed that in a series of 41 HIV-positive patients affected by HCC, 15 (35%) of them fulfilled the Milan criteria and could potentially have been treated with OLT as a curative intent. However, none of them underwent liver transplantation; only two underwent surgical resection with a 2-year survival of 41% in treated patients and 0% in untreated cases.

Since the introduction of HAART, the outcome of HIV infection has dramatically changed.

Patients with HIV have a substantially better long-term survival, and as a consequence, liver transplantation needs to be considered to treat HCC. Several studies found that most HIV-positive patients who undergo a liver transplant have a good long-term survival (Di Benedetto et al. 2008). Di Benedetto et al. (2008) reported on a series of 7 HIV-positive patients with HCC that, by fulfilling the Milan criteria, underwent OLT. After a mean follow-up of 232 days, the overall survival rate was 85.7%, and only one patient died; this individual had a functioning graft and no HCC recurrence and died of a myocardial infarction. Radecke et al. reported that out of five cases of OLT in HIV-infected cirrhotic subjects, two had stable liver function and non-progressive HIV infection under HAART, 61 and 23 months after OLT, respectively; unfortunately, in this report, three out of five patients died due to graft failure. Clinical posttransplant management of OLT in HIV-positive patients is doubtless more complex than in the HIV-negative counterpart. The main reported problem in these patients has been an earlier and more aggressive HCV recurrence (experienced in about 33% of patients), faster occurrence of hepatic fibrosis, a greater rate of rejection (from 33% to 38%) (Di Benedetto et al. 2012), and a higher incidence of tacrolimus toxicity.

The outcome of HIV-positive liver recipients depends in part on the immunological status of the patient at the time of OLT. There is a considerable agreement about the necessity of a full virological control of the underlying HIV infection before OLT: in fact, transplanted patients with higher CD4+ cell counts and undetectable HIV viral load display clinical courses similar to HIV-negative recipients. Di Benedetto et al. proposed some criteria to select HIV-positive patients with HCC for OLT: firstly, patients must completely fulfill the Milan criteria. They should also have an undetectable HIV viral load (<50 copies/mL) and a CD4+ cell count more than 200/mL. After OLT, HAART needs to be reinstated as soon as clinically possible, with the input of a multidisciplinary transplant team (surgeons, infective disease specialists, and oncologists) with great experience in the management of pharmacologic

Hepatocellular Carcinoma in HIV-Positive Patients, Table 1 Criteria for considering liver transplantation in HIV-infected patients (according to Di Benedetto 2008)

Liver disease criteria	
Child-Turcotte-Pugh score \geq B7; MELD score \geq 14	
<i>Milan criteria</i> ^a	
No more than three tumor nodules	
No nodule greater than 5 cm in diameter	
Absence of macroscopic portal vein invasion	
Absence of recognizable extrahepatic disease	
HIV infection criteria	
<i>Immunological criteria</i>	
None of AIDS-defining opportunistic infections in the previous year	
CD4 cell count >200 cells/ μ L or >100/ μ L in case of therapy intolerance	
<i>Virological criteria</i>	
Undetectable HIV viral load (<50 copies/mL) in the last 12 months or effective therapeutic options for HIV infection during the posttransplant period	
General criteria	
Favorable psychiatric evaluation	
Social stability	
No alcohol abuse for at least 6 months	
No drug consumption for at least 2 years (patients who are on stable methadone maintenance programs can be included and can continue on the maintenance program after the procedure)	
No extrahepatic malignancy	
No pregnancy	

^aPatients with HCC who are being considered for liver transplantation should not have a needle biopsy due to the significant rate of needle-track seeding leading to recurrence posttransplant

interactions between HAART and immunosuppressive agents (see Table 1). In conclusion, the latest evidence suggests that OLT should be considered as a possible therapy for patients with HIV and HCC. Accurate selection protocols for this approach that take into account HIV status, HCC stage, and other factors are essential (Wood 2012). At the present time, the key question is not *if* but *who* should be referred to liver transplantation.

In the palliative setting, recent data on the use of sorafenib in unresectable HCC/HIV-positive patients showed that this treatment along with concomitant HAART is safe and feasible and that the response rates are similar to general population (Berretta et al. 2013). Unfortunately the

Hepatocellular Carcinoma in HIV-Positive Patients, Table 2 HCC prevention

Primary prevention
Alcohol avoidance
Avoidance of injection drugs
Vaccination against hepatitis B
Secondary prevention
Six-monthly ultrasonography + AFP
Treatment of HCV and/or HBV coinfection
Tertiary prevention
No significant options

number of HCC/HIV-positive patients analyzed is small, and prospective and randomized trials are necessary to draw further conclusions.

HCC: Primary, Secondary and Tertiary Prevention

Prevention and early diagnosis are key points to the management of HCC, but, at present, there are no universal guidelines, especially when it occurs in HIV-positive patients (see also Table 2). Primary prevention in subjects with HIV should entail efforts to promote alcohol avoidance and when appropriate include strongly HBV. In fact, HCC was the first human cancer that was shown to be amenable to prevention using mass vaccination.

Secondary prevention should include regular exams aimed at early detection of HCC. The European Association for the Study of the Liver (EASL) has proposed guidelines describing patient selection and surveillance intervals for HCC screening in HIV-HCV and HIV-HBV-coinfecting individuals. Six monthly ultrasonography and alpha-fetoprotein (AFP) level measurements are the two methods most commonly used to screen cirrhotic patients for HCC. The use of AFP alone for early diagnosis of HCC in HIV-coinfecting patients is not recommended and may be suggested only where and if ultrasonography is unavailable. In fact, even though AFP values higher than 400 ng/ml are usually considered as diagnostic of HCC, false-positive AFP results can occur in HIV-infected patients as a result of HAART inducing substantial increases of AFP levels. The GICAT group observed a more advanced HCC at diagnosis in HIV-positive patients, apparently unrelated to a true delay in

diagnosis, and suggested shortening the interval for HCC screening in this patient population as hepatocarcinogenesis can be a more-rapid process in HIV-positive cirrhotic subjects.

Another point to consider in patients coinfecting with HIV is the treatment of HCV and/or HBV. Treatment with IFN and ribavirin may induce a persistent clearing of HCV viremia in 27–40% of HIV-HCV-coinfecting individuals and seems to reduce the risk of HCC in these cases. Moreover, HCV eradication in HIV-coinfecting patients results in a definitive improvement of liver function, and it seems to improve tolerance to antiretroviral agents. In particular, therapy with pegylated-IFN alpha plus ribavirin appears able to slow down the rate of liver disease progression, although the likelihood of achieving a sustained virological response (SVR) is lower in coinfecting persons than in those with HCV mono-infection. Based on available clinical results, 48 weeks of ribavirin and pegylated-IFN therapy at doses used for HCV mono-infected patients seems to be advisable. Unfortunately HCV therapy in HIV patients is not as well tolerated as in other patients; side effects include relatively more severe and frequent myelosuppression, more frequent anemia (especially in patients taking zidovudine), an increased risk of lactic acidosis, and a poorer response in patients with low CD4+ cell counts. HBV treatment also appears to substantially reduce HCC incidence in patients with severe cirrhosis, and there are reasons to suspect the same effect in HIV-coinfecting patients. Patients in whom treatment for both HBV and HIV is planned should receive therapies that are effective against both viruses, and regimens such as lamivudine plus tenofovir or emtricitabine plus tenofovir are preferred. As far as we know, no relevant data on tertiary prevention (which aims to reduce HCC recurrence after resection) are available in HIV-positive individuals.

Conclusion

Currently, in areas where HAART is available, a rapid increase of HCC incidence is occurring

among HIV-positive individuals. It is reasonable to hypothesize that in a few years, the burden of HCC in HIV-infected patients will increase in developing countries as well. Considering the more aggressive clinical behavior and progression of HCC in coinfecting patients, there is an urgent need for effective prevention programs, screening techniques, and specific management guidelines. Large, multicenter, randomized clinical trials are needed, in order to better define the criteria for surveillance and the effect of early diagnosis on outcome. In addition, important needs for future research include a further evaluation of the feasibility of liver transplantation and efficacy of new therapeutic agents.

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HIV "Auxiliary" Proteins

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Definition

In addition to *gag*, *pol*, and *env*, HIV and SIV genomes contain additional genes (*tat*, *rev*, *vif*, *vpr*, *vpx*, *vpu*, and *nef*) encoding for regulatory proteins. While Tat and Rev are required both in vitro and in vivo for virus replication, the Vif, Vpr, Vpx, Vpu, and Nef "auxiliary" proteins are

usually dispensable for virus growth in vitro, but these auxiliary proteins play essential roles in vivo for virus replication and AIDS pathogenesis through interaction and perturbations of cellular pathways and functions in HIV target cells.

Introduction

The genome of human lentiviruses (HIV-1 and HIV-2) contains more genes than the usual *gag*, *pol*, and *env* genes common to all retroviruses. Indeed, HIV-1 contains additional open reading frames called *tat*, *rev*, *vif*, *vpr*, *vpu*, and *nef* encoding for small regulatory proteins, while the HIV-2 genome has an additional *vpr*-related *vpx* gene but usually no *vpu*. While Tat and Rev proteins are absolutely required both in vitro and in vivo for virus replication, expression of Vif, Vpr, Vpx, Vpu, and Nef proteins is usually dispensable for efficient virus growth in several in vitro virus-replication systems and these proteins were thus first referred to as accessory or auxiliary proteins. However, their primordial role for virus infectivity and replication as well as in AIDS pathogenesis during the course of the natural infection was rapidly established. In spite of their small size, a plethora of effects and functions have been attributed to these regulatory proteins in vitro during virus replication in the target cells of HIV, CD4⁺ T lymphocytes, macrophages, and dendritic cells. It was known for many years that these proteins utilize and perturb basic cellular pathways leading to optimization of early or late essential steps of the virus life cycle. More recently, accessory proteins have progressively been shown to be involved in the counteraction of different innate antiviral cellular restriction factors that partially inhibit virus infectivity and dissemination in the target cells. Recent studies highlighted some cellular proteins targeted by the viral auxiliary proteins to perform their different tasks and counteract restriction factors, such as the apolipoprotein B mRNA-editing enzyme 3G (APOBEC3G), the bone marrow stromal cell antigen 2 (BST-2) also called tetherin, and the recently identified sterile-alpha motif and HD domain protein (SAMHD1). Their real roles and

functions during the course of natural infection are still largely enigmatic.

Here, an overview of the established knowledge concerning the multifunctional activities of HIV auxiliary proteins in focusing particularly on their interaction with restriction factors is presented.

Nef: An Essential Virulence Factor

Nef, abbreviated from negative factor, is a myristoylated protein of 27–35 kDa encoded by all HIV and SIV primate lentiviruses that is expressed early during the viral life cycle, suggesting an important role in creating a favorable environment for virus replication. Nef was initially so called as early *in vitro* studies showed its propensity to reduce HIV replication in established cell line, but subsequent *in vitro* and *in vivo* studies realized in macaques infected with Nef-deleted SIV as well as in transgenic mice expressing Nef and HIV-1-infected "humanized" mice identified Nef as a key factor for AIDS pathogenesis and unraveled its ability to promote virus spread. Despite its lack of enzymatic activity, Nef acts as an adapter to bind cellular factors and exert pleiotropic functions. Nef affects vesicular trafficking and signal transduction leading to prevention of superinfection, protection of infected cells from recognition by the immune system, reduction of the motility of infected T lymphocytes, and enhancement of the infectivity and replication of released virus particles (Malim and Emerman 2008; Abraham and Fackler 2012; Strebel 2013; Basmaciogullari and Pizzato 2014). Nef is a multifunctional protein able to interact directly with cellular components involved (i) in vesicular transport between endosomal membrane compartments of the endocytic pathway and (ii) in the control of several signaling pathways in HIV-1-infected cells. These Nef-induced alterations of intracellular trafficking and cell signaling pathways are related to the presence of specific motifs, reminiscent of interaction motifs found in cellular proteins, within the primary sequence of HIV-1 Nef. Two motifs of Nef have been extensively characterized:

a leucine-based motif (E/D₁₆₀xxxLL₁₆₅), found in a C-terminal flexible loop of HIV-1 Nef, and a poly-proline (P₇₂xxP₇₅) motif. While the leucine-based motif allows recruitment of clathrin-associated adaptor protein (AP) complexes that participate in the vesicular transport within the endocytic pathway, the poly-proline motif is required for interactions with cellular proteins containing SH3 domains, such as tyrosine kinases of the Src family (Abraham and Fackler 2012). AP complexes are heterotetramers constituted of two large subunits, a medium chain and a small subunit that play important roles in the selection of the cargo proteins, formation of clathrin-coated vesicles (CCVs), and vesicular transport between the different membrane compartments of the endocytic pathway. Therefore, some functions of Nef, such as cell surface downmodulation of certain membrane receptors, are specifically dependent of the Leu-based motif, whereas the integrity of the poly-proline motif is required for some other Nef effects, regarding alteration of signaling pathways observed in HIV-1-infected T cells and macrophages. Interestingly, Nef-mediated enhancement of HIV-1 infectivity and replication depends on the integrity of both Leu-based and poly-proline motifs.

It is now well documented that Nef acts on the endocytic pathway to disturb the intracellular trafficking and cell surface expression of several membrane receptors, including CD4, major histocompatibility class I and II (MHC-I and MHC-II) molecules, the CD28 co-stimulatory molecule, the DC-SIGN lectin expressed on dendritic cells, and the CCR5 and CXCR4 chemokine receptors involved in virus entry. The current molecular model for Nef-induced CD4 endocytosis suggests that Nef acts as a connector between CD4 and the clathrin-associated AP-2 complex at the plasma membrane. This connection results in the targeting of CD4 to clathrin-coated pits for rapid internalization via CCVs to reach internal early endosomes where CD4 is then rerouted to the lysosomal degradation pathway (Tokarev and Guatelli 2011). Whereas the recruitment of AP complexes is mediated by the leucine-based motif of Nef, the putative CD4-binding site is found in the N-terminal part of the protein. This

mechanism prevents cell superinfection and more surprisingly is required to maintain the full infectivity of the viral particles released.

Nef also downregulates the cell surface expression of MHC-I molecules, thus overcoming the antiviral cytotoxic T-lymphocyte response and the lysis by natural killer cells. Cell surface MHC-I downmodulation relies on the ability of Nef to connect MHC-I to the AP-1 complexes involved in the transport between the trans-Golgi network (TGN) and the early endosomes. Interestingly, two other Nef motifs are involved in MHC-I downregulation: the poly-proline motif and an acidic cluster-based sequence (EEEE), both motifs located in the N-terminal region of Nef. Although not fully understood, it was proposed that upon Nef binding, a putative tyrosine-based sorting motif found within the cytoplasmic tail of MHC-I is exposed allowing the interaction with AP-1 resulting in the retention of MHC-I in the TGN followed by its degradation in the lysosomes (Tokarev and Guatelli 2011).

Along Nef's potency to modulate cell surface protein expression, Nef can also alter signal transduction such as decreasing the threshold of T-cell activation creating a favorable environment for HIV-1 replication in CD4-positive T lymphocytes. By decreasing the cell surface expression of both CD4 and the CD4-associated lymphocyte-specific tyrosine kinase Lck, Nef induces a narrow T-cell antigen receptor (TCR) response at the plasma membrane while redirecting it to the TGN, resulting in the activation of the Ras-Erk pathway. Nef reroutes host cell signal transduction machinery away from the plasma membrane to assemble active intracellular signalosomes and fine-tune T-cell activation states via association with at least two independent signalosomes, the Nef-associated kinase complex (NAKC) and the complex formed by direct interaction between Nef and the serine/threonine kinase PAK2. In the context of the Nef-PAK2 complex, the viral protein associates with highly active PAK2 and alters the substrate specificity of the kinase. Phosphorylation of novel substrates such as the actin-severing factor cofilin allows Nef to reduce host cell actin dynamic and thus motility of infected T cells. The NAKC is a multiprotein complex

including kinases, the polycomb protein Eed, and protein interaction adaptors. Functionally, NAKC assembly triggers signaling events reminiscent of inside-out integrin signals leading to upregulated virus transcription as well as secretion of extracellular vesicles. Recent findings illustrate that these processes involve the PAK2-dependent phosphorylation of paxillin, thus providing a physical and functional link to the formerly established Nef-PAK2 complex. In the context of this complex, Nef recruits the proteases ADAM10 and ADAM17, and this results in the secretion of activated ADAM proteases in extracellular vesicles, leading to processing of the ADAM substrate TNF α in the target cells of these vesicles and contributing to the apoptosis of bystander T cells (Abraham and Fackler 2012). In macrophages, Nef's poly-proline motif interacts with high affinity with the SH3 domain of the protein tyrosine kinase Hck, an enzyme specifically expressed in myeloid cells such as monocytes and macrophages, thus activating the kinase activity, leading to alterations of the phagocytic and migration functions of infected macrophages (Basmaciogullari and Pizzato 2014). However, both poly-proline and leucine-based motifs of Nef are required for alterations of these macrophage functions, indicating that the Nef-induced perturbations of both intracellular trafficking and signaling pathways play additional roles in these processes.

A decade after HIV-1 discovery, the stimulation of Nef on virus infectivity and replication was revealed. The magnitude of this Nef-mediated enhancement of virus infectivity varies depending on the producer and target cell types and is comprised between 3- and 40-fold with the strongest effect observed when viruses are produced in lymphoid cell lines suggesting that a potential as yet to be discovered restriction factor may be present in these specific cells. Indeed, Nef effect on virus particle infectivity can only be observed if the regulatory protein is present in the producer cells during virus particle biogenesis. To date, though the exact molecular mechanisms remain largely unknown, it is admitted that Nef helps during the early phases of the viral life cycle. Following fusion with the plasma membrane of

the target cell, HIV-1 encounters a barrier consisting of the cortical actin network, and several lines of evidences suggest that Nef affects the actin cytoskeleton by controlling the incorporation of host factors, i.e., dynamin-2 and ezrin, to facilitate the viral penetration. Dynamin-2 is a GTPase responsible for the clathrin-dependent endocytosis and is required for viral infectivity. Ezrin belongs to the ERM family proteins and serves as a connector between the plasma membrane and the actin cytoskeleton. In the target cells, Nef has been documented to enhance cDNA synthesis by promoting the generation of reverse-transcription products suggesting that either the uncoating or the reverse-transcription steps are involved in this activity (Malim and Emerman 2008; Basmaciogullari and Pizzato 2014).

Vpr: An Essential Player of the Early Steps of Virus Replication

The viral protein R (Vpr) is a small regulatory protein (14 kDa) of 96 amino acids. As for Nef, its importance has been initially reported in vivo in rhesus macaques infected with SIV lacking the *vpr*, and the related *vpx* (see below) genes. These infected animals displayed a lower virus burden and did not consistently develop immunodeficiency disease in the absence of Vpr/Vpx expression (Guenzel et al. 2014; Planelles and Benichou 2009). Interestingly, Vpr is the only auxiliary protein specifically incorporated into viral particles via a direct interaction with the C-terminal p6 domain of the Pr55Gag precursor, indicating that Vpr plays critical roles during the early steps of the virus life cycle. In this regard, Vpr has been shown to influence the accuracy of the reverse-transcription process. The HIV-1 reverse transcriptase is an error-prone RNA-dependent DNA polymerase, and Vpr is able to modulate the mutation rate in both dividing and nondividing HIV-1 target cells, even if this activity is more pronounced in terminally differentiated infected macrophages. This role of Vpr was related to the recruitment of the nuclear form of uracil-DNA glycosylase (UNG2), an enzyme of the DNA

repair machinery able to remove the misincorporated uracil in DNA, into virus particles. Recent results suggest that incorporation of UNG2 into virions has a positive impact on HIV-1 infectivity and replication and positively influences the reverse-transcription process. However, several conflicting findings have been reported regarding the role of UNG2, arguing that the protein has rather a detrimental impact on virus replication and may act as a restriction factor. In this model, UNG2 could introduce a-basic sites in place of deoxyuridine created by the well-characterized HIV restriction factors of the APOBEC3 family (see below), leading to the degradation of the neo-synthesized viral DNA. The Vpr protein should induce the proteasomal degradation of UNG2 in virus-producing cells in order to prevent its recruitment into virus particles. The Vpr-induced degradation of UNG2 was related to the ability of Vpr to interact with the DCAF1 subunit of the Cul4a/DDB1 E3 ubiquitin ligase complex containing the damage-specific DNA-binding protein 1 (DDB1), Cullin-4A (Cul4A), and Cul4-associated factor 1 (DCAF1) as well as Rbx1, which would be responsible for targeting UNG2 to the proteasome for degradation (Guenzel et al. 2014; Romani and Cohen 2012).

Once the viral DNA is synthesized, Vpr could be involved in its transport toward the nucleus in association with other viral and host cell proteins to form the so-called preintegration complex (PIC). It was proposed that the PIC moves along the cytoskeletal microtubule filaments using the dynein/dynactin complex as a motor, leading to its accumulation in the perinuclear region close to the centrosome. It is not known whether Vpr plays an active role in the intracytoplasmic transport of the PIC or is only associated with the complex and plays a role later for nuclear membrane anchoring and translocation of the viral DNA into the nucleus (Guenzel et al. 2014). The nuclear envelope (NE) contains two concentric membranes in which are embedded nuclear pore complexes (NPC) consisting of aqueous channels allowing for selective transport between the cytoplasmic and nuclear compartments. The NPC corresponds to a 125 MDa structure consisting of 30 distinct

proteins, named nucleoporins (Nups). Through interaction with some nucleoporins (Guenzel et al. 2014), Vpr docks and accumulates at the NE and may be involved in active nuclear import of the PIC in nondividing cells, such as macrophages. Vpr may thus be responsible for the first step of viral DNA import by targeting the PIC to NPC, while other components of the PIC could trigger the next step of the nuclear translocation. In addition, it has been reported that this high affinity for components of the NE could be responsible for the induction by Vpr of herniations and dissociations of lamina and NE. These herniations provoke a blend of nuclear and cytoplasmic proteins suggesting that Vpr could impact nuclear membrane stability and consequently also facilitates the entry of the PIC through a non-conventional pathway (Planelles and Benichou 2009).

Finally, the best-characterized activity of Vpr is related to its ability to induce a cell cycle arrest in the G2 phase in infected cells. This cytostatic function of Vpr could be a strategy used by HIV to improve viral replication and protein expression. The biological significance of this cell cycle arrest during the natural infection is not well understood, but the HIV-1 LTR seems to be more active in the G2 phase, implying that the G2 arrest may confer a favorable cellular environment for efficient transcription of HIV-1 and viral replication in human CD4 T cells. Different and multiple interactions with cyclins and other key enzymes of the cell cycle have been described to explain the Vpr cytostatic effect. The first studies proposed a Vpr-dependent accumulation of the hyperphosphorylated form of the cyclin-dependent kinase CDC2 (the p34 cdc2/cyclin B complex) that would block the cell cycle prior to mitosis. As mentioned above, Vpr is present in the nucleus and at the NE where it can induce envelope bursting and disturb the nuclear lamina. The modification of the membrane structure would perturb the distribution of several nuclear factors and redistribute them to the cytoplasm, which would alter the cell cycle. In this nuclear context, Vpr could interact with the chromatin and also target and activate two critical sensors of the cell cycle (ATM/ATR, ataxia telangiectasia-mutated and

ataxia telangiectasia and Rad3-related proteins). The upstream events of the signaling pathway activated by Vpr, specifically how Vpr induces activation of the ATR/ATM kinases, were recently highlighted. It was first clearly established that Vpr connects the DCAF-1 (DDB1- and Cul4-associated factor) adaptor of the Cul4A ubiquitin E3 ligase for proteasomal degradation of a cellular target factor leading to G2 arrest. In this model, Vpr recruits both DCAF1 and this factor, which is ubiquitinated and degraded (Guenzel et al. 2014; Romani and Cohen 2012). Then, proteomic analysis finally allowed the identification of a new key target of Vpr, the SLX4 structure-specific endonuclease regulator complex playing a role in nucleic acid metabolism and in DNA repair. It was shown that Vpr activates this SLX4 complex through direct interaction with SLX4, leading to the recruitment of DCAF-1 and the PLK1 kinase. The activated form of the SLX4 complex will then cleave the DNA in association with the MUS81-EME1 endonucleases. Consequently, the activation of SLX4-bound MUS81-EME1 induces the cleavage of DNA intermediates and replication stress, leading to an arrest in the G2 phase in dividing cells (Brégnard et al. 2014). It is interesting to note that Vpr is also able to induce apoptosis in dividing cells and could be responsible, at least in part, for the drastic and critical depletion of CD4 T lymphocytes during the disease progression. Depending on the authors, this cytotoxic effect has been related to the direct consequence of the prolonged cell cycle blockage in the G2 phase induced by Vpr or to the recruitment of the mitochondrial transmembrane adenine nucleotide transporter (ANT) that leads to the classical apoptosis pathway (Guenzel et al. 2014).

Vpx: An Essential Protein for Virus Replication in Nondividing Target Cells

In addition to Vpr expressed by all primate lentiviruses, members of the HIV-2/SIV_{sm}/SIV_{mac} and SIV_{rsm}/SIV_{mnd-2} lineages also express Vpx, probably originated from gene duplication of the *vpr* gene, a 12 to 16 kDa protein, known to

outcome the restriction of HIV-2 and SIV infection in nondividing cells such as macrophages, dendritic cells, and resting CD4-positive T cells. Like Vpr, Vpx is also specifically incorporated into virions and was proposed to participate in the nuclear translocation of the newly synthesized viral DNA within the PIC in nondividing cells such as terminally differentiated macrophages. However, subsequent evidences indicated that Vpx could counteract and induce proteasomal degradation of a cellular restriction factor expressed in myeloid cells through interaction with the CUL4A E3 ubiquitin ligase. Using proteomic approach, SAMHD1 (sterile-alpha motif and HD domain protein I) was finally identified as a target of Vpx for the proteasome machinery (Laguette and Benkirane 2012). SAMHD1, an interferon (IFN)-induced factor in primary monocytes, was already known as associated with the genetic Aicardi-Goutières syndrome characterized by an elevated secretion of IFN- α and an abnormal activation of the immune system. SAMHD1 is an enzyme mainly displaying a deoxynucleoside-triphosphate (dNTP) phosphohydrolase activity and thus maintains low levels of the cellular dNTP pool below the levels required for productive reverse transcription leading to restriction of virus replication. The restriction activity of SAMHD1 is dependent on the phosphorylation status of the protein, and only the dephosphorylated form seems able to efficiently restrict virus replication. Since SAMHD1 is a substrate for phosphorylation by the cyclin-dependent kinase (CDK), such as CDK1, this could explain why SAMHD1 restriction is only efficient in differentiated nondividing cells (Strebel 2013). However, recent studies indicate that SAMHD1 also displays an exonuclease activity, not regulated by phosphorylation, for both single-stranded DNA and RNA and thus can restrict virus replication through damage of viral nucleic acids (Yang and Greene 2014). So far, it is still not clearly established which SAMHD1 enzymatic activity is required or whether both enzymatic activities participate in virus restriction (Yang and Greene 2014). Of note, in some SIVs devoid of a *vpx* gene but containing a single *vpr* gene, such as SIV_{agm}

and SIV_{mus}, the Vpr protein can counteract the restriction activity of their natural host, but Vpr from the HIV-1/SIV_{cpz} cannot.

In addition to SAMHD1, several lines of evidence suggest that other IFN-inducible antiviral factors might be counteracted by HIV-2/SIV Vpx proteins in myeloid target cells. Interestingly, the APOBEC3A, a member of the cytidine deaminase APOBEC3 family, is induced by IFN treatment, may display restriction activity for HIV-1 replication in myeloid cells, and is able to interact with Vpx. It seems that APOBEC3A stability is negatively affected by Vpx through targeting to proteasomal degradation. However, the exact role of Vpx for the control of APOBEC3A activity remains unclear (Schaller et al. 2014). Finally, the Vpx protein of HIV-2/SIV_{sm}/SIV_{mac} lineage certainly plays other functions during virus replication in other cell types than differentiated myeloid cells and resting CD4 T cells, such as activated primary T cells in which the lack of Vpx expression also leads to replication defects (Schaller et al. 2014).

Vif: An Essential Factor for Virus Replication

The viral infectivity factor (Vif) is a 23 kDa protein encoded by all primate HIV and SIV genomes but also by most of other lentiviruses. As evidenced in the SIV-infected macaque model, Vif expression is absolutely required for virus spreading *in vivo*. *In vitro*, it was revealed early that Vif was required for efficient HIV-1 replication in some established lymphoid and myeloid CD4-positive human cell lines, whereas some other cell lines are permissive for *vif*-deleted virus replication. However, the CD4-positive T lymphocytes and myeloid cells are non-permissive to HIV infection in the absence of Vif. Early studies also revealed that the requirement of Vif expression is primordial in virus-producing cells leading to release of virus particles defective for efficient replication in the target cells (Jónsson and Andrésdóttir 2013).

It was finally documented that Vif is essential for virus replication in primary target cells

because it prevents incorporation into released virions of the cellular restriction factors of the apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) family (Jónsson and Andrésdóttir 2013). The seven members of the APOBEC3 family (APOBECA-H) are cellular cytidine deaminase enzymes that catalyze sequence-specific cytidine deamination of single-stranded DNA. While APOBEC3G and APOBEC3F display the most potent restriction activity against primate lentiviruses, other members of the family could also exhibit antiviral activity against *vif*-deleted HIV. All the members of the APOBEC3 family are able to remove the amino group from the cytosine bases creating uracil residues in DNA that lead to the insertion of mutations in HIV DNA (Jónsson and Andrésdóttir 2013). By mutating cytosine into uracil in reverse transcribed (minus strand) HIV DNA, the enzyme induces G to A hypermutations in the second HIV DNA synthesized (plus strand) and makes the virus unable to replicate in the target cells. Furthermore, the newly appearing deoxyuridine in viral DNA could also enhance the cellular DNA repair machinery and simply provoke the definitive fragmentation of the viral DNA. As mentioned above, the uracil-DNA glycosylase (UNG2) could introduce a-basic sites in place of deoxyuridine created by the cytidine deaminase activity of APOBEC3 proteins leading to the degradation of the neo-synthesized viral DNA. Even if the restriction activity of APOBEC3 proteins is mainly related to their cytidine deaminase activity, some studies reported that APOBEC3G and 3F proteins may also exert antiviral activity independently of cytidine deamination through direct action on the reverse-transcription process (Strebel 2013).

It is now accepted that Vif counteracts APOBEC3G through the proteasomal machinery. Vif interacts both with APOBEC3G and the Cul5-based E3 ubiquitin ligase complex to target APOBEC3G for poly-ubiquitination and proteasomal degradation. The E3 complex involved in the proteasomal degradation contains the ElonginB, ElonginC, Cullin-5, and Rbx2. Other cellular factors, such as CBF β , a cellular transcription forming a heterodimer with the RUNX

transcription factors, also interfere with the Vif-induced APOBEC3 degradation mechanism. However, it is still unclear whether CBF β stabilizes the Vif protein folding or triggers Vif conformational changes in order to stabilize the interaction between Vif and the Cul5 E3 complex (Strebel 2013). Interestingly, there is also emerging evidence indicating that Vif can counteract APOBEC3G in a proteasome-independent mechanism. This alternative mechanism of Vif to block APOBEC3G encapsidation without degradation is supported by identification of APOBEC3G mutants that cannot be targeted to the proteasome for degradation but are still counteracted by Vif. In contrast, some Vif mutants with differential efficiency for APOBEC3G degradation still retain the same capacity to block APOBEC3G. Taken together, these observations indicate that Vif uses at least two different ways to hijack APOBEC3 proteins: a degradation-dependent and a degradation-independent mechanism (Strebel 2013).

Vpu: An Essential Protein for Virus Budding

Vpu, standing for viral protein unique, is a transmembrane type I protein of 16 kDa encoded exclusively by HIV-1 and its related SIVs, i.e., SIVcpz, SIVgsn, and SIVmon, but absent from HIV-2 and most of the SIVs. It is expressed later during the viral life cycle suggesting that it is playing a role during the late stages. Indeed, the two main functions assigned to Vpu are the down-regulation of the CD4 expression through proteasomal degradation of the receptor and the enhancement of virion release from the plasma membrane (Malim and Emerman 2008).

In cooperation with Nef (see above), Vpu-induced CD4 degradation is believed to block interaction of CD4 with envelope glycoproteins insuring the release of infectious viruses. Vpu interacts with neo-synthesized CD4 molecules and acts by retention and accumulation of CD4 in the endoplasmic reticulum (ER) followed by its targeting to the ER-associated degradation pathway. While the retention of CD4 requires the

interaction between the transmembrane domains of CD4 and Vpu, the determinants needed for its degradation by the proteasomal pathway are localized within the first alpha helix of the Vpu cytoplasmic domain. Proteasomal degradation of CD4 requires phosphorylation of Vpu on two serine residues situated in the conserved DSGxxS motif localized in the cytoplasmic tail of the viral protein. This phosphorylation induces the recruitment of the cellular beta-transducin repeat containing protein (β -TrCP) that belongs to the Skp1-Cullin-1-F-Box (SCF) E3 ubiquitin ligase complex. β -TrCP recognizes the phosphorylated substrate, here Vpu, allowing the recruitment of the SCF E3 ubiquitin ligase complex that mediates poly-ubiquitination of lysine and serine/threonine residues found in the cytoplasmic tail of CD4 for proteasomal degradation (Malim and Emerman 2008; Tokarev and Guatelli 2011; Strebel 2013).

Similarly to the CD4 downregulation activity, the Vpu-induced viral release has been observed early after HIV-1 discovery but remained uncharacterized during the first 20 years. A major finding was the identification of the cellular restriction factor counteracted by Vpu implicated in this block, the so-called tetherin as this host protein tethers the viral particles to the plasma membrane. Tetherin, also known as the bone marrow stromal antigen 2 (BST-2), is expressed in B cells, dendritic cells, T cells, and macrophages. Remarkably, tetherin is upregulated in response to type I IFN and shows a broad activity by inhibiting the release of various enveloped viruses such as retroviruses; hence, it belongs to the innate immune response against viral infection. Tetherin/BST-2 is a transmembrane protein that presents an unusual topology with a transmembrane anchor near its N-terminus and a glycosylphosphatidylinositol (GPI) lipid anchor at its C-terminus (Strebel 2013; Roy et al. 2014). The accepted scenario of viral particles entrapment provoked by BST-2 suggests that dimers of the protein anchored their N-termini into the cell membrane and their C-terminus GPI domains in the virion envelope. Of note, in HIV-2 and SIVs that do not contain a *vpu* gene, BST-2 is antagonized by determinants found in the HIV-2

surface envelope glycoprotein and in the SIV Nef protein (Strebel 2013).

The mechanism of Vpu antagonism appears to rely on misdirection of BST-2 from the plasma membrane to intracellular compartments followed by its degradation. However, in the CD4-positive T lymphocytes and macrophage primary target cells of HIV-1, the cell surface level of BST-2 is not decreased by Vpu, suggesting that the antagonism relies on the displacement of the restriction factor from the site of viral budding at the cell surface (Tokarev and Guatelli 2011; Roy et al. 2014). Similarly to CD4, Vpu induces the ubiquitination of BST-2 through phosphorylation of its conserved DSGxxS motif in a β -TrCP-dependent manner, but instead of targeting BST-2 to the proteasomal pathway, it redirects it to the lysosomal degradation pathway. Upon BST-2 ubiquitination, Vpu binds to the hepatocyte growth factor receptor-regulated tyrosine kinase substrate (HRS), a component of the endosomal sorting complexes required for transport (ESCRT) machinery involved in the generation of the multivesicular body late endosomal compartment and hijacked by HIV-1 during the budding process. Ubiquitinated BST-2 is recognized by the ESCRT machinery and misrouted to late endosomes for degradation in lysosomes (Roy et al. 2014).

In addition, recent evidence suggests that BST-2 is also able to sense HIV-1-infected cells, and it is hypothesized that Vpu counteracts this innate immune activity via interference with the BST-2 signaling cascade in a β -TrCP-dependent manner (Roy et al. 2014; Sauter 2014).

Conclusion

HIV auxiliary proteins play essential roles during the virus life cycle by rendering the cellular environment favorable to HIV-1 replication, dissemination, and persistence. Indeed, Vif, Vpu, and Vpr counteract restriction factors belonging to the innate immune response, and Nef has evolved to also affect the adaptive immune response ultimately leading to the evasion of the virus from the immune system. Since HIV discovery, lots of

efforts have been devoted to the development of therapeutics conducting to the design of the highly active antiretroviral therapy (HAART). Unfortunately, HAART can limit viral replication but neither completely eliminates the virus from infected patients nor avoids the deleterious depletion of CD4⁺ T cells. Hence, the need to unveil new potential targets seems obvious, and based on their preponderant role, the “essential auxiliary” proteins represent attractive targets for the development of novel antiviral strategies.

Cross-References

- ▶ [APOBEC3F/G and Vif: Action and Counteractions](#)
- ▶ [Budding](#)
- ▶ [Cellular Cofactors of HIV as Drug Targets](#)
- ▶ [Cofilin, Trafficking](#)
- ▶ [Counteraction of SAMHD1 by Vpx](#)
- ▶ [Nef/Env/Vpu/Tetherin](#)

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HIV and Sexual Violence

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Definition

Like HIV, sexual violence is a worldwide public health concern due to the extent of the problem and its multiple consequences to physical and mental health. Sexual violence occurs in all countries, to people of all ages and genders. Published rates of sexual violence from law enforcement and research are rough estimates since most occurrences are not reported due to fear, shame, and guilt on the part of the victims (Jewkes and Abrahams 2002). Researchers, service providers, policymakers, and law enforcement define sexual violence in multiple ways, and attempts have been made to decide upon uniform definitions (Saltzman et al. 1999). The World Health Organization (Krug et al. 2002) defines sexual violence as

Any sexual act, attempt to obtain a sexual act, unwanted sexual comments or advances, or acts to traffic, or otherwise directed, against a person's sexuality using coercion, by any person regardless of their relationship to the victim, in any setting, including but not limited to home and work. (p. 149)

Coercion includes all forms of unwanted aggression including psychological threats and physical intimidation to a person who can provide consent or taking advantage of a person who cannot provide consent due to intoxication or other form of mental incapacitation (Krug et al. 2002). As a result of this broad definition, a wide range of experiences can be classified as sexual violence including rape, attempted rape, sexual harassment, sexual coercion, sexual abuse, forced marriage, forced abortion, forced sexual initiation, denial of contraception or STD protection, genital mutilation, and sexual exploitation and trafficking (Krug et al. 2002; Stockman et al. 2010).

Additionally, sexual violence can occur in tandem with other forms of physical and psychological violence that are also associated with HIV risk and acquisition (Kouyoumdjian et al. 2013; Montgomery et al. *in press*; World Health Organization [WHO] 2001; Krug et al. 2002). Sexual violence can occur in a variety of settings by a variety of perpetrators. Frequent settings include the home, school, work, local neighborhood, health-care settings, and conflict settings. Likewise, perpetrators can range from strangers, intimate partners, relatives, colleagues, law enforcement, health-care providers, teachers, and fellow or opposing soldiers. Regardless of the setting or perpetrator, the mental and physical repercussions of sexual violence can be devastating and long lasting. Sexual violence can even result in death through HIV transmission, traumatic injuries, subsequent infections, suicide, and “honor” murders (Krug et al. 2002).

Intersection with HIV/AIDS

Many of the factors that increase risk for HIV are also associated with greater risk of experiencing sexual violence including alcohol and drug use, societal norms and beliefs about female oppression and male superiority, previous or childhood sexual violence, multiple sexual partners, poverty, and commercial, survival, and transactional sex work (Krug et al. 2002). The World Health Organization (2001) and extensive

peer-reviewed literature have examined the intersection between HIV and different forms of sexual violence including sexual assault (Draughon 2012), intimate partner violence (Gielen et al. 2007; Campbell et al. 2008; Kouyoumdjian et al. 2013), violence against women (Montgomery et al. *in press*; García-Moreno and Watts 2000; Maman et al. 2000; Manfrin-Ledet and Porche 2003; Meyer et al. 2011), and childhood sexual abuse (Senn et al. 2008). Across these reviews, several direct and indirect pathways between HIV and sexual violence have been identified. Sexual violence is directly related to HIV transmission through forced sexual intercourse with a person living with HIV. Risk of HIV exposure during unprotected nonconsensual sex is estimated to be higher than unprotected consensual sex because the likelihood of genital trauma is greater (Draughon 2012). HIV risk during sexual violence depends on the amount of physical force used, the extent and severity of the genital lacerations and abrasions, the location of the penetration (i.e., oral, anal, or vaginal), the amount of exposure to the virus through bodily fluids, and the age and gender of the survivor of the violent attack (World Health Organization 2004; Draughon 2012). Survivors of unprotected receptive anal rape are the most vulnerable to HIV transmission. Likewise, the vaginal tract of prepubescent girls can tear easily, which also puts them at increased risk for HIV transmission (Draughon 2012).

There are also several indirect pathways between sexual violence and HIV risk and acquisition (World Health Organization 2010). Survivors of sexual violence are more likely than individuals who have not experienced sexual violence to participate in HIV risk behaviors, including abusing alcohol and drugs, having multiple partners, having riskier sexual partners, participating in sex while using drugs or alcohol, engaging in transactional sex, and having unprotected sex with a partner who has an unknown or positive HIV status (World Health Organization [WHO] 2001; Krug et al. 2002; Manfrin-Ledet and Porche 2003; Koenig et al. 2004; World Health Organization [WHO] 2004; Senn et al. 2008). Furthermore, surviving sexual violence is associated with

higher sexually transmitted infection rates, which is associated with increased HIV risk (Gielen et al. 2007). Hypothesized reasons for this relationship include less sexual autonomy and poorer sexual risk negotiation skills. Additionally, survivors of sexual violence are more likely to report poorer mental health and psychological symptoms related to chronic anxiety, depression, and post-traumatic stress disorder (Senn et al. 2008). However, few studies have been able to test the temporality of these relationships, and therefore, causality has not been well-established (Campbell et al. 2008; Senn et al. 2008).

Implications

Given how HIV and sexual violence intersect, the World Health Organization suggests synergistically addressing sexual violence and sexual risk at multiple levels to amplify the effectiveness of HIV prevention programs (World Health Organization 2010). Behavior change interventions have taken place to assist HIV-positive and HIV-negative survivors of sexual violence and those at high risk for sexual violence (García-Moreno and Watts 2000; Sikkema et al. 2007; Senn et al. 2008; World Health Organization 2010; Chin et al. 2014). These interventions aim to reduce sexual risk by improving sexual risk negotiation skills, increasing consistent and correct condom use, and increasing HIV knowledge and awareness. Some concurrently aim to reduce drug and alcohol use, increase medication adherence, improve coping skills, address further risk of violence, and reduce traumatic stress.

Additionally, training on the intersection between sexual violence and HIV for law enforcement and health-care providers is recommended as an opportunity to aid survivors of sexual violence. For example, Sexual Assault Nurse Examiners are trained to deal with sexual assault and may be an avenue for reducing HIV risk through HIV-related counseling and education as part of post-rape care (Tufts et al. 2010). Another avenue is the use of post-exposure prophylaxis

immediately after potential HIV exposure (World Health Organization 2010). Screening for HIV or conversely for sexual violence during primary care visits or voluntary HIV-testing and counseling is another option that has not received much attention (World Health Organization 2010). Another option that may be especially effective is the use of programs that address the societal beliefs and norms about gender oppression and superiority which frequently support sexual violence, particularly against women (World Health Organization 2003, 2004).

Lastly, experiencing poverty and living in impoverished areas increase the risk of experiencing sexual violence and acquiring HIV (Goodman et al. 2009; Latkin et al. 2013). As a result, multi-level interventions that improve public awareness of the problem, economically and socially empower those most at risk for sexual violence, and promote legislation and policies that severely punish perpetrators of sexual violence regardless of the setting are all essential to address the devastating intersection between HIV and sexual violence (World Health Organization 2004).

Conclusion

Sexual violence and HIV intersect in many different ways on many different ecological levels. This intersection makes survivors of sexual violence a population at high risk for acquiring HIV. In addition to addressing the HIV-related and other health needs of survivors of sexual violence, national and international health agencies should make preventing both sexual violence and HIV top priorities. Public policymakers and HIV researchers should combat these co-occurring and mutually enhancing health and social problems in a comprehensive manner that addresses their multifaceted nature.

Cross-References

- ▶ [Coflin, Trafficking](#)
- ▶ [Housing as HIV Prevention](#)

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HIV and SIV, B-Cell Responses to

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Definition

Humoral immunity refers to the component of an immune response that is mediated by circulating antibodies. B cells secrete antibodies following differentiation into either short-lived plasmablasts, which can circulate, or long-lived sessile plasma cells that reside primarily in the bone marrow, but can also be found in mucosal tissues and the spleen. Classic memory B cells refer to cells that have encountered antigen, responded by undergoing somatic hypermutation

in the variable regions of their immunoglobulin genes with or without class switching (to IgG, IgA, or IgE) and returned to a resting state (Tarlinton and Good-Jacobson 2013). Upon reencounter with cognate antigen, memory B cells will rapidly respond by differentiating into plasma blasts/cells. HIV infection leads to numerous perturbations of the B-cell compartment that affect several developmental stages of maturation and differentiation. In the absence of antiretroviral therapy (ART), the resting memory B-cell compartment becomes depleted, and altered/activated memory B cells and plasmablast predominate. In advanced disease, there is an overrepresentation of immature/transitional B cells. The impact of these alterations on the humoral immune response to HIV is not completely understood, although recent advances in the investigation of antibodies derived from infected individuals have provided more insight into the dysregulation of this arm of the immune system.

Animal Models Used to Investigate HIV Infection and Associated Humoral Immunity

Studies on SIV in nonhuman primates, either in the setting of natural or induced infection, have helped advance the understanding of HIV infection and disease progression. Nonhuman primates offer several advantages, including being closely related to humans while providing the opportunity to study infection in natural and unnatural hosts that can be manipulated and followed from a known time point. However, there are also several limitations associated with SIV models for HIV disease (Brenchley and Paiardini 2011). Among these limitations are important differences between human and nonhuman primates that relate to immunophenotyping and functional attributes of B-cell subsets. For example, CD10 is one of the most useful markers to identify human immature/transitional B cells as well as germinal center B cells, yet there is no commercially available antibody to evaluate the expression of CD10 in nonhuman primates. Alternative markers of

immaturity such as CD24 and CD38, the latter of which is expressed in a complex and tightly regulated manner during human B-cell development, have not been well characterized in non-human primates. Furthermore, the reduced expression of the complement receptor CD21, an indication of B-cell abnormalities in HIV infection and other human disease settings (Moir and Fauci 2013), is a normal feature of a substantial fraction of memory B cells in uninfected healthy nonhuman primates. In contrast, memory B cells in SIV but not in HIV infection express significantly increased levels of PD-1, a marker of exhaustion that when blocked has been shown to improve the antibody response in treated animals. Finally, plasma blasts/cells, which are overrepresented in both HIV and SIV infection and associated with hypergammaglobulinemia, a hallmark of HIV/SIV infection, bear immunophenotypic features that are distinct to each species. These distinctions hamper the ability to compare differences in tissues and peripheral blood between the species, especially in the setting of SIV or HIV infection and disease progression. Finally, several HIV envelope proteins that are being used as probes to identify HIV-specific B cells with potent HIV-neutralizing potential have thus far not been extended to SIV infection. As an alternative, humanized mouse models have been developed and have provided important insight into the capacity of various HIV-specific antibodies to block HIV infection and/or restrict viral replication *in vivo* (Klein et al. 2013). As detailed below, the concept of passive immunization with recently described HIV-neutralizing antibodies has now been extended to SHIV models in nonhuman primates and HIV-infected individuals.

Early Phase of HIV Infection

It is very difficult to identify and investigate humans immediately upon HIV infection. However, a few studies have succeeded in describing early events following HIV infection with protocols that involve extensive and frequent screening of plasma in order to identify individuals who become positive for HIV RNA while negative

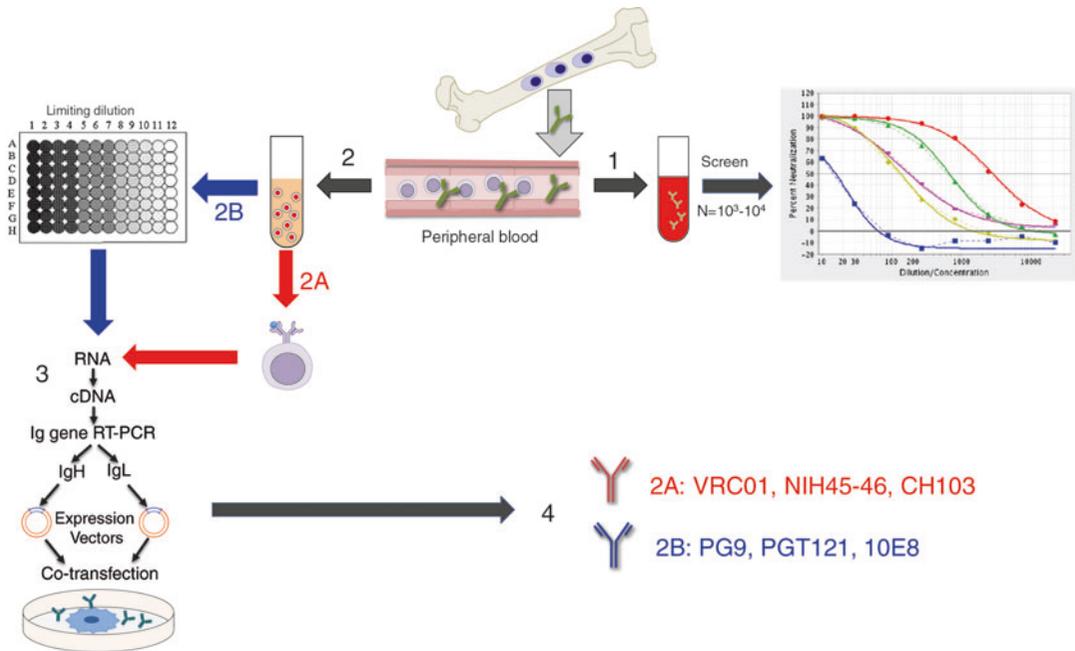
for HIV-specific antibodies. There is a ~12-day window between the time HIV RNA becomes detectable at ~10 days after transmission and the induction of humoral immunity against the virus, which begins with low affinity antibodies against structural proteins such as p24. During most acute viral infections, a strong innate immune response will precede and fuel the adaptive responses and generate a cytokine “storm,” of which the one induced by HIV infection is particularly strong (Cohen et al. 2011). Several of the cytokines induced during the acute phase of HIV infection target B cells; they include IP-10, IL-10, and IL-6. One of the corresponding early events of acute viral infections, as well as immunizations, is the transient appearance of plasmablasts in the peripheral blood at ~7 days postexposure, of which a variable percentage is pathogen-/immunogen-specific (Fink 2012). While there are no studies that have captured this burst of plasmablasts during acute HIV infection, these differentiated B cells are overexpressed in the peripheral blood during the early months of HIV infection.

Most of the plasmablasts found in circulation in acute/early HIV infection are not specific for the virus, likely reflecting the heightened level of nonspecific immune activation. The increased frequencies of plasmablasts in early as well as chronic phases of HIV infection are associated with hypergammaglobulinemia, suggesting that circulating plasmablasts are a source of these immunoglobulins. In this regard and despite the low frequencies of HIV specificity, circulating plasmablasts have been shown to be a source of antibodies against gp41, the transmembrane component of the HIV envelope, that appear in the blood a few weeks after transmission (Bonsignori et al. 2012). These early antibodies, which first appear complexed with virions, are non-neutralizing and do not drive escape mutations in the virus. Analysis of the gp41-reactive antibodies derived from circulating plasmablasts, as well as bone marrow plasma cells of recently HIV-infected individuals, revealed that they were highly mutated. However, these mutations are unlikely to reflect a response to HIV itself and more likely indicative of a polyreactive response

that arose from cross-reactivity with preexisting memory B cells, possibly against gut-derived antigens (Bonsignori et al. 2012). Polyreactivity is a common feature of HIV-specific antibodies, especially those directed against gp41, and it remains uncertain whether this feature is a desired or detrimental feature for neutralization (Klein et al. 2013; Bonsignori et al. 2012). Nonetheless, there is overwhelming evidence that the early antibody response against HIV is ineffective, and furthermore, once strain-specific neutralizing antibodies appear at approximately 80 days post-infection, escape mutations rapidly enable the virus to evade the autologous antibody response (Mascola and Haynes 2013). Paradoxically, it is the ongoing cat and mouse game that is thought to drive the induction of a broadly and potent neutralizing antibody response in approximately 20% of HIV-infected individuals, although the response provides limited clinical benefit to these individuals. However, it should be noted that while the antibody response is unlikely to contribute to long-term non-progression of disease, as has been shown for the CD8⁺ T-cell response in a certain percentage of ART-naïve individuals, there is nonetheless a possibility that antibodies can suppress viral replication and slow disease progression. In this regard, the majority of the highly potent and broadly HIV-neutralizing antibodies that have recently been identified were isolated from slow progressors (Mascola and Haynes 2013). As discussed below, passive immunization with such antibodies can significantly suppress viremia in HIV and SIV pre- and post-infection.

Chronic Phase of HIV Infection

The appearance of HIV strain-specific neutralizing antibodies coincides approximately with the transition to the chronic phase of infection. In the majority of ART-naïve individuals, this phase is associated with detectable viremia, accompanied by genetic diversification of HIV. At least a portion of this diversification is driven by antibody-mediated pressure. In turn, the heterogenic nature of the virus appears to also drive diversification of



HIV and SIV, B-Cell Responses to, Fig. 1 Strategies for isolating HIV-neutralizing antibodies. (1) Serum antibodies, most likely produced by plasma cells in the bone marrow, are screened for HIV-neutralizing activity. (2) Peripheral blood B cells are submitted to either (A) HIV-specific single B-cell sorting or (B) limiting dilution culturing. (3) The variable regions of immunoglobulin

heavy and light chains are amplified and cloned into vectors containing respective constant regions. The vectors are co-transfected into 293T cells, and supernatant containing the secreted antibodies is collected for analysis or purification. (4) Examples of HIV-specific antibodies and methods used to obtain the antibodies listed

the antibody response, which in a certain percentage of individuals (ranging from 10% to 30% depending on cohorts) leads to a broad and potent neutralizing antibody response (Klein et al. 2013; Mascola and Haynes 2013). Those individuals with the strongest responses, typically the top ~5% of cohorts, are referred to as “elite” neutralizers. It is important to note that this term refers to serologic activity and not to the clinical definition of elite non-progressors who are capable of maintaining aviremia in the absence of ART (Perreau et al. 2013). In addition, the HIV-neutralizing activity measured in the serum does not distinguish between an antibody response of single specificity and a response comprised of multiple specificities that in combination exhibit broad and potent neutralizing activity against the virus. With the advent of high-throughput technologies designed to screen large numbers of serum samples for neutralizing

activity against large panels of HIV isolates and advanced computational methods, there is now the capability of deciphering the components of neutralizing antibody responses without having to isolate each constituent (Kwong et al. 2013). However, monoclonal antibody reconstitution from single B cells remains the method of choice for definitive characterization of antibody activities. As described in more detail below, most of these single-cell analyses have been performed on memory B cells isolated from the peripheral blood of HIV-infected individuals whose serum had been prescreened for HIV-neutralizing activity.

High-throughput approaches that combine serum screening with single-cell dilution or isolation schemes have yielded a trove of new highly potent and broadly HIV-neutralizing antibodies (details in section “[Insight into Vaccine Development and Passive Immunization](#)” and Fig. 1). However, there appears to be few clinical

indicators, whether immunologic or virologic, that are predictive of individuals who are most likely to develop a potent and broad HIV-neutralizing antibody repertoire. Furthermore, even strong HIV-neutralizing antibody responses identified in elite neutralizers appear to be ineffective against their own virus. Nonetheless, the knowledge gained regarding the evolution of antibody responses in HIV-infected individuals and, most importantly, the identification of the germline antibodies that potentially initiated these responses are thought to be critical for the development of immunogens that could elicit similar response in uninfected individuals (Kwong et al. 2013). Such schemes could involve successive immunizations with different viral proteins, each designed to drive the memory B-cell response towards an increasingly more diverse and potent antibody response against HIV. This scenario would most likely involve memory B cells that had been elicited by the first immunogen being recruited to undergo further diversification in response to the next immunogen in the series. There is evidence from other pathogens, including influenza, that diversification and strengthening of virus-specific B-cell responses can occur over time, either from exposure to the pathogen itself or repeated vaccination with modified immunogens (Chiu et al. 2013).

There are several caveats to consider when developing an HIV vaccination scheme that is guided by the evolution of antibody responses in infected individuals. In a majority of ART-naïve individuals, their chronic phase of infection, while clinically asymptomatic, is accompanied by persistent immune activation that is manifested by several systemic alterations, including increased levels of inflammatory cytokines and other soluble factors. B cells are affected by chronic HIV viremia, as evidenced by persistent hypergammaglobulinemia and changes in circulating B-cell subsets, including antigen-experienced subsets. The circulating B-cell populations are quite distinct in HIV disease. Typically, resting memory B cells are depleted in HIV-infected chronically viremic individuals, whereas exhausted and activated memory B cells as well as short-lived plasmablasts are enriched when

compared to uninfected individuals (Moir and Fauci 2013). In addition, the presence of a persisting pathogen, such as HIV, is also associated with changes in secondary lymphoid tissues, including perturbations in the gut-associated lymphoid tissues (GALT) that likely fuel immune activation (Brenchley 2013). One of the more obvious consequences of HIV-induced immune activation is lymphoid tissue hyperplasia that is characterized by the presence of prominent germinal centers. While these histological observations were made decades ago on lymph nodes (Tenner-Racz and Racz 1995), it is only recently that hyperplasia in lymph nodes of HIV-infected individuals has been associated with increased numbers of CD4⁺ T follicular helper cells and germinal center B cells. Similar observations have been made in SIV-infected macaques. While there is some debate as to whether these conditions are helpful or harmful to the development of HIV-neutralizing antibodies (Ma and Deenick 2014), the persistence of virus is responsible for these changes and unlikely to be elicited and/or sustained following vaccination. Despite these differences, the advances made in understanding the antibody response in HIV-infected individuals and potential implications for vaccine development far outweigh the caveats mentioned here and elsewhere.

Advanced HIV Disease

Our understanding of B cells and the antibodies they secrete in response to HIV has advanced greatly over the past few years as it relates to the early and chronic clinically asymptomatic phases of infection. What has received less attention, and for good reason, is how such responses are affected by advanced HIV disease. Given the widespread availability of ART, even in developing countries, relevant observations on the effects of HIV disease progression on the antibody response to the virus are generally restricted to stored specimen or reinterpretation of earlier findings. The systemic changes that occur with advancing HIV disease would suggest a waning of immunity against the virus. Hyperplastic lymph

nodes become fibrotic, and the cell-cell interactions involved in maintaining immunity become restricted and impeded by increased collagen deposits (Zeng et al. 2012). These changes in lymphoid tissues are accompanied by notable systemic changes, including a reversal of hypergammaglobulinemia and increased levels of other soluble factors that are associated with hyperactivation. These declines are accompanied by increasing levels of factors associated with lymphopenia and loss of homeostasis, IL-7 being the most studied example. The paucity of memory B cells that begins in the early phase of HIV infection is exacerbated with advancing disease. As generalized lymphopenia progresses, immature/transitional B cells become more prominent in the peripheral blood and can be the major subset in individuals with CD4⁺ T-cell counts below 50 cells/ μ l. These B cells respond poorly to stimulation and are highly susceptible to intrinsic apoptosis (Moir and Fauci 2013). In this context, where CD4⁺ T-cell help is severely curtailed, B cells are unlikely to generate adequate responses against all pathogens, including HIV. At this stage of advanced disease, the circulating antibodies against HIV are most likely to originate from plasma cells that were seeding in the bone marrow in the years prior to disease progression. Despite these extreme alterations, recent studies conducted in SIV-infected monkeys indicate that even under conditions of severe lymphopenia, both cellular and humoral immunity can be enhanced by certain immunomodulatory interventions (Kulpa et al. 2013), suggesting that similar outcomes may be achieved in humans. In this regard, studies in advanced HIV disease suggest that the vast majority of individuals in advanced disease respond well to ART and can regain significant immunologic function (Deeks et al. 2013).

Effect of ART-Mediated HIV Suppression on B Cells

The vast majority of studies have shown that the reduction of HIV viremia by ART leads to a full or partial normalization of the B-cell compartment,

including an increase in resting memory B cells and a decrease in the more activated and differentiated subsets (Moir and Fauci 2013). However, there is also evidence that the reduction of HIV viremia by ART leads to decreased frequencies of HIV-specific B cells, indicating that at least part of B-cell immunity is sustained by ongoing viral replication. Nonetheless, there are several indications that it is not simply ongoing viral replication that drives HIV-specific B-cell responses, and little is known regarding the effect of decreased viremia on long-lived plasma cells, the cells in the bone marrow that are thought to be largely responsible for the antibodies that circulate in the blood. Furthermore, both the quantity and quality of B-cell responses are influenced by stage of infection and timing of ART. When ART is initiated in the early phase of infection, the resting memory B-cell compartment, both in terms of numbers and functional attributes, is better able to reconstitute itself than when ART is initiated during the chronic phase of infection (Moir and Fauci 2013). There are also indications that decreases in HIV-specific B-cell responses are not as precipitous in early treated individuals than in those individuals who initiate ART during the chronic phase of infection. Furthermore, whereas a majority of the HIV-specific response is contained within activated and exhausted memory B cells in untreated individuals, once HIV viremia is reduced by ART, these B cells disappear, whereas resting memory B cells, some of which are HIV-specific, are maintained or even expanded, especially in individuals who initiate ART early. However, these are preliminary observations that require further confirmation with analyses involving large and diverse cohorts of HIV-infected individuals. Nonetheless, these observations have implications for the overall immune competency of infected individuals and also for achieving sustained virologic remission where individuals would be able to stop ART and rely on their own immune system to prevent disease progression. While the antibody response alone is unlikely to prevent plasma viral rebound, a combination of humoral and cellular immunity with or without intermittent passive immunization may provide long-term “virologic remission” in

the absence of ART. It is in this unlikely yet possible future scenario that the maintenance of a competent memory B-cell repertoire against HIV would be very important.

Insight Into Vaccine Development and Passive Immunization

There are numerous virus- and host-related factors that have been identified as potential obstacles to the development of an effective HIV vaccine. While most of these factors are beyond the scope of this chapter, B cells of HIV-infected individuals and their derived antibodies have been a major focus of the renewed interest in an antibody-based vaccine. Prior to 2009, only a handful of HIV-neutralizing monoclonal antibodies had been characterized, all were derived from infected individuals, and all possessed unusual properties that were considered impediments relative to vaccine strategies (Kwong et al. 2013). Since 2009, several technical and computational advances have enabled the identification and synthesis of hundreds of HIV-specific monoclonal antibodies, many of which with breadths and potencies superior to the previous ones. The more recent crop of HIV-specific antibodies has been derived from circulating memory (IgG⁺) B cells of HIV-infected individuals, most of whom were identified by the screening of serum from a large number of individuals to identify those with broad and potent HIV-neutralizing antibody responses. These high-throughput screens, which test serum samples against panels of diverse HIV reporter strains, have led to the observation that approximately 20% of untreated HIV-infected individuals will develop a broad HIV-neutralizing antibody response during the chronic phase of infection.

Two approaches have been used to derive monoclonal antibodies from memory B cells of HIV-infected individuals (Fig. 1). One approach involves single-cell sorting and makes use of fluorochrome-labeled HIV envelope protein probes that bind to the B-cell receptor (BCR) of HIV envelope-specific B cells. One caveat to this approach is that the HIV envelope is known to bind non-BCR receptors on B cells. The variable

regions of the heavy and light chain immunoglobulin genes are then amplified, cloned into vectors that contain the constant elements of the human IgG1, and expressed/secreted in the human 293T cell line (Moir et al. 2011). Examples of antibodies derived with this approach include CD4 binding site-specific antibodies VRC01 and NIH45-46. The second approach involves performing limiting dilution followed by *in vitro* expansion of memory B cells isolated from pre-screened HIV-infected individuals. The end result is similar to that of the single-cell sorting approach in that monoclonal antibodies are derived from the clonally expanded B cells. Examples of such antibodies include PG9 and PG16 that recognize epitopes in the V1/V2 loops of gp120, the PGT series of antibodies that recognize peptidoglycan epitopes within the variable loops of gp120, as well as 10E8 that recognizes the membrane-proximal region of gp41.

A number of factors need to be considered when isolating HIV-specific antibodies from B cells of HIV-infected individuals. In the initial screening process, the antibodies being measured in serum likely originate from plasma cells that reside in tissues, either bone marrow or secondary lymphoid organs. However, the immunoglobulin genes that are isolated in the subsequent steps originate from memory B cells that circulate in the peripheral blood. There is an inherent assumption that the immunoglobulins secreted by plasma cells are related to those expressed on the surface of memory B cells as part of their BCR. This relationship remains largely unknown for HIV, whereas a direct link between antibodies secreted by plasma cells and the BCR of memory B cells has been observed for some pathogens, such as influenza, but not others (tetanus). Furthermore, current single-cell sorting strategies are somewhat limited by the nature of the probes and the ability of these probes to bind to HIV-specific BCRs at intensities that are clearly above background. Probes that identify CD4 binding site-specific B cells have been used with much success, but not all desirable epitopes, especially quaternary ones, within the HIV envelope can be targeted with this approach. Conversely, those antibodies derived from clonal dilution and *in vitro*

expansion will select those B cells that can survive and expand under the culture conditions used. In this regard, several subsets of B cells that are overrepresented in HIV-infected individuals and account for most of the HIV-specific response, including activated and exhausted memory B cells, are less likely to survive and expand during the *in vitro* selection process than resting memory B cells (Moir and Fauci 2013). Finally, there are concerns that the best neutralizing antibodies that have been derived from memory B cells of HIV-infected individuals involve an induction and maturation process that may be difficult to elicit in uninfected individuals following vaccination (Mascola and Haynes 2013). Several of the antibodies derived from infected individuals have properties that are not normally observed during an immune response, including polyreactivity and/or autoreactivity; long complementarity determining regions (CDR), especially CDR3 in the heavy chain; and extensively mutated variable regions in both heavy and light chains. There are also indications that mechanisms of tolerance and anergy are responsible for the rarity of certain antibodies that have potent neutralizing activities, including those directed against the membrane-proximal region of gp41 and antibodies that have long CDR3s. Nonetheless, the knowledge gained by investigating HIV-specific antibodies and B-cell responses in infected individuals has proven invaluable to the field and has prompted a search for vaccine strategies that would overcome the limitations described herein. As described above, one such strategy involves administering successive immunogens designed to direct the antibody response towards a desired outcome, whereas other strategies involve adjuvants and other immunomodulating agents designed to amplify responses or to overcome the negative selective forces of tolerance and/or anergy (Mascola and Haynes 2013).

The recent success in identifying highly potent and broadly HIV-neutralizing antibodies from infected individuals has also led to a renewed interest in passive immunization. The concept of preventing infection was first illustrated over a decade ago in the SIV model using relatively

large doses of neutralizing antibodies that were available at the time (Mascola and Haynes 2013). More recently, HIV viremia in infected individuals was decreased transiently, although again, this was with the older HIV-neutralizing monoclonal antibodies that were more weakly neutralizing when compared to the most recent ones described above. Over the past year, several studies performed in nonhuman primates and humanized mice have demonstrated that infection can be prevented, and viremia significantly reduced following infection, with relatively low concentrations of potent bNAbs, especially when several are administered in combination (Klein et al. 2013). In HIV-infected individuals, safety trials are being conducted with the latest generation of potent and broadly neutralizing antibodies, including VRC01. These trials are also designed to determine whether viremia can be decreased with these broad and potent HIV-neutralizing antibodies. The main purpose of these studies is to ultimately determine whether passive immunization can be used as a means of prevention or as a component of a combination strategy for maintaining HIV-infected individuals in remission.

Conclusions

Since 1983 when B-cell perturbations were first described in HIV-infected individuals, much has been learned regarding B-cell pathogenesis and hypergammaglobulinemia following infection. However, it is only with the recent emphasis on the antibody response to HIV in infected individuals that much progress has been made to better understand HIV-specific humoral immunity and to apply such knowledge towards the development of an effective antibody-based vaccine. While there is much to learn and much to do, this task does not seem as daunting and impossible as it did just a few years ago. As research efforts continue in this area, the outcomes are likely to provide new insight into the fundamentals of human immunology and possibly translate into new and ultimately successful approaches towards an effective HIV vaccine.

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HIV and SIV, CD4 T-Cell Responses to

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Definition

Immune responses to infections are a multifaceted interplay between different specialized cell types. These arms of the immune system are broadly divided into innate immunity, which is a rapid first line of defense that has limited specificity toward a particular pathogen, and adaptive immunity, which is delayed but develops with high specificity for pathogens, and once it is established, it has the potential for strong recall responses (immunological memory) (Abbas et al. 2012a). Successful cross talk between innate and adaptive immunity leads to effective immune responses and pathogen clearance. In HIV infection, however, defects in the function of both systems lead to ineffective immune responses that result into lack of viral control and chronic immune activation (Mandell et al. 2010).

CD4 T cells also known as T-helper cells (T_h) are cells of the adaptive immune system, and their main role is to orchestrate immune responses by fine tuning the cross talk between the innate and the adaptive immune systems (Abbas et al. 2012b). CD4 T cells are a subset of lymphocytes that develop and mature into the thymus, where a wide diversity in T-cell receptors (TCR) is generated. A given CD4 T cell expresses a single type of TCR, and it is the specificity of this TCR that determines what antigen the T cell can respond to. Naïve T cells then spread throughout the body and locate preferentially into lymphoid tissues, such as lymph nodes. CD4 T cells get activated when their TCR efficiently binds to an antigen presented on a major histocompatibility complex (MHC) class II molecule by an antigen-presenting cell (APC). This stimulation

of T cells by the TCR (primary signal) is modulated by a wide array of stimulatory or inhibitory secondary signals, including co-signaling surface molecules and cytokines (Abbas et al. 2012b). Upon antigen recognition and activation, CD4 T cells secrete cytokines that modulate function of other cells that contribute to innate immunity such as monocytes/macrophages, NK cells, and dendritic cells, which are highly effective antigen-presenting cells that bridge innate to adaptive immunity, as well as cells of the adaptive immune system such as B cells and CD8 T cells. When activated, undifferentiated interleukin-2 (IL-2)-secreting CD4 T cells will differentiate into several CD4 subsets that are categorized according to the cytokine they produce (Abbas et al. 2012a; Abbas et al. 2012b). The two main subsets that were initially described are the Th1 CD4 T cells, which secrete interferon- γ (IFN γ) and tumor necrosis factor- α (TNF- α) and modulate cell-mediated responses, and the Th2 lymphocytes, which predominately produce IL-4, IL-5, and IL-13 and regulate humoral immune responses. Another CD4 T-cell subset is the Th17 cells that secrete IL-17 and are important in regulating antibacterial and antifungal functions in the tissues. More recently, a new CD4 T-cell subset has been identified that resides in the lymph nodes and produces among others high levels of IL-21. This CD4 T-cell subset, also known as T-follicular helper (Tfh) CD4 T cells, modulates germinal center formation and provides help to B cells for the generation of antibodies (Abbas et al. 2012b). Newer data have challenged the traditional model of stably defined and separate CD4 T-cell lineages. CD4 T-cell differentiation appears to have a higher plasticity than initially thought, with many CD4 T cells presenting mixed phenotypes and functions and being influenced by their environment in body tissues.

CD4 T-Cell Depletion in HIV Infection

One of the first immunologic disorders characterized in HIV infection is the marked reduction in CD4 T-cells counts that leads to impaired immune functions and progression to AIDS. The loss of

CD4 T cells has been the focus of intense research that so far indicates a multifactorial basis: (1) direct destruction of HIV-infected CD4 T cells, (2) viral proteins that cause apoptosis of uninfected CD4 T cells, (3) abnormally high levels on immune activation that leads to activation-induced cell death of CD4 T cells, and (4) impaired renewal of the established pool of CD4 T cells (Levy 2007; Porichis and Kaufmann 2011). Recent studies have identified a novel mechanism of uninfected CD4 T-cell depletion where CD4 T cells that are abortively infected by HIV proceed to programmed cell death that is triggered by the abortive infection (Levy 2007). A massive destruction of memory CD4 T-cell populations, in particularly of those located in the gut-associated lymphoid tissue (GALT), occurs at the time of acute HIV infection, and this defect is only partially corrected by antiretroviral therapy (ART) (Mandell et al. 2010). Detailed tissue studies in nonhuman primates such as rhesus macaques infected by the simian immunodeficiency virus (SIV) have been particularly revealing in this regard. It is very likely that this extensive damage also cripple the HIV-specific CD4 T cell very early in infection. This defect in mucosal CD4 T-cell immunity is thought to play an important role in the ongoing translocation of bacterial products observed in HIV infection (Haase 2010). This phenomenon contributes to chronic immune activation with deleterious effects on the immune system and other complications such as accelerated cardiovascular disease (Mandell et al. 2010).

Consistent with this critical role played by the damage to the CD4 T-cell compartment in HIV pathogenesis, it was shown that elite controllers, who are rare individuals that achieve spontaneous control of HIV infection in the absence of ART, have the ability to maintain persistent functional memory T cells that are critical for long-lasting protection after exposure to pathogens, in contrast to individuals with progressive chronic HIV infection (chronic progressors or CP). Elite controllers preserve central memory (T_{cm}) CD4 T cells that have higher expression of IL-7 receptor. IL-7 is a critical cytokine that regulates T-cell homeostasis

and progressive, HIV infection is correlated with lower responsiveness to IL-7. Therefore, the higher expression of IL-7 receptor in CD4 T cells from elite controllers favors maintenance of these cells in contrast to chronic HIV-infected subjects that have CD4 T cells with low IL-7 receptor, which leads to reduced sensitivity to IL-7 and increased CD4 T-cell loss. Additionally, it was shown that in chronic HIV infection, deposition of collagen in lymphoid tissues resulted in reduced accessibility of T cells to IL-7 on the fibroblastic reticular cell network leading to enhanced CD4 T-cell depletion in these tissues. Administration of IL-7 in ART-treated subjects suggested a possible benefit of this cytokine as treatment to restore CD4 numbers in individuals whose immune system shows suboptimal restoration in spite of effective antiviral therapy, but clinical gains remain to be demonstrated.

Class II Recognition of CD4 T Cells

CD4 T cells utilize a heterodimeric cell surface protein, the T-cell receptor (TCR), to recognize foreign antigens bound to the human leukocyte antigen (HLA) class II molecules on antigen-presenting cells. The antigens parts presented on HLA molecules, or epitopes, are typically small chains of amino acids (peptides) that derived from proteins (Abbas et al. 2012a, 2012b). HLA class II molecules can accommodate larger peptides, 12–24 amino acids long, than class I molecules that are recognized by CD8 T cells and typically bind to epitope that are 8–11 amino acid long. The HLA molecules are highly diverse in human populations and genetically inherited from both parents. Their structure determines which antigen they can present which in turn can influence the ability of an individual to mount an effective T-cell immune response to a given pathogen or to develop specific diseases, e.g., the risk to develop certain types of autoimmune diseases. In the setting of HIV infection, some specific HLA class I alleles, in particular HLA-B*2705 and HLA*B5701/03, have been shown to be

associated with better outcome. In contrast, data on HLA class II alleles have been far less conclusive, although some studies suggest that some HLA class II molecules such as HLA-DRB1*13, HLA-DRB1*1502, and HLA-DQB1*06 are associated with immune control and/or more robust and functional HIV-specific CD4 T-cell responses (Porichis and Kaufmann 2011). This lack of strong association does not prove a weaker role for HIV-specific CD4 T-cell responses in viral control, as many CD4 T-cell epitopes can be presented by multiple HLA class II molecules, contrasting with a more specific binding repertoire for HLA class I molecules and CD8 T-cell responses. The fact that the same HIV epitopes can be broadly recognized by CD4 T cells in genetically diverse human populations may be useful for future development of an HIV vaccine with a broad population coverage.

Function of HIV-Specific CD4 T Cells

Although a number of studies in animal infectious disease models and humans suggest that effective HIV-specific T-helper responses will be critical both for control of viral replication in infected people and for development of an effective preventive vaccine, knowledge is currently still largely lacking on which specific functions are required for protective immunity (Porichis and Kaufmann 2011). Like in many other human studies, when investigations identify differences in characteristics of HIV-CD4 T-cell-specific responses in subjects who are able to maintain low levels of viremia as compared to people with progressive disease, it is difficult to tell apart factors that can contribute to better disease outcome from consequences of a better preserved immune system (Younes et al. 2003). In most infected individuals, HIV-specific CD4 T-cell responses are impaired early in the course of HIV infection. Some studies demonstrating preferential infection of HIV-specific CD4 T cells by HIV as compared to T-helper cells from other specificities, such as CMV, raised the concern that eliciting HIV-specific CD4 T-cell responses

by vaccines or immunotherapeutic strategies could fuel viral replication and have detrimental consequences for infected individuals (Douek et al. 2002). However, the initial notion that HIV-specific CD4 T cells are preferentially infected and as a consequence are depleted from the circulation is now being revisited since the large majority of HIV-specific CD4 T cells are not infected *in vivo*. Even though CD4 T-cell depletion is indeed a major reason for the collapse of the immune system at the later stages of HIV infection, it was shown that CD4 T cells lose their functionality before substantial CD4 T-cell depletion. The proliferative capacity of HIV-specific CD4 T cells was one of the first functions shown to be diminished in chronically HIV-infected subjects. It was later shown that the reduced proliferative capacity was a consequence of reduced IL-2 secretion by HIV-specific CD4 T cells. HIV-specific CD4 T cells in elite controllers have a greater capacity to proliferate *in vitro* compared to subjects with progressive HIV disease, suggesting a stronger capacity to mount secondary immune responses. Even though the magnitude of HIV-specific CD4 T cells as measured by IFN γ secretion did not correlate with viremia, elite controllers were found to have more HIV-specific CD4 T cells that are polyfunctional, i.e., are capable to produce multiple cytokines at the same time, than those of subjects with progressive HIV disease. However, detailed studies comparing HIV-specific CD4 T-cell responses in untreated individuals and people efficiently treated with ART showed that the functional profiles of the T-helper responses get closer to those of elite controllers once viremia is suppressed by therapy, suggesting that most of these differences are a consequence, rather than a cause, of viral control (Harari et al. 2004). Of note, a significant proportion of elite controllers maintain robust HIV-specific CD4 T-cell responses in spite of undetectable viral load (Ferre et al. 2010), whereas an attrition of these responses is seen once chronic progressors are put on therapy. This antigen dependence is classical of dysfunctional T-cell responses in chronic infection and has also been well described in animal models of chronic infection (Virgin et al. 2009).

Cytolytic CD4 T Cells

The majority of HIV-specific CD4 T cells in the blood produce IFN γ , TNF α , and IL-2, suggesting dominant antiviral Th1 responses in the absence of detectable Th2 cytokine secretion such as IL-4 or IL-13. Even though the main function of CD4 T cells is to orchestrate immune responses through cytokine secretion, recent studies have highlighted the ability of some CD4 T-cell subsets to kill infected cells either through direct cytolytic activity mediated by perforin or through Fas/Fas ligand-induced apoptosis. Cytolytic CD4 T cells have been observed in many chronic viral infections such as Epstein-Barr virus (EBV), hepatitis C virus (HCV), influenza virus, and cytomegalovirus (CMV). The most compelling data were derived from the lymphochoriomeningitis virus (LCMV) mouse model where LCMV-specific CD4 T cells with cytolytic activity killed virally infected cells *in vivo* and contributed to control of LCMV infection.

Cytolytic CD4 T cells have also been reported in HIV infection, although their importance in controlling the infection and the actual targets are still not well understood. Some of the first studies identified HIV-specific CD4 T-cell clones that showed potent killing capacity of HIV-envelope pulsed B cells *in vitro*. However, the fact that these cells were cultured for a long time *in vitro* in conditions that may favor certain functions raised the question whether HIV-specific CD4 T cells exist and could play a role *in vivo*. Ex vivo killing assays showed that both HIV- and SIV (simian immunodeficiency virus)-specific CD4 T cells have the ability to kill target cells pulsed with HIV or SIV peptides. Studies in SIV infection of nonhuman primate models suggested a preferential killing of macrophages over infected CD4 T cells that may arise by the high expression of HLA class II molecules on the surface of macrophages (Sacha et al. 2009). Infected CD4 T cells downregulate class I HLA molecules as a result of expression of viral proteins such as NEF and may therefore be more resistant to killing by cytotoxic CD4 T cells. Thus, cytolytic CD4 T cells might complement the activity of their CD8 T-cell counterpart for

specific infected cell subsets. A more recent study showed that cytotoxic activity of CD4 T cells, as measured by granzyme A, correlated with slower disease progression in HIV-infected subjects (Soghoian et al. 2012). This may suggest a role of cytolytic HIV-specific CD4 T cells in controlling infection or, as discussed above, may reflect a better preservation of some immune functions in subjects with favorable outcome.

T-Follicular Helper CD4 Cells

Another CD4 T-cell population that has gained focus in recent years is the T-follicular helper CD4 T-cell subset. Tfh cells reside in lymphoid follicles within lymphoid organs and exert their function within the germinal centers (GCs), which are the anatomic compartment where B cells and Tfh cells come in contact. Tfh cells are specialized CD4 T cells that provide help to antigen-specific B cells to support their differentiation and antibody class switching and secretion. They were initially identified by their high expression of B-cell zone homing CXC chemokine receptor 5 (CXCR5) (Abbas et al. 2012a). Subsequent studies characterized Tfh cells by the combined expression of CXCR5 with co-stimulatory molecules such as ICOS, CD40L, and the inhibitory receptor programmed cell death protein-1 (PD-1). Tfh cells in the germinal centers express high levels of the transcriptional repressor B-cell lymphoma 6 (BCL-6), and they secrete the cardinal cytokine IL-21.

T-follicular helper cells have drawn a lot of attention in the past few years due to their critical role in antibody development. Broadly neutralizing antibodies, whose generation is thought to be critical for any effective HIV vaccine, exist only in a small number of HIV-infected individuals. Their development requires the accumulation of a series of amino acids mutations during antibody maturation that occurs over an extended period of time. It is therefore very likely that vaccines aiming at eliciting broadly neutralizing antibodies need to accommodate strong, long-lasting Tfh responses that will help create functional germinal centers and provide constant help to B cells until

the generation and maturation of broadly neutralizing antibodies is achieved (Abbas et al. 2012a).

In HIV and SIV infections, a number of studies showed an accumulation of Tfh cells in the lymph nodes during chronic HIV and SIV infections. The increase in Tfh frequency was correlated with plasma viremia, the number of GCs, the frequency of plasma cells, and the onset of hypergammaglobulinemia suggesting a direct functional impact of Tfh cells on humoral responses during chronic HIV and SIV infection (Lindqvist et al. 2012; Petrovas et al. 2012). A recent study showed that even though Tfh cells frequencies are increased in chronic infection, they are dysfunctional, leading to deregulated help to B cells (Cubas et al. 2013). The increase in Tfh CD4 T-cell numbers contrasts with the general depletion of CD4 T-cell subsets occurring in HIV infection. Tfh cells were found to be susceptible to infection with HIV and SIV, and human Tfh were shown to support high levels of viral replication and contained more copies of HIV DNA than other CD4 memory subsets, suggesting that they may serve as preferential reservoir of the virus (Perreau et al. 2013). One of the explanations for the expansion of Tfh cells is that ongoing viremia may drive a Tfh cell differentiation of CD4 T cells. This hypothesis is supported by the fact that antiretroviral therapy reduces the frequency of HIV-specific Tfh cells. The effect of viral persistence could be further augmented by changes in cytokine levels that include increased IL-6 and reduced IL-2 production in chronic progressive HIV disease. Tfh cells express high levels of IL6 receptor and therefore are sensitive to IL-6 signaling that is one of the most potent inducers of Tfh differentiation. At the same time, IL-2 is a major negative regulator of Tfh differentiation, and the lack of IL-2 due to T-cell exhaustion along with high levels of IL-6 and ongoing antigenemia may be driving CD4 T-cell responses toward a Tfh phenotype.

The capacity to identify memory Tfh cells in the periphery (pTfh) is a golden fleece of current research since it would be an extremely valuable measurement to monitor responses to candidate vaccines. Many research groups have used several markers to identify pTfh, but there is currently no

consensus in the field, resulting in sometimes contradictory results in published studies. In HIV infection, a recent study identified PD-1⁺CXCR5⁺CD4⁺ T cells to be the population in peripheral blood most closely related to germinal center Tfh. The authors further subcategorized this subset and found that PD-1⁺CXCR5⁺CXCR3⁻ CD4 T cells have a transcriptional profile close to GC Tfh and provided better help to B-cell differentiation in *in vitro* assays than the other CD4 T-cell subsets tested. More importantly, they showed that this population correlated with development of broadly neutralizing antibodies against HIV in subjects with chronic infection (Locci et al. 2013). However, this correlation was established with the total frequencies of PD-1⁺CXCR5⁺ CXCR3⁻ CD4 T cells, not HIV-specific CD4 T cells within this population, whose detection appears very challenging with currently available assays. Thus, the contribution and the functional characteristic of peripheral HIV-specific Tfh CD4 T cells to foster development of broadly neutralizing antibodies remain unclear.

Regulatory T Cells

As discussed above, the main role of CD4 T cells is to orchestrate immune responses. Most CD4 T-cell subsets secrete cytokines to activate cells of the innate and adaptive immune system. However, an important function of CD4 T cells is not only to stimulate immune responses but also to regulate immune activation. The balance of immune activation and immune inhibition is critical in order to prevent excessive immune activation and thus protect the host tissues from immune-mediated damage. Regulatory T cells (Tregs) are CD4 T cell with suppressive effects (Chevalier and Weiss 2013). Their main role is to inhibit effector function of innate and adaptive immune cells (Abbas et al. 2012b). Tregs are characterized by high expression of IL-2 receptor alpha (IL-2R α or CD25) and transcription factor FoxP3. Although Tregs have been shown to play a critical role in suppressing autoreactive responses, their role in HIV infection is still controversial. Tregs could potentially be beneficial by inhibiting

excessive immune activation and the generation of new activated CD4 T cells that are vulnerable targets for the HIV virus. Conversely, they could be detrimental by inhibiting HIV-specific immune responses, thus contributing to ineffective viral clearance. Studies in the SIV macaque model indicated that early Treg response was associated with persistent inhibition of T-cell responses enhanced viral replication and disease progression (Chevalier and Weiss 2013). Tregs were shown to inhibit HIV-specific T-cell responses *ex vivo*. More recently, HIV-specific Tregs were identified using class II tetramer staining and flow cytometry, although their frequency was extremely low. Further studies are needed to specify their function. Initial work looking at the frequency and numbers of Tregs in the periphery of HIV-infected subjects also showed sometimes contradictory results. More recent studies rather suggest an increase in the frequency of Tregs in peripheral blood. However, the main anatomic compartment where Treg exert their function is in tissues, in particular in the gut-associated lymphoid tissues (GALT), where a tight control of inflammation is critical given the constant exposure to the gut bacterial flora (Nilsson et al. 2006). Studies in the SIV macaque model and subsequently in HIV-infected human subjects showed enhanced frequencies of Tregs in the lymph nodes and tonsils. Whereas specific modulation of Treg responses by therapeutic interventions in HIV infection might have a beneficial effect is currently unknown.

T-Cell Exhaustion

In HIV infection, like in other chronic viral diseases, there is a progressive loss of T-cell functions, also known as T-cell exhaustion, which leads to ineffective immune responses and defective viral clearance. Virus-specific T-cell proliferation and IL-2 secretion are among the first functions to be impaired, whether other effector functions such as IFN γ secretion are lost at later stages of the disease (Wherry 2011). T-cell dysfunction is a result of extrinsic negative regulatory pathways, such as immunoregulatory cytokines, e.g., IL-10, and cell-intrinsic inhibitory pathways,

such as inhibitory receptors on the surface of CD4 T cells.

One of the first inhibitory pathways implicated in CD4 T-cell dysfunction was the PD-1 receptor. PD-1 is an inhibitory molecule of the B7:CD28 family and modulates the signal given by the TCR. It is upregulated on HIV-specific CD4 T cells, and its expression was correlated with markers of HIV disease progression, positively with the viral load and negatively with the CD4 count (Day et al. 2006; Porichis et al. 2011). Blockade of the PD-1 pathway *in vitro* using blocking antibodies restored proliferation and cytokine secretion by HIV-specific CD4 T cells. Interestingly, blockade of the PD-1 pathway was found to be effective, although to a lesser extent, in ART-treated subjects suggesting a potential role of immunotherapeutic interventions targeting this pathway as an adjuvant to antiviral treatment to improve HIV-specific immune responses (Porichis et al. 2011). More recently, PD-1 was shown to regulate function of Tfh cells, and blockade of the PD-1 pathway restored HIV-specific Tfh help to B cells resulting to enhanced HIV-specific immunoglobulin production *in vitro* (Cubas et al. 2013).

Besides PD-1, a complex network of inhibitory receptors has been identified as contributing to HIV-specific CD4 T-cell dysfunction. At the same time, as PD-1 was identified as one of the major inhibitory pathways, another member of the B7:CD28 family, the inhibitory receptor CTLA-4, was also shown to play a critical role in CD4 T-cell dysfunction. Similar to PD-1, CTLA-4 is upregulated on HIV-specific CD4 T cells and mediated a reversible T-cell dysfunction (Kaufmann et al. 2007). However, in contrast to PD-1 that regulates both CD4 and CD8 T-cell responses, CTLA-4 was shown to be specific for CD4 T-cell responses as it had lower expression on HIV-specific CD8 T cells and had minimal impact on their dysfunction. Further studies identified showed that coexpression of PD-1, CTLA-4, and another co-inhibitory receptor, TIM-3, was linked to more exhausted HIV-specific CD4 T cells. Further investigations confirmed that the HIV-specific CD4 and CD8 T-cell subsets are regulated by different set of inhibitory coreceptors: in contrast to HIV-specific CD8

T cells, CD4 T responses do not express significant levels of the inhibitory molecules CD244, LAG-3, and CD160 (Porichis et al. 2011).

Chemokines and cytokines are also major immunoregulatory pathways and in combination with the inhibitory receptors form a complex network that regulates HIV-specific CD4 T-cell function. IL-10 is a cytokine shown to play a critical role in immunopathogenesis of HIV infection. IL-10 plasma levels are increased in HIV infection and correlate with markers of disease progression. Even though IL-10 is produced by multiple cell subsets, monocytes appeared to be the major producers in the setting of HIV infection. Blockade of IL-10 pathway *in vitro* restores proliferation and cytokine secretion by HIV-specific CD4 T cells. However, IL-10, through dampening of chronic immune activation, may also have positive effects in HIV infection. Recent studies showed a link between IL-10 and the PD-1 pathway, where triggering of PD-1 on the surface of monocytes resulted in enhanced secretion of IL-10 (Said et al. 2010). It is important to note that inhibitory pathways can have qualitatively different effects on CD4 T-cell functions. As a consequence, combining PD-1 and IL-10 blockade *in vitro* has a strong additive impact on some HIV-specific CD4 T-cell functions but not others. In the cancer field, tremendous progress has been made in the clinical use of inhibitory pathway blockade (such as PD-1 and CTLA-4) to restore T-cell functions of exhausted cells and combat tumors. Clinical trials will be needed to assess whether such interventions could be a useful adjunction to standard antiviral therapy in HIV-infected patients, in particular to improve CD4 T-cell functions or help reduce viral reservoirs.

Conclusion

Effective HIV-specific or SIV-specific CD4 T-cell responses are likely important for long-standing control of viral replication once infection is established and will likely be critical to elicit protective immune responses by a preventive HIV vaccine. HIV-specific CD4 T-cell responses have been the focus of renewed research efforts after early hopes that vaccine trials emphasizing

generation of virus-specific CD8 T cells failed to show efficacy. Although important research efforts are still needed to identify protective components of the T-helper response against HIV, progress has been made to characterize in-depth these responses and their link to evolution of HIV disease. It is very likely that potent HIV-specific CD4 T-cell responses will need to act in concert with other arms of the adaptive immune responses such as antibodies and CD8 T cells as well as effective stimulation of innate immunity will be required to design effective preventive vaccines and, possibly, therapeutic vaccines to achieve HIV cure in infected people.

Cross-References

- ▶ [Cellular Immune Response to HIV-2 Infection](#)
- ▶ [Central Memory CD4 T cells](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [Long-Term Nonprogressors and Elite Controllers](#)
- ▶ [PD-1](#)
- ▶ [Role of Regulatory T Cells During HIV Infection](#)
- ▶ [T Follicular Helper Cells in HIV Infection](#)
- ▶ [Tim-3](#)
- ▶ [Th17 Cells](#)

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HIV and SIV, CD8 T Cell Responses to

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Definition/Introduction

HIV infection is associated with widespread infection of both circulating and tissue CD4 T cells,

with initial viral loads peaking at five to ten million HIV RNA copies/ml plasma within weeks of exposure. This is followed by a rapid decline in plasma virus to a median “viral set point” of around 30,000 HIV RNA copies/ml. Importantly, studies in humans infected with HIV and monkeys infected with SIV indicate that CD8 T lymphocytes are pivotal to this dramatic decline in viral load, yet the progressive dysfunction of the CD8 T lymphocyte population over time leads to a subsequent increase in viral load and progression to AIDS. CD8 T lymphocytes are a white blood cell subset of the adaptive immune system, which recirculate between the blood and peripheral lymphoid organs. The main role of CD8 T lymphocytes is to recognize and kill infected target cells that harbor intracellular pathogens; therefore, those targeting HIV are defined as HIV-specific CD8 T cells. Understanding the potent antiviral function of HIV-specific CD8 T cell responses and the factors that lead to their dysfunction has important implications for the development of therapeutic and prophylactic HIV/AIDS vaccines.

Frequency of HIV-Specific CD8 T Cells

The emergence of HIV-specific CD8 T cells is strongly associated with the initial decrease of HIV viral load to set point, and several studies have shown that HIV-specific CD8 T cell responses can also control HIV replication during chronic infection. Yet, the correlates of a protective HIV-specific CD8 T cell response remain poorly defined (Walker and McMichael 2012). Early studies focused on the quantity of CD8 T cell responses in HIV-infected persons. Importantly, in vitro assays analyzing their lytic function (including chromium release assays, viral suppression assays) or ex vivo binding to HLA class I tetramers (tetrameric complexes of peptide-HLA, bound to a labeled streptavidin bead) consistently detected robust CD8 T cell responses in the peripheral blood of HIV-infected individuals. In some HIV-infected individuals, the percentage of HIV-specific CD8 T cells could be 10–20% or more of the total CD8 T cell population, yet

control of virus was not consistently observed in these individuals. Collectively, these studies were in general agreement that there are no significant differences in the frequencies of HIV-specific CD8 T cells between “HIV controllers” (chronically infected individuals who can spontaneously control HIV in the absence of antiretroviral drugs) and “HIV progressors” (chronically infected individuals who progress to AIDS in the absence of antiretroviral drugs) (Walker and Yu 2013). Furthermore, detailed characterization of the specificity of CD8 T cell targeting of different HIV peptides (using IFN γ ELISPOT assays) has also failed to correlate the magnitude or breadth of HIV-specific CD8 T cell responses with better control of viremia. Together, the findings from numerous laboratories indicate that HIV-specific CD8 T cell activity is influenced by quality rather than merely the quantity of the response alone. Consequently, efforts have focused on the search for the key qualitative determinants of HIV-specific CD8 T cell efficacy.

Function of HIV-Specific CD8 T Cells

Cytotoxic T Cells

A key function of CD8 T cells is to recognize pathogen-derived peptides presented by HLA class I molecules on the surface of infected cells and kill those infected cells by cytolytic/cytotoxic mechanisms, thereby eliminating the intracellular pathogen. Therefore, CD8 T cells are often referred to as “cytotoxic T lymphocytes” (CTL) in the scientific literature. In order to kill the infected cell, CTL cells must adhere tightly to the infected cell, with the formation of an “immunological synapse” at the site of contact. Activated CTL then release their granule contents by exocytosis, which induces cell death of the infected target cells. The two main types of granule protein released by activated CTL are granzymes and perforin. In the setting of HIV infection, several studies have suggested that CTL in “HIV controllers” demonstrate a superior ability to upregulate perforin (ex vivo after antigen stimulation and in vitro proliferation) and that lytic granule loading and delivery of granzyme B from CTL into

infected target cells were statistically more pronounced (Migueles et al. 2008). This is concordant with studies demonstrating that CTL from “HIV controllers” are better able to inhibit HIV replication. However, it is worth noting that many of these studies are conducted in in vitro culture conditions, which may not represent the ex vivo activity of these cells. Direct measures of cytotoxic activity have been difficult to achieve, especially in a physiologically relevant setting. Further research is required, but the ability of CTL from HIV controllers to upregulate perforin and enhance their cytotoxic capacity may represent the functions most clearly associated with immune control.

Polyfunctionality

Another important attribute of HIV-specific CD8 T cell antiviral activity is the mixture of cytokines and chemokines released. The ability of HIV-specific CD8 T cells to proliferate upon antigen encounter, a property allied to the production of the cytokine IL-2, has been associated with HIV control. In addition, it has been shown that HIV-specific CD8 T cells secreting TNF α and IFN γ cytokines in tandem, rather than IFN γ alone, have enhanced cytolytic activity. Recently, several investigators have used multiparameter flow cytometry to analyze multiple effector functions simultaneously and have thus identified “polyfunctionality” as a strong correlate of CTL antiviral efficiency (Betts et al. 2006). “Polyfunctionality” typically describes the capacity of these cells to degranulate (CD107a) and simultaneously produce multiple cytokines and chemokines including IFN γ , TNF α , IL-2, and MIP-1 β . These studies found that polyfunctional secretion by HIV-specific CD8 T cells was significantly more frequent in “HIV controllers” than in “HIV progressors,” whose CD8 T cells largely secreted only IFN γ . Thus, these groups recorded an inverse correlation between polyfunctionality and viral load. However, it is not clear whether polyfunctionality is a cause or consequence of immune control. More recent studies comparing chronically infected HIV individuals (with prolonged antigenic exposure in the absence of antiretroviral drugs) and HIV individuals on

antiretroviral treatment (with drug-suppressed viral loads and consequently low antigen levels) have suggested that polyfunctional HIV-specific CD8 T cell profiles may simply reflect the consequence of a better-preserved immune system, rather than the cause.

Functional Avidity and TCR Usage

Another CD8 T cell effector function that has received much attention is “functional avidity,” the affinity of the TCR for peptide presented by HLA. Several authors have demonstrated a correlation between enhanced functional avidity of HIV-specific CD8 T cell responses and a reduction in HIV viral load. It has been theorized that high levels of antigen sensitivity enable HIV-specific CD8 T cells to recognize peptide-HLA complexes at low density, readily triggering effector functions such as perforin, granzymes, and cytokines, which correspond to rapid and effective killing capacity against infected target cells. However, this remains controversial with many others finding no correlation between functional avidity and functional output. Other factors may also be important, including the specific TCR clonotype usage, with some TCR clonotypes associated with enhanced effector functions and superior HIV control.

CD8 T Cell Exhaustion

During the course of HIV infection, like in other chronic viral infections, CD8 T cell functions such as cytokine secretion, cytotoxicity, and proliferation diminish gradually. This progressive loss of CD8 T cell effector functions is referred to as “immune exhaustion.” These dysfunctional HIV-specific CD8 T cells were described early in the HIV epidemic and have been associated with the inability of the immune system to clear HIV infection.

Programmed death-1 (PD-1) was one of the first inhibitory receptors to be implicated with immune exhaustion. PD-1 is an inhibitory molecule of the B7:CD28 family that is expressed on both CD8 and CD4 T cells, among other immune cells. PD-1 expression on HIV-specific CD8 T cells is positively correlated with HIV viral load and progression to disease (Day

et al. 2006), although observations suggest that these phenotypic changes are the end result of persistent antigenic stimulation, rather than the direct cause of high viral loads. Importantly, blockade of the PD-1 pathway (in rhesus macaque monkeys chronically infected with SIV) resulted in the recovery of virus-specific CD8 T cell responses, including improved cytokine secretion and enhanced proliferation. These studies not only demonstrated that blockade of PD-1 signals can reverse immune dysfunction but also demonstrated the potential efficacy of blocking inhibitory pathways in future immunotherapeutic approaches to restore T cell function. Further research on inhibitory pathways such as PD-1, in addition to immunoregulators LAG-3 and TIM-3, is needed to better understand how immune exhaustion contributes to HIV pathogenesis and whether blocking of such pathways could be useful in addition to standard antiretroviral therapy in HIV-infected individuals.

CD8 T Cells and HLA Class I

CD8 T cells recognize and kill cells that harbor nonself proteins and thus are a major defense mechanism against invading viruses. To initiate antiviral activity, CD8 T cells first require a signal from the infected cell. This signal is a virus-derived peptide displayed by a major histocompatibility molecule (MHC) class I molecule on the infected cell surface. Each T cell expresses a unique T cell receptor (TCR) capable of recognizing viral peptide bound to MHC molecules. Classically, the T cell paradigm dictates that CD8 T cells recognize short viral peptides of 8–11 amino acids in length bound to MHC class I molecules (while CD4 T cells recognize longer peptides of 12–24 amino acids bound by MHC class II molecules). In humans, MHC molecules are referred to as human leukocyte antigens (HLA). The HLA locus is the most polymorphic region of the human genome. HLA molecules are genetically inherited from both parents, which each individual inheriting up to six different HLA class I molecules (two HLA-A, two HLA-B, and two HLA-C; homozygosity can be

present, making the total number of distinct class I molecules less than six).

Interestingly, numerous cohort studies have shown strong associations between the expression of certain HLA class I molecules and disease outcome (Walker and Yu 2013). In particular, some HLA class I genes are linked with individuals who succumbed quickly to AIDS, while other HLA class I genes are associated with individuals called “HIV controllers” who spontaneously maintain low viral loads, in some cases for decades, without requiring antiretroviral drug treatment. Most of these associations are in HLA-B alleles, most notably with expression of HLA-B*57 and/or HLA-B*27 associated with better control of HIV in “HIV controllers” and expression of HLA-B35 variants associated with faster progression to AIDS-like disease (Goulder and Walker 2012). In particular, a large-scale genome-wide association study (GWAS) enrolling nearly 1,000 “HIV controllers” and almost 3,000 “HIV progressors” detected four single-nucleotide polymorphisms (SNPs) that are associated with HIV control or lack of control (International HIV Controller Study 2010). All four SNPs were located in the HLA region of chromosome 6. In-depth analysis of this region has revealed that the SNPs are tagging specific amino acids in the HLA-B binding groove that influence the binding of viral peptides for recognition by HIV-specific CD8 T cells, in addition to tagging other amino acids that influences regulation of HLA-C expression.

Overall, these strong associations between HLA class I expression and viral control implicate an important role for HLA class I-restricted HIV-specific CD8 T cells in the setting of HIV infection. However, it is important to note that these data suggest that even in persons with so-called “protective” HLA-B alleles (such as B*57 or B*27) and with favorable amino acids in the HLA-B peptide-binding groove, the majority of these individuals will become “HIV progressors,” rather than “HIV controllers.” Indeed, host genetics can currently explain only 23% of the variation in HIV viral load at the population level.

Specificity of HIV-Specific CD8 T Cells

The ability of T cells to distinguish between different viral proteins or viral peptides is defined as the “T cell specificity.” The specificity of HIV-specific CD8 T cell responses varies greatly between HIV-infected individuals (due to genetic differences in HLA molecules), yet is highly predictable once the HLA type of the individual is known. The specificity of HIV-specific CD8 T cell response also differs during the stage of viral infection. In the earliest stages of acute HIV infection, the HIV-specific CD8 T cell response in the blood is narrowly directed against only a handful of HIV peptides, predominantly derived from Env and Nef (which are among the most variable HIV proteins) (McMichael et al. 2010). These HIV-specific CD8 T cell responses are linked with the dramatic decline in HIV viral load to set point, indicative that these narrowly directed responses are at least partially effective. The HIV-specific CD8 T cell response then broadens over time, with a median of 14 peptides simultaneously targeted by HIV-specific CD8 T cells in chronic infection.

In chronic infection, multiple studies have shown that CD8 T cells dominantly target peptides derived from Gag (which is a highly conserved HIV protein), which has been associated with lower HIV viral load. In particular, a large study of chronically infected individuals found that broader Gag-specific CD8 T cell response was associated with lower HIV viral load, while conversely broader Env-specific CD8 T cell responses were found in persons with higher HIV viral load (Kiepiela et al. 2004). In addition, studies of HIV controllers expressing “protective” HLA alleles (such as HLA-B*27 and B*57) have shown that CD8 T cell targeting of specific peptides in the structurally important Gag protein can result in the selection of viral escape mutants with reduced viral fitness, facilitating immune control. Collectively, these studies repeatedly suggest that the specificity of the CD8 T cell response plays a key role in chronic HIV infection, with responses to the highly conserved Gag protein associated with superior immune control.

Viral Escape from HIV-Specific CD8 T Cells

During the acute and chronic phases of HIV infection, the targeting of HIV-infected cells by HIV-specific CD8 T cells exerts a strong inhibitory effect on viral replication. The strong CTL-driven selective pressure on HIV may consequently induce the emergence of mutations in the initial infecting virus, termed “escape mutants,” that enable the mutant virus to escape CTL recognition. The first example of escape was proposed in 1991, yet this was not generally accepted until further examples of CTL escape in natural infection were demonstrated (Borrow et al. 1997). Numerous papers have subsequently been published identifying HIV and SIV escape mutants and also evaluating the underlying mechanisms of escape.

The most well-established mechanism of HIV escape is mutation of amino acids within the targeted peptide that binds to MHC class I. Mutations within the epitope can result in a loss of binding between the peptide and MHC complex and/or impair the interaction of the TCR for its peptide-MHC complex. In addition, mutation within a peptide and also in the flanking region of the peptide may result in altered peptide processing and presentation. The propensity of HIV to mutate in response to CTL selection pressure is not random but rather occurs at specific positions in the viral sequence. This strongly suggests that there are functional and structural constraints on virus sequence variability, with some escape mutations imparting a detrimental “fitness cost” on HIV replication resulting in less-fit viruses (as commonly observed in Gag protein in “HIV controllers”). In contrast, other escape mutations may confer a “selective advantage” allowing the virus to effectively escape the host immune response. Importantly, many of these mutations become unstable upon transmission from an HIV-infected donor to a new recipient, with the virus frequently reverting to the original “wild-type” sequence in the recipient (in the absence of ongoing immune selection pressure). The timing of escape may vary greatly, with some

escape variants detected within days of acute HIV infection, while others occur late in chronic infection and may be associated with clinical decline.

Overall, there is considerable evidence of viral escape during acute and chronic HIV infection, which is driven by HIV-specific CD8 T cell selection pressure. Although some mutations result in a less-fit virus with impaired replicative ability (particularly mutations occurring in Gag), other mutations may have no fitness cost on the virus and may actually help to drive global HIV sequence evolution.

Immune Activation of Nonspecific and HIV-Specific CD8 T Cells

One of the hallmarks of HIV disease progression is chronic immune activation. Chronic immune activation refers to an overtly overactivated state of the immune system, which is characterized by increased expression of activation markers on T cells, hyperactivation of B cells (often in conjunction with hypergammaglobulinemia), and increased levels of proinflammatory cytokines. Immune activation of T cells is defined by the expression of specific activation markers on T cells (most notably CD38 and HLA-DR), increased T cell turnover, and spontaneous T cell death referred to as “apoptosis.”

Importantly, immune activation of memory CD8 T cells is one of the strongest predictors of how quickly an HIV-infected individual will progress to AIDS (in the absence of antiretroviral treatment). Numerous studies have shown that high frequencies of activated CD8 T cells (expressing CD38) are associated with faster disease progression. However, this does not imply that CD8 T cells are detrimental in the setting of HIV infection; instead, it suggests a paradoxical role. The majority of highly activated cells are not HIV-specific CD8 T cells, but rather represent CD8 T cells of undetermined specificity, most likely CD8 T cells specific to other microbes or self-proteins that are vulnerable to bystander immune activation. HIV-specific CD8 T cells clearly play a major role in limiting viral spread,

but after years of continued HIV replication and repeated antigenic stimulation, a proportion of these HIV-specific CD8 T cells will be driven to a state of hyperimmune activation, irreversible exhaustion, and T cell death by apoptosis. Immune activation and the resulting loss of some of these HIV-specific T cells therefore likely accelerate the onset of disease progression to AIDS, even though the majority of HIV-specific T cells remain functionally active, albeit less effective against HIV.

The cause of immune activation is still largely unknown; yet it is likely multifactorial. For activated CD8 T cells, one favored explanation is “microbial translocation,” whereby the destruction and depletion of CD4 T cells from the mucosal lymphoid tissue during primary HIV infection lead to increased “leakiness” of the gut membrane, allowing for microbial products to translocate from the gut, thus enhancing the amount of antigenic stimuli that both CD8 and CD4 T cells are exposed to (Brenchley et al. 2006). In addition, HIV may mediate a direct negative effect on T cell activation (by accessory proteins such as Nef), and coinfection or reactivation of pre-existing viruses and bacteria such as cytomegalovirus, CMV, is also likely to play a role. Understanding the mechanisms that give rise to immune activation represents an area of continued investigation that has important implications in the clinical treatment of HIV-infected individuals (to limit disease progression) and in the setting of prophylactic and therapeutic vaccines where the focus is to induce beneficial T and B cell responses that remain effective against HIV.

Importance of CD8 T Cells in Controlling HIV and SIV

It is widely believed that HIV-specific CD8 T cells play a key role in controlling HIV and SIV infection. Strong evidence for this stems from several key observations (Walker and McMichael 2012):

1. The rapid drop in plasma HIV viral load very early in acute infection coincides with the initial detection and expansion of the

HIV-specific CD8 T cell response, which may exceed 10% of all circulating CD8 T cells.

2. Detection of viral escape mutations in the first days and weeks following acute HIV infection implies that CD8 T cells are mounting strong selective pressure on the virus. Furthermore, the detection of viral escape mutants that can evade HIV-specific CD8 T cells is frequently linked to disease progression in HIV-infected individuals.
3. The expression of particular HLA class I alleles restricting HIV-specific CD8 T cell responses is associated with different clinical outcomes. Most notably, a delay in AIDS progression in infected individuals carrying HLA-B27 and HLA-B57 is observed, while the accelerated onset of AIDS is associated with HLA-B35.
4. Arguably, the most compelling evidence for the importance of CD8 T cell responses in controlling HIV has come from animal models. In the rhesus macaque model, the depletion of CD8 cells (by anti-CD8 antibody infusion) during acute SIV infection resulted in high viral loads and rapid progression to AIDS-like disease and death. In animals in which the infusion of monoclonal antibody was halted, CD8 T cell numbers rebounded, and a resulting decline in viral load was observed, thus attributed to immune control mediated by SIV-specific CD8 T cells.
5. Interestingly, while the vast majority of HIV-infected individuals progress to disease in the absence of antiretroviral drug treatment, a very small percentage of HIV-infected individuals (~1%) are able to spontaneously control HIV viral loads to almost undetectable levels in the absence of antiretroviral drugs for many years and do not progress to AIDS-like disease (Walker and Yu 2013). These HIV-infected individuals have been termed “HIV controllers” or long-term nonprogressors (LTNPs). (The definition of “HIV controllers” can also be subdivided into “elite controllers” who have viral loads of less than 50 HIV RNA copies/ml and “viremic controllers” who have viral loads of 50–2,000 HIV RNA copies/ml.) Importantly, multiple studies suggest that the

superior immune control observed in “HIV controllers” may be linked with improved functions of their HIV-specific CD8 T cells (such as proliferation and cytotoxicity) and the HLA class I alleles that restrict these HIV-specific CD8 T cell responses.

Importance of CD8 T Cell Responses in Prophylactic HIV Vaccines

Although the CD8 T cell response to HIV infection is often considered to be “too little, too late” to control HIV infection, there is significant hope in the HIV research field that CD8 T cells elicited by a prophylactic vaccine may be able to suppress viral load to such low levels that it would confer a strong beneficial effect in the HIV-infected individual, limiting immunopathogenesis and/or reducing the risk of transmission (in the absence of antiretroviral drugs). Vaccine-induced T cell responses would not be expected to prevent initial HIV infection. Instead, strong vaccine-elicited responses could potentially intercept HIV infection near the site of entry to prevent systemic viral spread to other areas, or eradicate HIV particles “hiding” in viral reservoirs. Toward this goal, T cell responses have been evaluated in depth in the development of prophylactic HIV vaccines (both in the context of T cell-inducing vaccines and also for T cell- and B cell-eliciting vaccines).

Initial tests of HIV vaccines are conducted in nonhuman primate (NHP) models, typically rhesus macaque monkeys, who are challenged with the simian equivalent of HIV called SIV. Although studies vary greatly (in the species of monkeys used, the SIV strain, the route of viral challenge, the vaccine regimen, and the vaccine constructs used), several key observations have emerged. Firstly, vaccines can induce SIV-specific CD8 T cells, which upon viral challenge can rapidly expand up to tenfold in magnitude and frequency. Secondly, in some (but not all) studies, vaccine-induced CD8 T cell responses can reduce the peak viral load and/or reduce viral set point (by up to two logs), which is likely to limit progression to AIDS-like disease and prolong the survival of the animals. Thirdly, at least one

study has demonstrated that vaccine-induced CD8 T cells can progressively clear SIV infection from the blood and tissues, although it should be noted that the HLA restriction and recognition characteristics of vaccine-induced CD8 T cells appear to be highly unconventional (Hansen et al. 2013; Ranasinghe and Walker 2013). Collectively, there is wide variation in the results of these NHP studies, and arguably, the critical question remains whether the vaccines tested in NHP models with SIV challenge are going to be more or less effective in protecting humans from HIV infection.

To date, only three HIV vaccines have been tested in phase 2b or phase 3 efficacy trials in humans (with numerous others failing to demonstrate sufficient safety at phase 1 or sufficient immunogenicity at phase 2, or currently awaiting testing). The first of these HIV vaccines, VAX003, was not designed to elicit CD8 T cell responses; instead, it was designed to elicit only antibodies and showed no efficacy in HIV-negative individuals at high risk of HIV acquisition. In contrast, the second trial, phase 2b Merck STEP trial using adenovirus 5 (Ad5) vectors with HIV Gag, Nef, and Pol inserts was designed to induce HIV-specific CD8 T cells in HIV-negative individuals at high risk of HIV acquisition. The vaccine elicited detectable HIV-specific CD8 T cells in 73% of vaccinated individuals, yet there was no evidence of vaccine-induced protection (either in preventing HIV acquisition or reducing post-acquisition viral load at peak or set point) (Buchbinder et al. 2008). Post hoc analysis suggests that the magnitude and breadth of these CD8 T cell responses may have been too weak, potentially still “too little, too late,” to achieve protection in this vaccine, although the vaccine did show some evidence of exerting selection pressure on the virus in those who became infected. The STEP trial was a clear negative result in the pursuit of an HIV vaccine. Yet, it serves to shed more clues on the different strategies that could be used for future HIV vaccines, such as the use of different vectors (adenovirus, canarypox, cytomegalovirus, etc.) to modulate the CD8 T cell responses induced, and a renewed focus on developing vaccines that induce both T cells and antibodies.

Importantly, in addition to these two efficacy trials, a third trial did suggest vaccine-induced protection from HIV acquisition. The US Military HIV Research Program (MHRP) RV144 phase 3 efficacy trial in Thailand involved a combination regimen of a recombinant canarypox virus with booster doses of a clade A/E gp120 glycoprotein vaccine in HIV seronegative persons at low risk of HIV acquisition. The vaccine group showed a 31% reduction in HIV acquisition when compared to the placebo group. This demonstrates for the first time that an HIV vaccine can reduce HIV acquisition. However, the observed reduction in HIV acquisition was modest and only just above the borderline of statistical significance ($p = 0.04$, with a $p = 0.05$ statistical cutoff), and no significant difference in HIV viral load was observed between vaccinees who became infected and placebo groups. This vaccine was primarily designed to elicit antibodies, which are likely to have mediated the prevention from acquisition in almost a third of vaccinees, yet investigations are still ongoing to determine precise immune correlates of protection (Kim et al. 2015).

The next scheduled efficacy trials in the HIV/AIDS vaccine field are primarily focused on building on the results of the Thai RV144 trial. The MHRP is currently running RV305 in Thailand, which seeks to evaluate the effects of re-boosting the volunteers who participated in the initial RV144 study. In addition, a consortium known as the Pox-Protein Public-Private Partnership (P5) was formed to coordinate further clinical tests of RV144-like vaccine strategies in Thailand and South Africa. A new phase III efficacy trial using a clade B/E boost is expected to start in Thailand in 2016/2017. Trials in South Africa testing the original clade A/E boost, and separately a new ALVAC/protein clade C boost, are also expected to start in 2016/2017. In addition, other vaccine constructs including NYVAC, DNA, and protein will also be tested by P5 in multiple locations across Southern Africa. The results of these trials are not expected for several years; however, there are also over 20 other HIV/AIDS vaccine trials at earlier stages of development (phase 1 and 2 and preclinical testing),

including several that aim to elicit only T cells, only antibodies, or both T cells and antibodies. Collectively, the highly varied design and implementation of these vaccine trials will be instrumental in discerning the most efficacious vaccine-induced immune responses and vaccine strategies required for preventing HIV acquisition.

Conclusions

As with other viral infections, the host immune response mounts a vigorous adaptive immune response to HIV, with clear evidence that HIV-specific CD8 T cells exert an antiviral effect that contributes to lowering viral load to a quasi set point (Walker and McMichael 2012). Unfortunately, there is no clear evidence that this T cell response ever completely eradicates natural infection. A combination of progressive CD8 T cell dysfunction in response to ongoing viral replication, together with immune escape through mutations, leads to a gradual increase in viral load in untreated persons. However, the finding that some persons known as “HIV controllers” are able to maintain low to undetectable viral loads for years in the absence of antiviral therapy suggests that CD8 T cell responses might be meaningfully enhanced in infected persons and might contribute to protection from disease progression if induced by immunization (Walker and Yu 2013). Intriguingly, the finding that a CMV vector expressing SIV proteins was able to induce potent and persistent effector memory CD8 T cell responses that protected macaques from infection (Hansen et al. 2013) suggests that CD8 T cells may also have an important role in preventive HIV vaccines.

Cross-References

- ▶ [Cellular Immune Response to HIV-2 Infection](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV Antigen Processing and Presentation](#)
- ▶ [HIV-1 Mutational Escape from Host Immunity](#)
- ▶ [HIV-1 Transmission Blocking Microbicides](#)

- ▶ Long-Term Nonprogressors and Elite Controllers
- ▶ Mucosal Immunity to HIV-1
- ▶ Viremic Nonprogressors

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HIV Antigen Processing and Presentation

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Definition

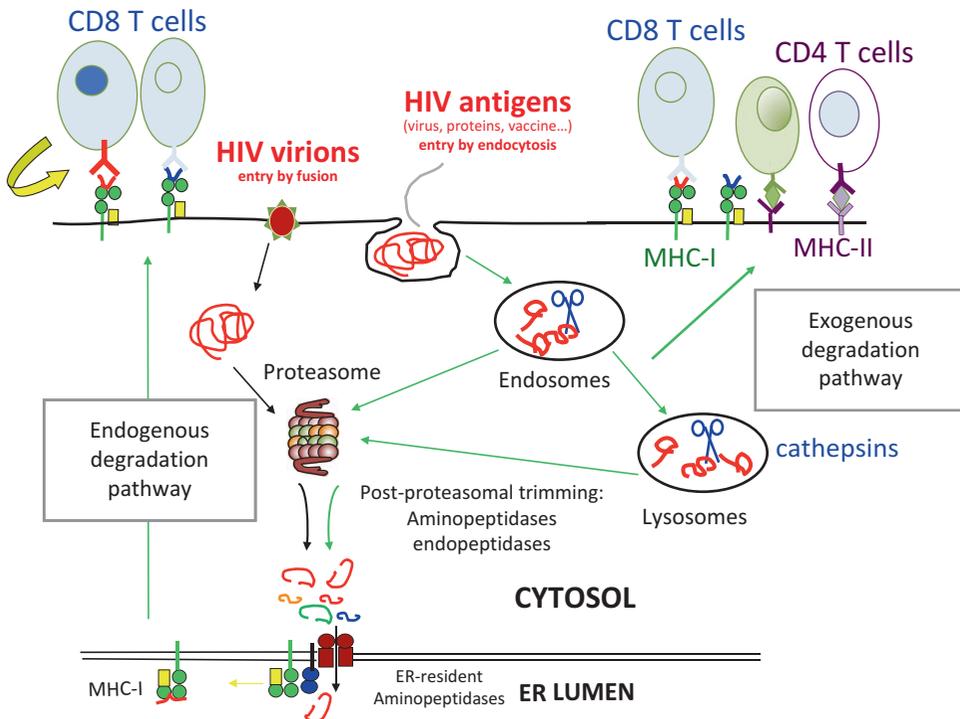
Immune T cells detect and clear cells infected by pathogens such as bacteria or viruses, because infected cells present peptides derived from the intracellular degradation of the pathogen. Antigen processing consists of the multi-step intracellular degradation of self- and pathogen-derived proteins into short peptides loaded onto major histocompatibility complex (MHC) molecules and displayed at the cell surface. MHC-I complexes display peptides mostly 8–11 amino acids (aa) long to CD8 T cells and MHC-II display longer peptides (mainly 12–18 aa) to CD4 T cells. While pathogen-specific CD8 T cells are cytotoxic T cells killing infected cells, CD4 T cells primarily provide help to maintain CD8 T cell responses and antibody responses against pathogens. The processing of pathogen-derived antigens into epitopes and their presentation by dendritic cells is the first step leading to the priming of pathogen-specific CD8 and CD4 T immune responses in the context of infection or vaccination. Antigen processing and presentation enables the immune system to monitor the intracellular content of cells, detect pathogens, and fight infection. Understanding antigen processing and presentation in the context of HIV infection is critical to identify epitopes presented by HIV-infected cells, to define protective immune responses, to elucidate the mechanisms of immune escape, and to improve the design of vaccine immunogens.

Overview of the Protein Degradation Pathways

Protein degradation constantly occurs in healthy or infected cells as it allows for the regulation of cellular activities through the removal of specific proteins, to eliminate misfolded or defective proteins, and to recycle amino acids to make new proteins and display peptides at the cell surface for immune monitoring (Blum et al. 2013; Yewdell 2007).

The endogenous processing pathway refers to the degradation of proteins located inside cells, mostly endogenous proteins but also proteins from pathogens entering in the cytosol or newly synthesized pathogens (Fig. 1). Polyubiquitinated proteins or misfolded proteins are degraded intracellularly by proteasomes located in the cytosol and nuclei. Proteasomes are the only proteases that can both unfold and degrade proteins and are therefore the first involved in protein degradation. They produce peptides ranging from 2 to

30 aa and often define the C-terminus residue of epitopes due to frequent cleavage sites after hydrophobic residues, the most frequent C-terminal anchor residues of MHC-I epitopes. 20S proteasomes are barrel-shaped proteases made of four rings of seven subunits each, where the two central rings each contain three catalytic subunits cleaving after hydrophobic, acidic, or hydrophilic residues. The core can be capped with two lids forming the 26S proteasome. In the presence of interferon gamma, the constitutive catalytic subunits can be replaced by immunocatalytic subunits leading to immunoproteasomes (Blum et al. 2013). Lymphoid cells such as T cells or dendritic cells contain mostly immunoproteasomes and some constitutive proteasomes; in other tissues, the majority of proteasomes are constitutive proteasomes. Whereas in other tissues constitutive proteasomes are the major type of proteasomes. Immunoproteasomes tend to produce longer peptides with more hydrophobic C-termini (fitting most MHC molecules)



HIV Antigen Processing and Presentation, Fig. 1 Processing and presentation of MHC-I and MHC-II epitopes to T cells

that may be better suited for the production of epitopes. Thymic tissues contain proteasomes with unique catalytic subunits forming thymoproteasomes that may permit the production of a variety of peptides suitable for positive and negative selection during thymic education. After proteasomal degradation peptides can be further degraded in the cytosol by endopeptidases and aminopeptidases (cleaving from the N-terminal side of peptides). Peptides of at least 8 aa long and usually shorter than 16 aa that bear adequate anchor residues for the transporter associated to antigen processing (TAP) will translocate into the endoplasmic reticulum (ER), where they can be further trimmed by ER-resident aminopeptidases (ERAP1 and ERAP2) before or after loading onto MHC-I. MHC-I-peptide complexes released from the ER traffic to the cell surface and are displayed for immune monitoring. Although many peptidases have been identified and involved in the processing, the number of peptidases involved in degradation and order in which they cleave a given substrate is not well defined.

In addition to the endogenous pathway, the exogenous pathway leads to the degradation of extracellular proteins, pathogens, or fragments of dead cells taken up by phagocytosis, endocytosis, or macropinocytosis (Yewdell 2007) (Fig. 1). Exogenous substrates are degraded by cathepsins in endosomes and lysosomes (Blum et al. 2013). Degradation products can transfer back in the cytosol for further degradation and cross-presentation by MHC-I. In professional antigen-presenting cells constitutively expressing MHC-II (macrophages, dendritic cells, and B cells), degradation products reach a specialized phagocytic compartment called MIIC containing MHC-II molecules bound to an invariant chain Ii and where cleavage of Ii and loading of peptides onto MHC-II trigger the traffic of MHC-II-peptide complexes to the cell surface. MHC-II can also present endogenous antigens such as membrane proteins degraded in lysosomes or during autophagy when cytosolic and nuclear antigens are engulfed in autophagosomes fusing with lysosomes for degradation and MHC-II presentation. The distinction between endogenous and exogenous

pathways is becoming more tenuous as the processing of some MHC-II epitopes of exogenous and endogenous antigens involves lysosomal enzymes and proteasomes, and the processing of MHC-I epitopes from exogenous and endogenous antigens sometimes involves peptidases from several compartments.

Factors Driving the Efficiency of HIV Epitope Production

HIV can enter cells through fusion at the plasma membrane or be phagocytosed, while after integration of the provirus, newly synthesized HIV proteins will assemble in the cytosol into new virions (Fig. 1). Thus peptidases in multiple compartments may contribute to degrading HIV during its replication cycle. So far proteasomes, TPPII, TOP, nardilysin, and ERAP1 have been involved in the processing of some HIV epitopes. There is no extensive knowledge of all peptidases involved in the processing of HIV epitopes due to the lack of throughput tools to follow epitope production and presentation. Immunoproteasomes and proteasomes are likely to be the starting point of degradation of HIV proteins and be involved in the processing of most MHC-I epitopes. However, the contribution of post-proteasomal peptidases in the cytosol and in the ER is less well defined and probably variable according to peptide lengths and sequences. Each category of post-proteasomal peptidases has catalytic specificities defining cleavable substrates. For instance, aminopeptidases cleave peptides shorter than 16 aa from the N-terminal side and cleave only 10 residues with good efficiency. TPPII is an endopeptidase that cleaves longer peptides up to 32 aa and could cleave long peptides released by proteasomes. Peptidases are involved in producing peptides for loading onto MHC but can also destroy peptides and limit epitope presentation (Blum et al. 2013). Thus the sequence of the antigen and cellular peptidases encountered by antigens will define the pool of peptides available for loading onto MHC molecules and shape the specificity of immune responses during infection.

HIV proteins are unevenly immunogenic; Gag, specifically p24, and Envelope contain the highest density of T cell or antibody immune responses while proteins such as Tat or Vpr contain fewer responses (Llano et al. 2013). Searches for potential MHC-I epitopes throughout the HIV proteome are performed by identifying potential anchor residues in HIV proteins allowing binding to specific MHC complexes and show many more potential epitopes than known T cell responses in HIV-infected persons. One possible early bottleneck limiting the diversity of immune responses is the degradation patterns of HIV proteins and the subsequent limited production of peptides compatible with loading onto MHC molecules. In vitro degradation of HIV Nef with purified proteasomes showed production of a larger number of fragments in epitope-rich areas with many containing hydrophobic C-terminal residues. Degradation of long HIV peptides of 24–35 aa or full proteins in cytosolic extracts also showed variability in fragments with some protein areas producing mostly 8–11 aa long peptides (compatible with MHC-I loading), some producing mostly longer fragments (possibly indicative of slower degradation rates), and some producing mostly peptides <7 aa too short to be loaded onto MHC-I. The higher proportion of 8–18 aa long fragments produced in epitope-rich areas and the complete degradation of some other protein areas suggest that degradation patterns contribute to shaping the immunogenicity of proteins by providing selected protein fragments that may be displayed by MHC molecules.

Complete or incomplete proteins are degraded in fragments eventually trimmed into epitope precursors and epitopes. Both production and degradation of fragments will define peptide availability for MHC-I and MHC-II peptide presentation. Degradation experiments of proteins or long peptides by purified peptidases (Tenzer et al. 2009) or cellular extracts (Le Gall et al. 2007) showed that HIV epitopes within a given protein (even for overlapping epitopes) are sequentially and unevenly produced. Computational analysis of motifs flanking epitopes showed that the efficiency of epitope production is driven by surrounding motifs creating good or poor

cleavage sites for peptidases that will impact the time at which an epitope is produced. Mutating residues flanking poorly processed epitopes toward motifs enriched around efficiently processed epitopes (as long as they are processed by the same categories of peptidases) accelerated the production of the epitope (Le Gall et al. 2007; Tenzer et al. 2009). In addition to the efficiency of epitope production, the sensitivity of a peptide to degradation before binding to MHC may affect the amount of peptides available for T cell recognition. Whereas it was thought that short peptides are quickly degraded in the cytosol, a study on HIV peptide stability in the cytosol highlighted variability in peptide half-lives (<10 s to >60 min), a property that extends beyond HIV peptides (Lazaro et al. 2011). Whereas most HIV peptides were unstable, about 15% of them displayed unusually high stability in the cytosol. Distinct motifs were enriched in stable and unstable peptides, and exchanging motifs between peptides switched their stability ranking. Intracellular peptide stability contributed to the amount of peptides available for display by MHC-I and T cell recognition. Altogether, in vitro degradation data of HIV proteins by purified peptidases or cellular extracts have provided a wealth of information on steps and sequence signatures linked to efficient or inefficient epitope production. In conjunction with studies on peptide affinity for HLA molecules or T cell receptor (TCR) and the identification of TCR repertoire, unveiling the mechanisms of HIV protein degradation into epitopes will define factors underlying the variable immunogenicity of HIV proteins. Nonetheless several questions remained to be answered. CD4 T cells and monocytes present different levels of antigen processing activities that affect the kinetics and amount of epitope production. The relative hydrolytic activities of all HIV-infectable cell subsets (CD4 T cells, monocytes, macrophages, dendritic cell subsets) have not yet been compared in primary cells from the same donors. However, if they are heterogeneous as demonstrated for CD4 T cells and monocytes in humans, this may affect the production and display of HIV epitopes. The diversity of degradation pathways and peptidases in these cells might increase the diversity of

peptides presented by HIV-infected cells, either the diversity of HIV peptides presented or the relative amount of each peptide. The direct and unbiased identification of epitopes presented by MHC-I or MHC-II after HIV infection has not yet been achieved due to technical difficulties related to the cytopathic effect of the virus on cells and the very high number of cells required for MHC-bound peptide isolation by mass spectrometry. One group identified self-derived peptides in HIV-infected cells secreting MHC molecules (Hickman et al. 2003), an important first step toward the identification of peptides presented by cells. It will be critical to expansively identify peptides naturally presented by MHC molecules of HIV-infected cells in order to identify immune responses able to efficiently recognize and clear infected cells.

HIV Antigen Processing and Hierarchy of HIV-Specific Immune Responses

HIV-specific T cell responses are extensively studied with regard to peptide and HLA specificity (i.e., specific subtypes of MHC-I or MHC-II molecules), frequency and magnitude of interferon gamma production by T cells in HIV-infected persons, proliferation and cytokine production, killing capacity, TCR sequence, and specificity, but much less in terms of HIV epitope processing and presentation. Numerous HIV-specific CD8 and CD4 T cell responses have been identified in large populations of HIV-infected persons. Immunodominant responses correspond to the most frequent T cell responses in a population sharing the restricting HLA or, when defined at the individual level, as the T cell response producing the most interferon gamma. Narrow immunodominance of CD8 T cell responses in acute HIV infection contrasts with broader immune responses in chronic infection. The association of certain HLA with spontaneous control of HIV and the presence of multifunctional T cell responses indicate an important role of T cell responses in the outcome of HIV infection. However, factors driving the elicitation of immune responses associated with

control or progression of the disease or driving immunodominance are not well defined. The most detailed studies on immunodominance come from mouse models using artificial antigens such as ovalbumin or pathogens with little genetic variability and show that immunodominance is multifactorial and depends on either of these factors alone or in combination: protein degradation, affinity of peptides for TAP binding leading to peptide transfer into the ER, binding to MHC or the TCR, as well as TCR repertoire in the human population (Yewdell 2006). The presentation of a peptide by its cognate HLA is the earliest and mandatory step required to trigger an immune response. Thus both timing and amount of peptides presented by cells will likely play an important yet understudied role in the hierarchy of T cell responses established during infection or vaccination. Degradation of long HIV peptides or proteins with purified proteasomes or cytosolic extracts containing cytosolic peptidases identified a link between efficiency of epitope production and immunodominance of the corresponding T cell response. However these studies have been performed on a limited number of epitopes, and other factors such as binding affinity to MHC or to the TCR may be more influential in shaping immunodominance of certain epitopes. Since subdominant rather than dominant CD8 T cell responses correlate with lower viral loads in HIV-infected persons, and since immunodominance hierarchy naturally established during infection never leads to clearance of infection, it is now accepted that a HIV vaccine should elicit immune responses breaking natural immunodominance. Assessing the contribution of antigen processing to immunodominance is therefore important to manipulate immunogen design and to orchestrate epitope presentation in such way that natural immunodominance of HIV-specific immune responses will be broken.

Immune Escape Through Impaired Epitope Production

HLA-restricted mutations in HIV genome (i.e., mutations found only in persons sharing a given

HLA) induced by immune pressure are observed during the acute and chronic phases of infection. They can be found within and outside epitopes throughout most of the HIV proteome except a few highly conserved areas. HLA-restricted mutations in HIV can impact viral fitness, either reducing replicative capacity of the virus or compensatory mutations reestablishing viral fitness. They can also prevent immune recognition through mutations at anchor residues of epitopes either preventing or reducing binding to MHC molecules or reducing binding to the TCR of CD8 T cells. Additionally mutations outside epitopes can modify the degradation patterns of protein into epitope precursors and epitopes. Mutations within epitopes can create cleavage sites and destroy epitopes before presentation. Identifying factors driving viral evolution and predicting upcoming mutations for given combinations of HLA will permit to better design vaccine eliciting immune responses driving viral evolution toward unfit variants. However the high number of mutations and HLA combinations in the population preclude testing all mutations and assessing their potential role in immune escape. Recent studies took an indirect approach by first determining motifs that can be cleaved or not by families of peptidases involved in epitope processing to identify signatures of impaired epitope presentation (Zhang et al. 2012). Ten poorly cleavable motifs and ten well cleavable amino acids by aminopeptidases, which trim peptides from the N-terminal side, were identified. The presence of a poorly cleavable residue flanking an epitope reduced epitope production measured by mass spectrometry in *in vitro* degradation assays increased the production of N-extended peptide less well suited to be loaded onto MHC, and accordingly diminished its endogenous processing and presentation to epitope-specific CD8 T cells measured by killing assays. Conversely, switching a flanking residue toward a more cleavable flanking residue enhanced epitope production and presentation to epitope-specific CD8 T cells. Interestingly, the analysis of all HLA-restricted mutations in a population of 1,200 HIV-infected persons showed a significant

enrichment – at the population level – in residues poorly cleavable by aminopeptidases flanking several HIV epitopes. Another study showed that the intracellular stability of peptides prior to loading onto MHC-I contributes to defining how much peptide is available for display to T cells. The analysis of 167 HIV peptides showed highly variable intracellular stability, and the computation analysis of combinations of residues in stable and unstable peptides identified motifs in either category of peptides. Again natural HLA-restricted mutations found in HIV-infected persons tend to evolve toward more cleavable motifs reducing peptide presentation. Thus mutations outside epitopes leading to modified degradation patterns and reduced production of optimal epitopes are probably occurring more frequently than originally thought during viral evolution under immune pressure.

Altogether, these results show that the combined experimental and computational analyses of HIV protein degradation and epitope production, a better understanding of the specificity of cellular peptidases, and the capacity to analyze binding affinity of peptide variants to MHC molecules will permit the prediction of mutations leading to impaired peptide presentation and the contribution of various mechanisms of immune escape to viral evolution at the population level.

HIV Antigen Processing and Vaccine Immunogen Design

The ultimate goal of HIV research is to develop vaccines that will protect from infection (prophylactic vaccines) or to boost immunity in HIV-infected persons to clear infection or at least reduce viral load to undetectable levels (therapeutic vaccines). A commonly accepted approach to vaccine design would combine a potent antibody response to prevent establishment of infection and sustainable T cell responses to control viral replication (Burton et al. 2012). Challenges faced by vaccinologists include the genetic diversity of HIV, our lack of understanding of protective immunity (since there is no case

of natural clearance of infection), the establishment of viral reservoirs that cannot be eliminated with antiretroviral therapy, and our inability to induce B cells making specific neutralizing antibodies (Picker et al. 2012). Various types of immunogens (proteins, peptides, nucleic acids), viral vectors (non-replicative or attenuated persistent), vector-less system (nanoparticles carrying immunogen), and adjuvants are being tested in cellular studies, preclinical studies in animals, or in clinical trials (Johnson et al. 2013; Parks et al. 2013). The most encouraging results in recent preclinical studies used either chimeric SIV immunogens expressed by an adenoviral vector (Barouch et al. 2013) or an attenuated rhesus CMV viral vector expressing complete SIV proteins (Hansen et al. 2013). These two successful approaches may have provided immunization conditions that deeply differ from those established by natural infection, breaking immunodominance and broadening immune responses. The mechanisms underlying priming of immune responses leading to clearance (CMV study) or protection (chimeric immunogen study) are unknown. We still do not comprehend how the viral vector selected to express the immunogen (or the use of vector-less nanoparticles carrying HIV peptides or proteins), the design of the immunogen, the choice of adjuvants, and modes of vaccination impact the elicitation, hierarchy, specificity, and functionality of immune responses elicited by HIV/SIV vaccines. A shared goal of all vaccination approaches is the elicitation of sustainable immune responses clearing infected cells or blocking viral spread. Studies on HIV antigen processing and presentation will be instrumental to identify epitopes presented by infected cells, to rationally design immunogens leading to the presentation of adequate epitopes (for instance, by defining the optimal order of selected SIV/HIV fragments for chimeric immunogens, the addition of linkers facilitating the liberation of peptides from the immunogen), and to test epitope processing from immunogens prior to *in vivo* studies in relevant cells. Regardless of the vector used for vaccination, the immunogen will be processed in APC for priming of immune

responses. It is necessary to ensure through in-depth studies of antigen processing that – in the context established by the specific vaccination strategies discussed above – the immunogen will be degraded into MHC-I and MHC-II epitopes corresponding to sustainable protective immune responses able to recognize infected cells.

Conclusion

The degradation of HIV proteins into MHC-I or MHC-II epitopes is the earliest event leading to immune recognition. Compared to the wealth of knowledge on HIV-specific immune cells, little is known about HIV antigen processing and presentation. These early events will define the timing and amount of epitopes presented by HIV-infected cells. The identification of HIV peptides presented by infected cells should be an important goal of the current HIV antigen processing research as it will permit the complete definition of the landscape of peptides during infection, possibly leading to the identification of additional immune responses efficiently recognizing and clearing infected cells. Various motifs within and outside epitopes define the sensitivity of a given sequence to degradation, the kinetics of epitope production, and the overall efficiency of epitope presentation. Conversely, studies on HIV antigen processing also allowed a better understanding of the mechanisms of immune escape and highlighted the multiple ways HIV evolves to block or limit epitope presentation through mutations affecting various steps of epitope processing. Understanding the contribution of various mechanisms to viral evolution (viral fitness, compensatory mutations, drug resistance, antigen processing mutations) will inform how to identify immune responses of the virus toward unfit variants while allowing efficient recognition of infected cells. Finally, uncovering mechanisms of HIV peptide processing presentation may provide ways to check proper processing of vaccine immunogens into epitopes in relevant cells and if necessary to modify sequences to optimize presentation of HIV epitopes during vaccination.

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HIV Cancers in Resource-Limited Regions

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Definition

About 29 of 33 million persons living with human immunodeficiency virus (HIV) or the acquired immunodeficiency syndrome (AIDS) (PLHA) worldwide in 2009 reside in resource-limited countries. The burden of cancer in this population is substantial, although there is much we still do not know about the epidemiology of various HIV-associated tumors in these regions. Access to life-extending combination antiretroviral therapy (cART), which has been rapidly expanding since 2000, has improved survival to nearly normal life expectancy, including in resource-limited countries where about 30% of PLHA are receiving cART. The improvements in survival with HIV, however, are occurring at the expense of increased incidence of chronic comorbidities including cancer in PLHA on cART. Sparse data about the risk of cancer in PLHA in resource-limited countries complicates efforts to evaluate this concern. Cancer is diagnosed in about 30% of PLHA in developed countries. Cancers in PLHA are historically categorized as “AIDS-defining” cancer (ADC) or “non-AIDS-defining” cancers (NADCs). ADCs include Kaposi sarcoma (KS); aggressive non-Hodgkin lymphoma (NHL), including Burkitt lymphoma (BL); and invasive

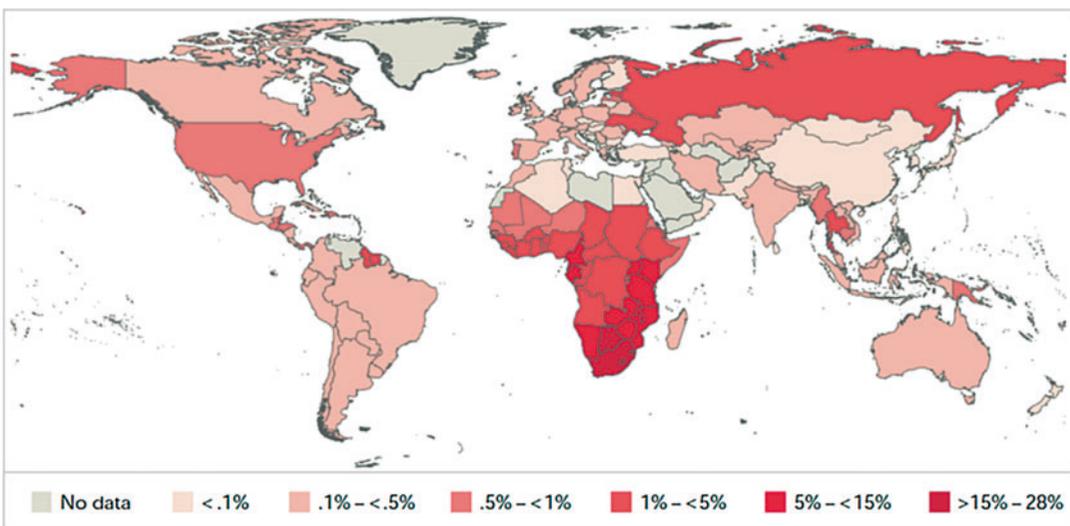
cervical cancer. These cancers have a viral etiology and are more likely to be associated with the degree or duration of immunosuppression. NADCs include Hodgkin lymphoma and anogenital, liver, and lung cancer. Most of these cancers have a viral etiology or are associated with lifestyle factors, e.g., cigarette smoking or injection drug use, which are more prevalent in PLHA. These cancers often occur in spite of sustained immune restoration. Thus, NADCs have assumed greater importance as PLHA survive longer and ADCs proportionately decrease. Cancer is diagnosed in fewer than 5% of PLHA in resource-limited countries. This rate is likely a gross underestimate and is also affected by competing mortality from endemic infectious diseases such as malaria and tuberculosis. The low access to diagnostic services in low-resource settings may also mean that a notable fraction of cancers remain undiagnosed in PLHA. Increasing access to affordable cART in resource-limited settings is likely to rapidly reduce infectious comorbidities such as tuberculosis and thus amplify the importance of cancer in PLHA. Studies of PLHA in resource-limited countries are needed to characterize the additional cancer burden in PLHA to inform public health policy and knowledge about cancer etiology in different populations.

Introduction

In 2009, 28.8 million PLHA were living in resource-limited countries, including 22.5 million in sub-Saharan Africa and 6.3 million in countries in South Asia, Southeast Asia, East Asia, and Central and South America (History of the AIDS Epidemic) (Fig. 1) (2009). The risk for cancer in PLHA in resource-limited countries has not been well described, with fewer than 5% of PLHA in resource-limited countries being diagnosed with cancer (► [Cancers Related to HIV](#)). This low rate is most likely a gross underestimate because of competing mortality due to common infections like malaria and tuberculosis and because of lack of specialized hospitals that can diagnose and treat cancer and cancer registries that can collect and analyze incidence data on various cancers that are seen in the region. For example, only four countries in sub-Saharan Africa had registries of high quality sufficient for inclusion in the *Cancer Incidence in Five Continents* monograph published by IARC in 2002. Thus, the relationship between HIV and cancer in the resource-limited countries remains poorly described.

Understanding the impact of HIV on cancer in populations residing in resource-limited countries is important for public health and science

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HIV Cancers in Resource-Limited Regions, Fig. 1 Global prevalence of HIV in 2009 (UNAIDS Report)

(► [Cancers Related to HIV](#)). In 2008, the International Agency for Research on Cancer (IARC) estimated that 12.7 million new cancers were diagnosed worldwide, 56% – or seven million new cases – were in resource-limited countries, and about 23% of those cancers could be attributed to infections (de Martel et al. 2012). Also, the profile of cancers occurring in resource-limited countries varies considerably from region to region, in part, because these regions span a broad range of prevalent infections; lifestyle factors; genetic, environmental, and dietary backgrounds; and different demographic distributions. No prospective studies have been conducted to investigate cancer risk in PLHA in resource-limited countries. In developed countries, the evaluation of cancer burden in PLHA has been facilitated by linking the records of population-based HIV/AIDS registers to population-based cancer registers, but this is difficult to implement in most resource-limited countries because of the lack of electronic registries. Some data has accumulated from case series reports and a few case-control studies that have been published. The case series suggest an increase in the number of cases or a change in the clinical pattern of disease, but they are not useful in quantifying risk or identifying cofactors associated with cancer in PLHA. Case-control studies have provided some insights into risk, but they are few and often have been small. The results from the studies are described in detail below. For these reasons, more quantitative and accurate studies of HIV-associated cancers in resource-limited regions are urgently needed.

AIDS-defining Cancers

Kaposi Sarcoma

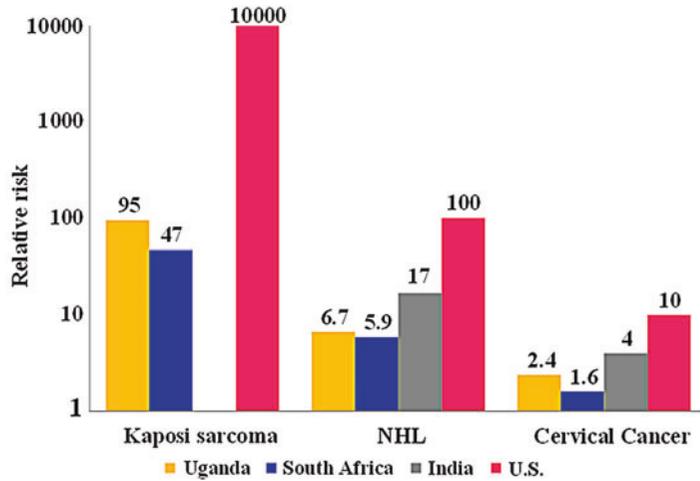
KS was endemic in sub-Saharan Africa before the onset of the AIDS epidemic. The epicenter of KS was around the Congo-Nile Watershed in Equatorial Africa, and the incidence fell with distance away from this epicenter. In the early 1980s, clinicians working in Uganda and Zambia noted an increase in the number of KS cases attending their clinics and a change in the clinical course of the

disease (1988). The changes in the clinical picture and epidemiology resembled the pattern of epidemic KS cases that had recently been described in men who have sex with men in the USA and Europe (► [Epidemiology of AIDS-Related Malignancies](#)). Subsequent studies on site in Africa confirmed that epidemic, but not endemic, KS in Africa was associated with AIDS. In contrast to patients with endemic KS, whose clinical course was indolent and who developed lesions mostly on the feet or hands, patients with epidemic KS often developed widely disseminated lesions almost anywhere on the body, including internal body organs and mucosal surfaces, and often had a rapidly fatal course (► [Presentation and Pathogenesis of Kaposi Sarcoma](#)). The median age of patients with epidemic KS was also lower, corresponding the median age of patients with AIDS, and the male-to-female sex ratio was close to 3:1, in contrast to 10:1 reported for endemic KS.

The incidence of KS has increased in the general population of countries in sub-Saharan Africa with a substantial HIV epidemic. For example, in Uganda, one of the first countries to be touched by the HIV epidemic, KS incidence rate per 100,000 increased 12-fold in men and 218-fold in women during 1995–1997 as compared with 1960–1966 (Mbulaiteye et al. 2011). It is currently the first or the second commonest cancer in men, women, and children in Uganda. Similar changes have been reported in other countries in Equatorial Africa, including Zimbabwe, Zambia, and Tanzania, although precise estimates of the change in the incidence are not available. The risk of KS is increased 22–95 times in persons with HIV compared to unaffected persons (Fig. 2) (Mbulaiteye et al. 2011). For example, in Rwanda, close to the epicenter of endemic KS, the risk of KS is increased 35 times in persons with HIV compared to unaffected persons. Similarly, elevated risks have been reported in South Africa (22–47 increased risk with HIV). The impact of HIV on KS risk appears to be higher in children. One study conducted in Uganda reported that the risk of KS was 95 times higher in children (≤ 15 years) with HIV compared to unaffected children. While the fold increases in the risk of KS in Africa are

HIV Cancers in Resource-Limited Regions,

Fig. 2 Relative risk of AIDS-defining cancers (Kaposi sarcoma, non-Hodgkin lymphoma, and cervical cancer) in people living with HIV in three different resource-limited countries and in the USA as compared to unaffected populations in the same countries (Mbulaiteye et al. 2011)



lower in PLHA than in the USA, these estimates probably grossly underestimate the impact of HIV because of premature mortality due to more common illnesses, such as malaria and tuberculosis, due to underdiagnosis, and higher background incidence rate of KS in the general population in Africa.

An essential risk factor for KS is infection with Kaposi sarcoma-associated herpesvirus (KSHV) (► [Kaposi's Sarcoma Associated Herpesvirus](#)), which was discovered by Moore and Change in 1994 (Boshoff 2000). Serological studies conducted in Africa, where KS is endemic, have confirmed, as expected, that KSHV is common, with antibodies detected in 20–80% of persons tested. The highest prevalence is found in populations residing in the Congo-Nile watershed, the epicenter of endemic KS, where 50–90% of adults have KSHV antibodies. The risk of KS is associated with the presence of KSHV antibodies or DNA in peripheral blood and with severe T-cell immunosuppression. In resource-limited countries, the use of cART is associated with a substantial reduction in the risk of KS (► [Management of Kaposi's Sarcoma](#)). The globally coordinated efforts aimed at interrupting the spread of HIV/AIDS and the reducing mortality from AIDS in sub-Saharan Africa have dramatically increased access to cART, from a few hundreds a few years ago to close to four million PLHA in 2009 (2009), mostly using the more affordable regimens based on non-nucleoside

reverse transcriptase inhibitors (NNRTIs). Significant increase in life expectancy has already been reported among PLHA using cART in sub-Saharan Africa, and the incidence of KS is falling, although less precipitously than was observed in developed countries. The reasons for the apparent lower protection of cART against KS in patients in Africa are unclear, but they may include poor adherence, the effects of coinfections on immunosuppression, or other unknown factors. There is some evidence suggesting that anti-HIV regimens containing NNRTI may provide less protection against KS than regimens containing protease inhibitors, although some retrospective studies suggest there is no difference and this remains a controversial area. KS has also been reported to develop shortly after the initiation of cART, suggesting that KS may occur as a complication of the immune reconstitution syndrome (Jaffe et al. 2011). These observations suggest that unlike in developed countries, where KS has become less of a concern with the introduction of cART, KS is likely to continue to be a major problem of PLHA in sub-Saharan Africa because of the significant overlap of KSHV and HIV infections in the population. Thus, public health messages in Africa should stress the link between HIV and KSHV and highlight the opportunity for screening and early detection of KS and linkage to HIV care.

While KS can occur in Asian PLHA, it is distinctly uncommon. Only a handful of cases

have been reported in India, although the country has more than 1.5 million PLHA who include a substantial proportion of men who have sex with men. KS was not observed among 137 HIV-positive patients with cancer treated from 2001 to 2005 at the Tata Memorial Hospital in Mumbai, the largest tertiary cancer referral medical center in India (Biggar et al. 2009). The reasons for the low rate of KS in India are unclear. KS also is uncommon in Thailand. Since KSHV infection is required for KS to develop, the low rate of KS suggests low prevalence of KSHV. However, there is little known about KSHV seroprevalence; in one study, the seroprevalence was about 7% in India (Ablashi et al. 1999). Why KSHV would be uncommon in India is unclear. In Africa, KSHV infection occurs in early childhood, and prevalence increases rapidly with age in both sexes, particularly in poorer households without access to clean water. Transmission is probably via saliva exchange. Why KSHV has not spread in India or other areas of Asia, despite a large population living in poor socioeconomic conditions and lacking access to clean water, raises interesting questions about the biology of the virus versus human hosts in Asia. KSHV infection can occur among Asians because infection and KS have been reported in populations on the west coast areas of India, which have had many centuries of immigration and trade interaction with east and central Africa. Although data are sparse, there is evidence that half or more of adults in those regions are KSHV-infected and KS is also endemic. Studies conducted in PLHA and in the general population to confirm the low prevalence of KSHV and KS in Asia could provide insights about virus-host pathobiology. These studies might reveal, for example, pockets of KSHV infection in remote rural areas of India or Asia, which when they overlap with HIV might be associated with increased risk of KS.

In contrast to India, KS is diagnosed frequently in men who have had sex with men in Latin American countries, including Brazil, Columbia, and Argentina. In these countries, one-quarter of KS cases are AIDS defining, and three-quarters are diagnosed after AIDS onset (Yoshioka et al. 2004). The risk of KS has been linked to

infection with KSHV, which can be transmitted through male-male sexual contact (Boshoff 2000).

Non-Hodgkin Lymphoma

The risk for NHL in resource-limited countries is considered to be lower than in resource-limited countries, with notable exceptions. For example, Burkitt lymphoma (BL), which was first described by Denis Burkitt in 1958 in African children and linked to Epstein-Barr virus (EBV) (► [Epstein-Barr Virus](#)), is endemic in sub-Saharan Africa (► [Burkitt and Burkitt-Like](#)). In 1982, BL was one of the aggressive lymphomas that heralded the AIDS epidemic (► [Epidemiology of AIDS-Related Malignancies](#)). Not surprisingly, scientists wondered whether all or some of endemic BL cases might also be AIDS associated. Studies conducted on site in Africa dispelled this notion. Those studies demonstrated, as has been shown by studies conducted since, that most instances of BL in sub-Saharan Africa are HIV negative. The prevalence of NHL was 2.8% (compared to 6% in developed countries) in a necropsy study of among 247 persons aged 14 years or older who died of HIV-related disease and underwent post-mortem diagnosis at a hospital in Abidjan, Côte d'Ivoire, during 1991–1992 (Lucas et al. 1994). This included 1.6% with visceral NHL and 1.2% with primary cerebral lymphoma. Childhood BL was not observed among 78 autopsies performed on children in the same study. In the absence of well-conducted prospective studies, the results from this autopsy study provide the best data about the impact of HIV on NHL in Africa, where underdiagnosis may contribute to the low rates of NHL. A study of 26 NHL cases among persons aged 16 years or older diagnosed between 1992 and 1996 in Kenya found that 19 of the cases were diagnosed in HIV-positive persons. This number was higher than the investigators had expected, based on historical patterns before the HIV epidemic. The median age of the HIV-positive BL cases was 35 years, which is higher than the peak age of 5–9 years observed in endemic BL.

Data from population-based cancer registries comparing the incidence of NHL before and after

the onset of the AIDS epidemic support the notion that the NHL incidence may be increasing during the AIDS era. The incidence increased about three times for all NHL and about four times for pediatric BL, in the general population covered by the Kampala Cancer Registry between 1961 and 1971 and 1997 (Mbulaiteye et al. 2011). While these results support the notion of a positive impact of HIV on NHL, the lack of HIV status results on the NHL cases prevents firm conclusions.

Data from case-control studies conducted at large tertiary hospitals have provided a mixed picture. The results are compatible with a null or a modest impact of HIV with the estimates of increased risk ranging from 2.2 to 46.2 times compared to unaffected populations (Fig. 2). The largest study conducted in South Africa looked at 154 histologically confirmed adult NHL cases and compared them to 4,399 adult hospital controls drawn from cancers unrelated to HIV among the men or vascular conditions among the women. This study reported a fivefold increase in HIV infection in the NHL cases (95% CI 2.7–9.5) (Mbulaiteye et al. 2011). The results from an HIV/AIDS-cancer record linkage study conducted in Uganda observed that NHL incidence was increased seven times in persons with HIV compared to the general population (SIR 6.7, 95% CI 1.8–17) (Mbulaiteye et al. 2006), but no independent histological verification of NHL was done. The impact of HIV on BL varies from 2.1 to 46. The uncertainty about the degree of impact of HIV on BL is due to the inclusion of cases diagnosed clinically or with local histopathology without confirmation by expert hematopathologists. The possible exception is the result from a study conducted in South Africa, where the BL subtype observed is sporadic. Compared to children with other cancers, children with BL were 46 times more likely to be HIV positive, based on the finding that 13 of 33 cases were positive. The impact of HIV on BL in South Africa is more similar to the impacts reported in developed countries than in Africa (Mbulaiteye et al. 2011). The contrasting impacts of HIV on BL according to the subtype of BL are intriguing and prompt the question whether the association with HIV is different

between endemic and sporadic BL (► [Burkitt and Burkitt-Like](#)). Some of the reasons for the relatively low impact of HIV on BL include premature mortality from competing causes like malaria or tuberculosis or due to underdiagnosis of BL. If HIV increases the risk of BL, then the success in reducing mortality from preventable infections like tuberculosis and malaria may more clearly reveal the impact of HIV on BL. If the risk for BL is increased, approaches to increasing surveillance may have to be considered. In addition, questions about the proper treatment of HIV-positive children with BL will have to be considered.

Studies in Asia and Latin America also suggest that NHL risk is increased in persons with HIV. In India, the proportional incidence of NHL in persons infected with HIV was increased 17 times in men and ten times in women, based on a study conducted at Tata Memorial Hospital. NHL was the most common type of cancer reported in this study, accounting for 38% of the cancers seen in HIV-infected males and 16% of all cancers in HIV-infected women. The apparently lower risk of NHL in women than men is due to a large proportionate contribution from cervical cancer. Data from population-based cancer registries in Thailand suggest that the incidence of NHL has almost tripled from 1989–1991 to 1999–2001 (Sriplung and Parkin 2004). The incidence increased most steeply (11% per year) for diffuse/high-grade lymphoma, which is an ADC (Sriplung and Parkin 2004). The risk of NHL was elevated 34 times among 3,554 PLHA in Hubei Province in China followed from 2004 to 2007 compared to the general population.

Data from Latin America are scanty, but NHLs have been reported in persons with HIV and show a strong association with EBV in different countries, including Brazil (Sampaio et al. 2007). The low rates of NHL in PLHA from resource-limited countries are likely due to the gross underestimate of the real risk due to competing mortality (Access to Care). Under-ascertainment and/or misclassification of cases likely contributes. Conclusions about the impact of HIV on NHL await results from studies using sound epidemiological and laboratory design.

Cervical Cancer

Cervical cancer is the leading cancer and cause of cancer death in women in the resource-limited countries (► [Cervical Cancer](#)) (Sylla and Wild 2012). The principal cause is infection with carcinogenic human papillomavirus (HPV) via sexual contact (► [Human Papillomaviruses](#)). Thus, an association between HIV and invasive cervical cancer might be expected in populations where there is significant overlap of both infections because of shared routes of transmission (► [Epidemiology of AIDS-Related Malignancies](#)). An additional biological association might be expected because immunosuppression by HIV would impair the women's ability to clear their HPV infection, and it might act synergistically with HPV oncoproteins E6 and E7 to facilitate progression to invasive cervical cancer in HIV-/HPV-coinfected women. Consistent with this expectation, the prevalence of cervical intraepithelial neoplasia (CIN), which reflects infection of the cervical epithelium with HPV, with or without cellular abnormalities, is increased with HIV infection, and the prevalence or incidence of HPV or CIN is inversely associated with CD4 counts. However, the evidence for the association between invasive cervical cancer with HIV infection in resource-limited countries is controversial. The incidence of invasive cervical cancer in the general population in Uganda increased about threefold from 1960–1966 to 1991–2006 (Mbulaiteye et al. 2011), based on data from the Kampala Cancer Registry, but this magnitude of increase is small and could be due to effects of temporal changes, e.g., urbanization, better access to medical care, or improved case reporting. In Zimbabwe, which has also been severely affected by the HIV epidemic, no significant change in the incidence of invasive cancer in the general population was seen between 1990–1992 and 1993–1995 despite a sharp increase in the HIV prevalence among women during the same period. Moreover, the clinical presentation of invasive cancer has not dramatically changed during the AIDS era. The null or small increase (1.6–8-fold) in the risk of invasive cervical cancer in HIV-infected women has been confirmed by findings from case-control studies.

In Uganda, HIV was associated with a nonsignificant 1.6-fold increase in invasive cervical cancer in a hospital-based case-control study conducted in five hospitals in Kampala, the capital city of Uganda (Fig. 2). The risk was similar but statistically significant in a hospital-based study on Johannesburg in South Africa. A record linkage study of the women registered in the AIDS Support Organisation in Uganda with a local cancer registry reported a 2.4-fold increase in invasive cervical cancer incidence in HIV-infected women compared to women in the general population (Mbulaiteye et al. 2006). The highest risk of invasive cancer was reported in a case-referent study conducted in referral hospitals in Côte d'Ivoire and Benin from October 2009 to October 2011. The prevalence of HIV was eight times higher in women with invasive cervical cancers compared to other cancers.

Because African countries have only begun implementing cervical screening programs, the small impact of HIV on the risk of invasive cancer in sub-Saharan Africa may be largely due to competing causes of mortality in HIV-infected women. This explanation would be consistent with reports of elevated risk of CIN in HIV-infected women, but small impact with invasive cervical cancer, which takes up to 10 years to progress from initial lesion to invasive cancer. Despite the small impact, the prevention of invasive cervical cancer must remain a priority in many African countries. Recently established HIV treatment cohorts provide suitable opportunities to not only gather information about cancer risks but also introduce innovative approaches that link HIV services to cancer screening, early detection, and treatment services (► [HIV Prevention and Women](#)).

The impact of HIV on cervical cancer in India, the country with the world's highest number of cervical cancer cases, was slightly stronger (Biggar et al. 2009). The prevalence of HIV was four times higher in women with invasive cervical cancers compared to other cancers. The impact might be 68-times higher in HIV-positive women compared to unaffected women in other countries, based on results from one study in Hubei Province in China during 2004–2008

(Mbulaiteye et al. 2011). These results, while not confirmed yet, justify continued concern about the potential association between invasive cervical cancer and HIV in resource-limited countries.

Non-AIDS-Defining Cancers

Squamous Cell Carcinoma of the Conjunctiva

The diagnosis of squamous cell carcinoma of the conjunctiva (SCCC), a rare cancer of the ocular surface linked to exposure to ultraviolet light, has been linked to HIV infection in African countries close to the equator (► [Conjunctival Carcinoma](#)). This association was first reported in Uganda, where a curious doctor noticed an increase in the number of cases presenting with SCCC at eye clinics in Kampala. A formal study of 48 patients with SCCC and matched to 48 patients with benign conditions attending the eye clinics at Mulago Hospital revealed HIV infection in 75% of the cases compared to 19% among the controls (Mbulaiteye et al. 2011). Studies conducted in Uganda and Malawi suggest a tenfold higher risk of SCCC in HIV-infected individuals than in the unaffected individuals (Mbulaiteye et al. 2011). Consistent with this high risk, the SCCC incidence rates have increased 15 times in the general population between 1960 and 1997 (Mbulaiteye et al. 2011). This increase corresponds to the increase in the fraction of eye tumors that are due to SCCC, which increased from 23.5% to 71% among the men and from 0% to 85% among the women between 1960 and 1997. The strong association between SCCC and HIV has prompted a search for infectious etiology, focusing on mucosal high-risk and cutaneous HPV. However, in spite of the initial excitement about the possibility of discovering a novel infection or association, no clear etiologic agent has yet emerged.

An increase in the risk of SCCC has not been observed in South Africa (Mbulaiteye et al. 2011), despite a substantial HIV epidemic in that region and advanced medical services. The absence of impact in South Africa, which lies in the southern hemisphere far away from the equator, suggests that the HIV impact may be mediated by

ultraviolet light-mediated damage to the surface of the eye.

The risk for SCCC in other resource-limited countries has not been quantified, but reports of cases in HIV-infected people in India suggest that it occurs (Biggar et al. 2009).

Hodgkin Lymphoma

While studies conducted in developed countries suggest an excess risk of Hodgkin lymphoma in HIV-infected persons (► [Hodgkin Lymphoma](#)), the association with HIV in sub-Saharan Africa is less clear (Mbulaiteye et al. 2011). In Uganda, Newton et al. found that two of the four adults with HL (50%) were HIV seropositive compared to the (21%) HIV seropositivity observed among other cancers not known to be related to an infectious etiology. The risk of Hodgkin lymphoma was increased 1.4-fold in a case-referent study conducted in Johannesburg in South Africa (OR 1.4, 95% CI 1.0–2.7), and it was 1.6-fold when follow-up analysis was conducted with larger numbers (95% CI 1.0–2.7). The risk was increased 5.7-fold in a record linkage study conducted in Kampala in Uganda (OR 5.7, 95% CI 1.2–17) (Mbulaiteye et al. 2006), which is compatible with an adverse impact of HIV on Hodgkin lymphoma risk. Similar to other cancers, the risk of Hodgkin lymphoma may be grossly underestimated because of the lack of pathology diagnosis in most countries in sub-Saharan Africa. It is interesting that some studies, but not all, conducted in resource-limited countries suggest that the risk of Hodgkin lymphoma may be increasing in patients who are started in cART. If this pattern is confirmed, then studies conducted in sub-Saharan Africa patients who are initiated on cART may reveal an increase in risk in patients started on cART.

In India, a study of cancer patients at the Tata Memorial Hospital observed a fourfold increase in the proportional incidence of Hodgkin lymphoma among HIV-positive men and twofold increase in women, which was not statistically significant.

Liver Cancer

Hepatocellular carcinoma (HCC) is one of the most common cancers in sub-Saharan Africa (Sylla and

Wild 2012), with most cases attributed to hepatitis B and/or C virus infections. Studies conducted in sub-Saharan Africa have confirmed the expected high frequency of coinfection between HIV and chronic HBV and HCV infections in many countries in sub-Saharan Africa (Sutcliffe et al. 2002). However, an impact of HIV on liver cancer has not been demonstrated (Mbulaiteye et al. 2011) in the studies conducted in South Africa (OR 0.8, 95% CI 0.4–1.7) or Uganda (OR 1.2, 95% CI 0.3–4.2) (Mbulaiteye et al. 2011). Data from population-based cancer registries have demonstrated continuing stable rates during the HIV epidemic, consistent with the lack of an impact from HIV. Recently, a case-referent study conducted in referral hospitals in Côte d’Ivoire and Benin has reported an increased risk of liver cancer in their series including 1,017 cancers (OR 2.7, 95% CI 1.1–7.7). Liver cancer has a long induction period, and the observed risk may be low because of competing causes of mortality in HIV-infected patients in Africa. Thus, conclusions about the associations with HIV will have to wait for better data coming from the new consortia established to provide treatment in sub-Saharan Africa and may also increase as more HIV-infected patients get access to anti-HIV therapy.

A study of cancers in a cohort of HIV patients at the Zhongnan Hospital of Wuhan University in Hubei Province in China observed an elevated risk of liver cancer (SIR 6.0, 95% CI 2.6–12.2) (Mbulaiteye et al. 2011). While these results must be confirmed, they suggest that the risk of liver cancer may be significantly increased in HIV-positive patients in China, perhaps because of coinfection with HBV and/or HCV (Mbulaiteye et al. 2011).

The Role of Infections as Drivers of Cancer in PLHA

The onset of the HIV epidemic, heralded by cancers with suspected infectious etiology, focused attention on the role of infections and immunity on cancer risk. Although it was initially suspected that HIV might directly influence the risk of some cancers, it is now well established that HIV lacks oncogenes, which are needed to directly influence the risk of cancer in PLHA. The consensus is that

HIV primarily influences the risk of cancer indirectly via suppression of T-cell immunity (► [CD4 T Cell Depletion](#)), which then permits reactivation of latent oncogenic infections such as KSHV and EBV. This indirect mechanism has been supported by studies showing both the degree and the duration of immunosuppression measured by deficit in CD4 T cells correlate with the risk of KS and aggressive NHLs. However, not all cancers associated with infection show this expected pattern, suggesting that the relationship between HIV and cancer risk is multifactorial. The studies are limited in number and duration and have few outcomes, precluding any confident comment on risk factors, which might affect cancer risks in PLHA. The patterns of diagnosed cancer in resource-limited countries differ from those in the developed countries. Thus, the cancer profile and the risk factors for cancer in PLHA in resource-limited countries will differ from those in developed countries (Table 1). More than 50% of the 12 million cancers diagnosed globally are in resource-limited countries and about 30% are attributable to infection. The overlap between cancer and HIV among the 29 million PLHA in resource-limited countries is unknown, but even a small relative risk increase of cancer among PLHA has important public health consequences. The study of PLHA in the resource-limited countries could lead to the discovery of novel infections or novel associations with cancer. The discovery of KSHV and of a polyomavirus associated with Merkel cell carcinoma in patients with HIV/AIDS demonstrates the potential for such discoveries (► [Merkel Cell Virus](#)).

Conclusions

While cancer is not currently considered a common clinical problem in PLHA in resource-limited countries, this is likely to change as access to affordable cART improves and premature mortality from other causes is reduced (Access to Care; ► [cART and Supportive Care](#)). In the West, cancer is responsible for up to one-third of deaths in PLHA. The burden of cancer has increased in spite of the reduction in risk of

HIV Cancers in Resource-Limited Regions, Table 1 Summary of associations with selected cancers among people living with HIV/AIDS (PLHA) in developed and resource-limited countries

Cancer sites with increased risk	Relative risks in developed countries	Correlated with duration or degree of CD4 loss in PLHA	Tumor-associated co-viral infections	Incidence in developing relative to developed countries	Fold increase in PLHA in developing countries	Additional comments
AIDS-defining cancers						
Kaposi sarcoma	>1,000	++++	KSHV	Varies by region; very high in Africa	20–600 in Africa	Reported occasional in Asia
Non-Hodgkin lymphoma	20–350	+++	EBV	Low	2–46	
Cervical cancer	2–20	–	HPV	Very high	2–68	
Non-AIDS-defining cancers						
Squamous carcinoma of the conjunctiva	10–15	–+	?HPV	Very high	10	Reported in Africa, rare elsewhere
Hodgkin lymphoma	3–18	Inverse	EBV	Low	2–7	
Liver					<1	
Anus	20–50	–	HPV	Low	?	Little information
Vulva and vagina	4–8	–	HPV	High	?	

ADCs in PLHA principally because of a shift from ADC to NADCs. Projecting the future impact of HIV on cancer in resource-limited countries is fraught with difficulty given the lack of reliable data on cancer and HIV, but the negative aspect of the increasing risk of cancer in PLHA is a real concern. New efforts are needed to quantify the risk and identify opportunities to implement early cancer detection and treatment programs through linkage of HIV services to cancer care ([► Observational Cohorts Highlights of Evidence and Research](#)).

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HIV Compartments and Viral Rebound During Treatment Interruption

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Definition

One of the main challenging characteristics of HIV is its extensive genetic diversity. This diversity is apparent across geography as represented by differing clades, but on a smaller scale exists within an individual. Viral phylogenetic studies within the same individual have shown diversity and divergence of the HIV virus in the multiple cell types and tissues analyzed. These subpopulations are known as viral quasispecies (Blackard 2012). The local environment present in the tissues influences the evolution of the quasispecies and subsequently impacts cellular tropism (i.e., higher affinity to macrophages from brain-derived virus), affects the response to antiretroviral therapy (ART), and ultimately, could affect eradication strategies.

A compartment has been defined as an anatomical site in which the virus is present, and there is limited exchange of viral genetic information with

other sites and may contain compartment-specific viral sequences (Eisele and Siliciano 2012). A reservoir, on the other hand, is characterized by cells or tissues that restrict viral replication and preserve replication-competent virus for a long period of time. It has more stable kinetics than the main pool of actively replicating virus. A sanctuary site is a compartment defined by limited penetration of ART (Nickle et al. 2003). These terms are not mutually exclusive, and some anatomic sites might function as one or more of these at the same time.

Introduction

The introduction of ART has dramatically changed the survival and prognosis of people living with HIV (Rodger et al. 2013). Initial analysis of the viral dynamics after initiating ART identified an initial rapid decay phase (half-life of 0.5–2 weeks) and a second slower decay phase (half-life of 1–4 weeks); early estimates based on these decay characteristics estimated that 2–3 years of therapy would be necessary to eliminate HIV from all its compartments (Perelson et al. 1997). We now know that there are two additional decay phases; a third one with a half-life of approximately 39 weeks and a fourth one with an estimated infinite half-life (Coffin and Swanstrom 2013). Recent estimates predict that close to 73.4 years of therapy might be necessary to eradicate the HIV reservoir within an individual (Siliciano et al. 2003), and new direct sequencing studies suggest that the size of the reservoir could be 60 times greater than initially proposed based on data from quantitative viral outgrowth assays (Q-VOA) (Ho et al. 2013).

It has been apparent that despite being on suppressive therapy, most patients have detectable plasma viremia when using more sensitive tests (i.e., HIV-RNA single-copy assay [SCA]). The significance of this is still unclear, but it has been shown to be a predictive factor of future virological failure and HIV drug resistance (Santoro et al. 2014; Maggiolo et al. 2012).

The source of these virions remains controversial summarized by two main hypotheses. The

first one posits that residual viremia stems from a low degree of ongoing viral replication. The second hypothesis proposes that the viremia represents a release of virions from the latent cellular reservoir, without infecting new cells owing to the presence of ART. Intensification of therapy, such as adding an integrase inhibitor, seems to have had no demonstrable reduction in residual viremia. This finding supports the theory that residual viremia might originate from the latent reservoir and not from ongoing viral replication (Gandhi et al. 2010), although there are two reports contradicting these findings (Hatano et al. 2013; Buzón et al. 2010).

The stability of this latent reservoir, mainly in resting memory CD4 T cells, allows the virus to persist despite potent ART and rebound after discontinuation of therapy.

Viral Rebound During Treatment Interruption

Treatment interruption leads almost invariably to viral rebound regardless of ART duration (Davey et al. 1999). Once therapy has been discontinued, time to detectable viral rebound is determined by two main factors; initially, the time to initiate viral replication by the latent reservoir (influenced by drug washout and time to viral reactivation), and second, the time from replication to viral load detection (Pinkevych et al. 2015). Several studies have shown a median time of 4 weeks to viral rebound (using a threshold of 200–400 copies/ μ L) after stopping ART and a median of 50 weeks to achieve pre-ART viral load levels (Li et al. 2016; Hamlyn et al. 2012).

The length of this period is influenced by several factors such as time of initiation of ART (acute vs. chronic HIV infection) (Steingrover et al. 2008), duration of ART prior to treatment interruption (Marconi et al. 2008), cd4 nadir (Hamlyn et al. 2012), and reservoir size (Li et al. 2016).

There is a small proportion of patients (5–15%), called posttreatment controllers (PTC), who remains virologically suppressed for several years after treatment interruption. Usually, they

have received very early and effective ART for several years before treatment interruption. These patients have been described in several studies (Hocqueloux et al. 2010; Sáez-Cirión et al. 2013; Sthör et al. 2013) and differ from elite controllers in several ways: there is not an overrepresentation of protective HLA class I alleles (HLA B*27 or HLA B*57), and they have ineffective HIV-specific CD8+ T-cell responses and low levels of T-cell activation. The exact mechanism has not been fully elucidated, but factors associated with PTC are a longer duration of ART, a female gender (Sthör et al. 2013), and a small viral blood reservoir (Sáez-Cirión et al. 2013).

Sources of Viral Rebound

Analyses of structured treatment interruption studies (STI) after long-term suppressive ART have shown that the origin of the rebound virus seems to be from long-lived latently infected resting CD4 T cells (Joos et al. 2008). More in-depth analyses have shown no significant changes in diversity, divergence, or phylogenetic structure on the HIV subpopulations when comparing pre-therapy to post-therapy virus, further supporting the idea that the reservoir becomes established before ART initiation and persists in long-lived cells that are subsequently the source for the rebound virus (Kearney et al. 2014, 2015). A recent study showed that the origin of this rebound virus seems to be from reactivation of many latently infected cells from multiple sites (Rothenberger et al. 2015).

HIV Compartments

As described above, viral compartments are characterized by a restriction of the viral gene flow between cells or tissues. The different factors thought to influence the establishment of a compartment are the following: cell types susceptible to HIV infection, immune pressure, local concentration of antiretroviral drugs, levels of inflammation/immune activation, and coinfections, among others (Blackard 2012).

To date, the most well-established HIV tissue compartments are the central nervous system (CNS), genital tract, gut-associated lymphoid tissue (GALT), and lymph nodes. Despite our extensive knowledge of viral dynamics in peripheral blood, there are limited studies on the dynamics existent in these different tissue compartments (Svicher et al. 2014).

CNS

The CNS represents an anatomic compartment as well as a pharmacologic sanctuary with very distinct characteristics. Its relative immune-privileged state permits an immunologic selective pressure on the virus that is distinct from lymphoid tissues. Additionally, viral quasispecies adapt within this compartment in order to replicate efficiently in macrophages further diverging these populations from peripheral blood (Gonzalez-Perez et al. 2012). The compartmentalization of the CNS is apparent when comparing HIV viral sequences between the blood and the brain; changes have been noted in the envelope (*env*) (Dunfee et al. 2006), *pol* (Dahl et al. 2014), *nef* (Olivieri et al. 2010), and long terminal repeat (LTR) sequences (Ait-Khaled et al. 1995). Certain variations in the HIV *env* sequences have shown relationship with HIV-associated dementia (HAD); this is believed to be due to increased neurotoxicity of these brain-derived variants (Power et al. 1998).

In the CNS, HIV replicates primarily in macrophages. There are four types of macrophages: the perivascular macrophages, meningeal macrophages, and macrophages of the choroid plexus and the microglia. The perivascular macrophages and microglia have been found to be the major reservoir for HIV, as well as the primary source of persistent inflammation in the brain (Zayyad and Spudich 2015). The role of astrocytes as a reservoir in HIV infection is less clear. HIV-infected astrocytes have been identified, but it is unclear if these cells can reactivate and produce infectious virions. Nonetheless, these cells produce tat protein, a potent neurotoxin associated with neuronal damage (Churchill and Nath 2013).

Regarding antiretroviral (ARV) penetration of the blood–brain barrier, a CNS penetration

effectiveness (CPE) rank was developed in order to categorize the current ARVs based on the ability to achieve optimal concentrations in the CNS. These categories were established after evaluating each ARV's chemical properties, concentration in the cerebrospinal fluid (CSF) from clinical studies, or effectiveness in mitigating HIV-associated neurocognitive disorders (HAND). The three categories are those with (1) the lowest CNS penetration, (2) an intermediate penetration, and (3) the highest penetration. Individual drugs are then assigned a value of 0, 0.5, or 1, respectively (Letendre et al. 2008). Evidence regarding the utility of this score to target CSF viral escape or HAND remains controversial, with some studies advocating for a benefit (Canestri et al. 2010), while others showing no benefit of an ART regimen with a high CPE score (Caniglia et al. 2014).

Lymph Nodes (LN)

Within lymph nodes, the majority of HIV-infected CD4⁺ T cells are found in the follicular mantle of the germinal center (GC) and the paracortex. The germinal center itself mainly contains follicular dendritic cells (FDC) that bind free virions via their complement and antibody receptors (Haase 1999).

HIV infection within the LN gets established in the very early stages of infection and, in untreated individuals, represents the main source of virus production and storage (Pantaleo et al. 1991). Unfortunately, despite the initiation of ART and suppressed plasma viremia, LN contain higher titers of viral RNA than other tissues (Horiike et al. 2012), and they are likely a key reservoir that contributes to viral rebound after treatment interruption (Rothenberger et al. 2015).

It is possible that LN could also represent a viral sanctuary, as ART (TDF, FTC, ATV, DRV, EFV) drug penetration is lower in lymphoid tissues compared to the blood cells (Fletcher et al. 2014), although this remains to be confirmed.

Genital Tract

HIV is transmitted predominantly via the sexual route, and semen is considered the major vehicle for transmission in both women and MSM

(Svicher et al. 2014). Current ART has significantly decreased the risk of sexual transmission by decreasing the HIV RNA in plasma and seminal fluid to undetectable levels. Nonetheless, HIV shedding in semen has been detected in some men receiving suppressive ART, highlighting the importance of the genital tract as a distinct compartment (Gianella et al. 2013).

It has been shown that HIV characteristics between the blood and semen differ in aspects such as the envelope and protease genes, drug resistance, and lack of correlation between the blood and seminal plasma RNA viral loads. Besides, there is evidence of different HIV quasi-species between seminal leukocytes and seminal plasma (Craig and Gupta 2006). The mechanism for compartmentalization within this site is not known, but higher levels of cytokines and chemokines and therefore inflammation and T-cell activation have been found in the seminal compartment (Politch et al. 2012). This inflammation and T-cell activation increases HIV replication and infection of new cells through several mechanisms. Cytokines such as IL-6 result in an increase of HIV transcription and post-transcription steps, a proliferation of HIV-infected cells, and a reduction in HIV immune response. Expression of CCR5 on CD4⁺ T cells leads to an increased susceptibility of these cells for HIV infection and traffics these cells to areas of highest HIV infection within tissues (Golden et al. 1992; Blackard 2012). The genital female tract has documented compartmentalization as well, and factors such as a higher CD4 count are associated with a higher degree of compartmentalization and changes in the density of gp120 glycosylation (Craig and Gupta 2006; Kemal et al. 2003).

Elevated levels of genital pro-inflammatory or chemotactic cytokines (MIP-1 α , MIP-1 β , IP-10, IL-8, MCP-1, IL-1 α , IL-1 β , IL-6, and TNF- α) have been associated with an increased risk of HIV acquisition (Masson et al. 2015). A major source of elevated inflammatory cytokines in the genital female tract seems to be sexually transmitted infections (STI) and bacterial vaginosis (BV) even if they are asymptomatic (Masson et al. 2014).

Herpes simplex type 2 (HSV-2) infection has been demonstrated to have an important synergy with HIV as both increase the risk of acquiring the other. It is believed that HSV-2 increases the risk of HIV-1 infection through disruption of the epithelial barrier and recruitment and persistence of inflammatory cells that are susceptible to HIV-1 (CCR-5 expressing CD4 T cells, dendritic cells) in the genital tract (Barnabas and Celum 2012).

GALT

Within weeks after primary HIV infection, there is massive depletion of CD4⁺ memory T cells in the lamina propria of GALT; this significant depletion likely reflects the high frequency of lamina propria CD4⁺ T cells that express CCR5 and are in an activated state (Eisele and Siliciano 2012). The initiation of ART does not rapidly reverse this process, and higher frequencies of HIV-proviral DNA are detected in this compartment compared to blood despite long-term suppressive therapy (Chun et al. 2008).

The role of GALT as a compartment is still controversial. Several studies have shown no genetic differences or viral evolution between the HIV-1 sequences in the blood compared to the gut (Svicher et al. 2014).

HIV Reservoir

The reservoir has been recently redefined as an HIV-infected cell population that allows the existence of replication-competent HIV-1 in patients on optimal ART in the order of years (Eisele and Siliciano 2012). This new definition emerged to reflect the increasing efforts toward identifying a cure and eradication of the reservoir. However, the reservoir is already present in a small fraction of resting CD4⁺ T cells (<0.05%) before ART initiation. The reservoir is extraordinarily stable, and it seems to have minimal decay after several years of suppressive therapy (Siliciano et al. 2003).

Latency

Viral latency is a state of reversible, nonproductive HIV infection of individual cells. HIV establishes latency in cells through two different

mechanisms: pre-integration and post-integration latency. Pre-integration latency usually happens when the virus infects resting CD4⁺ cells; however, due to blocks on the cell cycle in this state, the viral genome is unable to migrate to the nucleus and integrate to the host genome. This reservoir is considered to be very labile, with a half-life of 1–6 days. Post-integration latency occurs when HIV infects activated CD4⁺ T cells and integrates into the host DNA and the cells survive long enough to revert back to a resting state as memory cells (Blankson et al. 2002). This reservoir gets established very early during the primary HIV infection and remains stable despite viral suppression with ART (Finzi et al. 1999).

The most well-characterized cellular reservoir in HIV-1 infection is the small pool of latently infected resting memory CD4⁺ T cells; among which, the subset of central memory (T_{CM}) and transitional memory (T_{TM}) CD4⁺ T cells are infected in higher proportion compared to effector memory (T_{EM}) or naïve (T_N) T cells (Chomont et al. 2009). During treatment, the frequency of these latently infected cells has been noted to be low, between the ranges of 0.03–3 infectious units per million (IUPM) (Siliciano et al. 2003). The factors that contribute to the stability of this latent reservoir have not been fully elucidated, but are thought to be due to the intrinsic stability of the resting memory CD4⁺ T cells, cryptic (cell-to-cell) transfection, and homeostatic proliferation (Chomont et al. 2009; Rong and Perelson 2009). More recently, another mechanism has been postulated for the persistence of this reservoir and involves clonal expansion of HIV-infected T cells influenced by insertion of the provirus in specific genes (i.e., MKL2 and BACH2) contributing to the expansion and persistence of the host cell (Maldarelli et al. 2014).

Other proposed cellular reservoirs are the monocyte–macrophage cell lineage, dendritic cells (DCs) and follicular dendritic cells (FDCs). These cells have been shown *in vitro* to support HIV replication and have long half-lives permitting an ideal candidate for composing part of the reservoir.

The monocyte–macrophage cell lineage could represent an important second reservoir for HIV

given their longevity and limited cytopathic effects in these cells. Monocytes (Sonza et al. 2001) and macrophages (Cribbs et al. 2015) have been shown to harbor HIV-proviral DNA despite undetectable plasma viremia. However, their role in latency and as a reservoir is still being studied.

Measuring the HIV Reservoir

One of the greatest challenges to eradicating HIV is detecting changes in the reservoir after experimental interventions. Measuring the HIV reservoir generally refers to quantifying the pool of latently infected T cells in the body. To date, there is no perfect method to measure this reservoir, as the latently infected T cells are disseminated throughout the body and concentrate differently in the lymphoid tissues (Rouzioux and Richman 2013). Each technique will give us different information about the reservoir.

Viral outgrowth assay (VOA): currently considered the gold standard assay for measuring the size of replication-competent HIV reservoir, but with a number of drawbacks. This measures the frequency of resting CD4⁺ T cells that produce infectious virus after a single round of maximum *in vitro* T cell activation. Limiting dilutions of resting CD4 T cells are stimulated with the mitogen phytohemagglutinin (PHA); released viruses are expanded by addition of CD4⁺ T lymphoblasts from healthy donors. Afterwards, the number of culture wells at each dilution that are positive for HIV-1 p24 antigen (via ELISA) is determined. These results are extrapolated to calculate the number of latently infected cells per million resting CD4⁺ T cells (infectious units per million, IUPM) (Ho et al. 2013; Eisele and Siliciano 2012). The main disadvantages of this assay are that it is labor-intensive and time-consuming, requires a large volume of blood (120–180 ml), and relies on the number of resting CD4 T cells isolated.

Molecular Measure of the Reservoir

HIV genetic sequences can be found within infected cells (i.e., the reservoir) in a variety of forms: HIV DNA (integrated, nonintegrated, and

circular) and HIV RNA (spliced and unspliced) (Lewin et al. 2011).

Total cell-associated HIV DNA (CA-DNA): CA-DNA (integrated, nonintegrated, and circular) is detected through PCR. The PCR-based assays are far cheaper and faster than the VOA. However, the main problem with this assay is that it cannot differentiate between cells infected with defective virus and viable provirus, overestimating the size of the reservoir when compared to the VOA. Furthermore, it cannot distinguish between integrated and nonintegrated HIV DNA.

Integrated HIV DNA: for this assay, an *Alu*-LTR PCR is used with the capacity to quantify only integrated HIV DNA.

2-LTR circles: 2-LTR circles are episomal forms of nonintegrated HIV DNA and a marker of recent infection. PCR is also used to detect them.

CA HIV RNA: viral RNAs transcribed from the integrated provirus give origin to multiply spliced RNAs (ms), unspliced RNAs (us), and incompletely spliced (is) RNAs. The origin of these transcripts in patients on suppressive ART can be from latently infected cells, reactivated cells, or newly infected cells. This CA-RNA is considered to be a biomarker of the “active viral reservoir” (Pasternak et al. 2013). Similar to HIV-DNA measures, this assay cannot distinguish between replication-competent and replication-incompetent virus.

RT-PCR for poly(A) tail of HIV-1 mRNA: this assay targets specifically the viral mRNA generated only after host transcription via downstream primers consisting of oligo dTs followed by nucleotides complementary to the last few nucleotides before the poly(A) tail (Shan et al. 2013). It is also used to detect residual viremia. The advantage of this technique is to eliminate the host read-through of HIV DNA.

Tat-/Rev-induced limiting dilution assay (TILDA): this assay measures the frequency of cells with multiply spliced HIV RNA in unstimulated and stimulated cells.

SCA: a very sensitive PCR assay that detects the presence of residual viremia with a limit of detection as low as 0.2 copies/ml plasma depending on the input volume (Eriksson et al. 2013).

Conclusion

Our inability to eradicate HIV from its compartments and reservoir represents the major impediment to an HIV cure. During acute HIV infection, the virus disseminates via the blood stream to various organs and tissues within weeks. It also establishes latency in a small pool of long-lived CD4⁺ T memory cells, which has precluded early initiation of ART from completely eradicating infection and explains viral rebound after stopping ART. In addition, HIV compartmentalization represents a unique opportunity for the virus to grow in environments with different immune pressures, drug concentrations, and levels of inflammation/immune activation that further drive its diversity and divergence.

A better understanding of these interactions might permit the development of more targeted therapeutic strategies.

Cross-References

- ▶ [Anatomic Compartments as a Barrier to HIV Cure](#)
- ▶ [Central Memory CD4 T Cells](#)
- ▶ [HIV Life Cycle: Overview](#)
- ▶ [HIV Reservoirs in Lymph Nodes and Spleen](#)
- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [Post-treatment Controllers](#)

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HIV Coreceptor Tropism in Different Reservoirs

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Definition

For *Retroviridae* like human immunodeficiency viruses (HIV), the latent reservoir consists of inactive forms of the provirus that is the DNA version of the viral genome integrated at a random position in the host genome. After infection, HIV rapidly disseminates through the body and HIV reservoir is consisted of one or multiple viral reservoirs (cell types or tissues). Despite the development of successful treatment of HIV-infected individuals and the capacity to reduce HIV in the blood to undetectable levels, it has not been yet possible to eradicate HIV from the body due to the persistence of viral reservoirs. CD4+ T cells are thought to be the most significant cellular reservoir for HIV-1, but other reservoirs can contribute to virus persistence (Brenchley et al. 2004). Furthermore, the

establishment of viral persistence and latent reservoirs could also depend on the HIV tropism and differential expression of coreceptor on the surface of cells.

HIV-1 entry into CD4+ T cells is a multiple step process. First, the external envelope glycoprotein (gp120) of HIV-1 binds to the CD4 receptor on the surface of the CD4+ T cell. Binding of the coreceptor leads to conformational changes in HIV-1 gp41, followed by fusion of the viral membrane and the host cellular membrane and the release of the viral particle contents into the cytoplasm. The HIV-1 infection is then limited mostly to cells that express CD4 (a member of the immunoglobulin superfamily) and appropriate coreceptors. The identification of CCR5 and CXCR4 as main HIV-1 coreceptors for entry of HIV-1 into target cells was one of the most important discoveries in the HIV-1 field that significantly advanced the understanding of virus-host interactions. The human receptors CCR5 and CXCR4 are members of (or closely related to) the chemokine receptor family. These receptors are expressed on a wide range of cells that circulate in the blood, but the primary target for HIV-1 infection is the proliferating CD4+ T cell.

The regions of gp120 implicated in the interaction with coreceptors are variable V1/V2 and V3 loops. Coreceptor selectivity is mainly determined by genetic sequences within gp120, particularly on a highly variable and structurally flexible region termed “V3” involved in coreceptor binding (Jensen and van't Wout 2003). This V3 loop of HIV-1 gp120, a disulfide-linked loop of approximately 35 amino acids, makes direct contact with the coreceptor and plays a dominant role as determinant of coreceptor usage. Several amino acid substitutions in V3 loop (e.g., at positions 11 and 25) and total net charge of V3 loop frequently determine viral coreceptor usage. The V3 loop has now long been known to be a major determinant of cell tropism and now receptor use. The HIV viruses were then categorized as R5-tropic (ability to interact with R5 coreceptor) or X4-tropic viruses (ability to interact with X4 receptor). The R5X4-tropic viruses have the ability to use both coreceptors.

There is an increasing interest in the coreceptor tropism implication, including in reservoirs, in the HIV disease progression, to better understand the physiopathology of the disease and also because an antiretroviral class uses a novel mechanism of action by blocking attachment between HIV-1 gp120 and the human CCR5 receptor.

Tropism in PBMCs (Peripheral Blood Mononuclear Cells)

Proviral DNA in PBMCs may be considered as a potential alternative source of viral genetic material for tropism testing in patients with low or undetectable viral load (Soulie et al. 2012). The percentage of R5-tropic viruses determined in DNA varied between approximately 48% for multi-treated patients with undetectable or detectable HIV-1 viral load and 92% for non-treated patients in primary infection (Soulie et al. 2011; Frange et al. 2009; Bon et al. 2015). This proportion was similar to that previously evidenced for HIV-1 tropism in plasma. In some studies, tropism determination was performed both in proviral DNA and plasma RNA (at the same time or delayed time), and discordance rate ranged from 4.8% to 10%. Thus, there is a relatively high concordance of HIV tropism in RNA and DNA (Soulie et al. 2012). Some studies suggested that the composition of HIV-1 quasi-species in patients with sustained undetectable viral load on antiretroviral treatment can evolve with an increase of CXCR4 virus in DNA (Delobel et al. 2005).

The presence of X4-tropic viruses in PBMCs in chronically infected patients, with an R5-tropic variant in plasma, could represent the emergence of an archived virus (distinct env sequences in compartment and plasma) (Rozera et al. 2009). This is consistent with the presence of archived proviruses which might not correspond to the most prevalent variant in plasma as distinct resistance patterns were found in PBMCs and plasma.

Tropism in the Central Nervous System

The macrophages in the central nervous system, and other tissues, are an important cellular reservoir for HIV infection. R5 viruses are

predominant in tissues in which monocytes/macrophages lineage cells are prevalent like in brain tissue (Peters et al. 2007). Indeed, most HIV strains isolated from individuals with HIV encephalitis and AIDS dementia are R5-tropic viruses, which are consistent with the central role of macrophage in brain infection (Soulie et al. 2009). X4-using viruses mainly infect T lymphocytes, although some X4-tropic isolates could also infect macrophages and microglia.

The ability of drugs to cross the blood-brain barrier is a limitation for HIV treatment. An antiretroviral treatment with a low penetration in the central nervous system (CNS) can allow a residual HIV replication in the compartment; this can be related to cognitive impairment and HIV genetic variation including tropism changes. It has been shown that maraviroc (a CCR5 antagonist) achieved concentrations within the EC(90) range in cerebrospinal fluid (Tiraboschi et al. 2010). Another way to improve the drug penetration could be new drug delivery technologies.

Tropism in the Male and Female Genital Compartment

The male genital secretions are implicated in the majority of HIV transmission. Viruses present in the seminal fluid include X4 or R5 strains that preferentially infect lymphocytes and macrophages according to their coreceptor usage, respectively (Curran and Ball 2002). Several genetic studies have predicted viral tropism in the genital mucosae or secretions with a viral genetic compartmentalization between blood and this compartment associated with different coreceptor usage (Andreoletti et al. 2007). The mechanism for coreceptor switching in women's secretions is not known, but assumptions are made that X4-infected cells are originated from peripheral blood. The R5-tropic viruses are more readily transmitted than X4-tropic viruses, probably due to the selective expression of the R5 cell-surface protein on the target cells in the genital mucosa. This compartmentalization for the male or female genital tract could have an impact on transmission and could be due to divergent immune responses in these compartments.

Tropism in the Gut-Associated Lymphoid Tissue

The gut-associated lymphoid tissue is the largest lymphoid tissue in the body and contains a high level of CD4 cells that express CCR5 coreceptor (van Marle et al. 2007). It was evidenced that HIV persists in the gut-associated lymphoid tissue even in the presence of successful antiretroviral therapy. A recent study used sequence analysis of the *env* C2-V3 region to clearly demonstrate a lack of compartmentalization of HIV-1 quasi-species between blood and gut. This argues that the gut mucosa does not appear to serve as a sanctuary site for HIV-1 replication and that free exchange of HIV-infected cells takes place between gut and blood (Imamichi et al. 2011). Then, the tropism should be similar between these two compartments.

Conclusion

It is now unquestionable that the evolution of R5-tropic to X4-tropic strains occurs in vivo in HIV patients (about 50% of symptomatic individuals illustrate the capacity of HIV-1 to switch coreceptors) and that the presence of X4 viruses clearly increases the risk of disease progression. Furthermore, compartmentalization of the viruses has been shown in some cases, evidenced by HIV-1 tropism, genetic evolution, or distinct patterns of resistance. Depending on the compartment, the presence of the coreceptors and the immune selective pressure could be different. Independent HIV variation in different body compartments has been now well documented and also suboptimal drug penetration in tissue that can participate in HIV replication within compartments. In this way, there is a need to develop drugs with a good penetration into compartments to have a good pressure on HIV and to avoid viral evolution.

Cross-References

- ▶ CXCR4, Coreceptors
- ▶ Global NeuroAIDS

- ▶ HIV Life Cycle: Overview
- ▶ HIV-1 Transmission: Influence of Bodily Secretions
- ▶ Overview of HIV CNS Infection
- ▶ Tropism and Properties of the HIV-1 Envelope Glycoprotein in Transmission

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HIV Counseling and Testing, Prevention of HIV

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Definition

HIV testing and counseling (HTC) is the process that individuals and couples undergo to learn if they are infected with the human

immunodeficiency virus (HIV). A blood, saliva, or urine sample is taken from the person and tested for HIV antibodies or antigens, depending on the type of test being used. Counseling is an interactive and personalized dialog between the person or couple seeking the test and the provider. Certain kinds of HTC, such as voluntary counseling and testing (VCT), provide counseling before and after an HIV test. Other models of HTC, such as provider-initiated testing and counseling (PITC), offer pretest information and posttest counseling based on the test result and are integrated with routine clinical services. In addition to the diagnostic value of HIV testing, learning one’s HIV status through HIV testing and counseling has been shown to motivate behavior change among people who test negative for HIV to prevent acquisition of the virus and among those who are positive to reduce their risk of transmission of the virus. It is also critical to know one’s HIV infection status in order to appropriately link individuals who test positive with HIV care such as antiretroviral therapy (ART) and also triage preventative interventions such as medical male circumcision, antenatal prophylaxis, and pre-exposure prophylaxis. The World Health Organization emphasizes that mandatory or coerced HIV testing is never appropriate and that the provision of HIV testing and counseling must adhere to what is known as the five Cs – consent, confidentiality, counseling (at a minimum, pretest information and posttest counseling), correct test results, and linkages to care (WHO 2012a).

Introduction

At the time the first licensed HIV test became available in 1985, there were no treatments for HIV available and learning one’s HIV status was viewed as a virtual death sentence. During the years immediately after HIV testing became available, positive test results diagnosed within the health system were frequently reported to insurance companies, government agencies, and employers resulting in significant social harm to those tested. These social harms amplified the already existing stigma surrounding HIV and

toward the groups initially most affected, including men who have sex with men and injecting drug users. As a result, there were concerns that people at heightened risk of HIV would donate blood in order to learn their status through blood banks rather than through the health-care system. Given that the HIV test could often not detect the virus during the 6-month window period when an infected person is developing antibodies, people seeking an HIV test result through blood banks could have jeopardized the safety of the US blood supply. The US government responded to this dilemma by supporting the development of HIV testing sites where individuals could learn their HIV status anonymously. Shortly afterwards, the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) developed official guidelines on HIV testing. These guidelines emphasized that HIV testing needs to be a confidential process and should always occur together with HIV prevention counseling (CDC 1985, 1987; WHO 1990). Ultimately, voluntary counseling and testing (VCT) emerged as a model of HTC that includes pre- and posttest client-centered counseling focused on HIV prevention (CDC 1994; Divine et al. 2001; WHO 1993). VCT rapidly became the HTC model promoted internationally as an HIV prevention tool in the mid-1990s (UNAIDS 1997, 2000).

Since then, multiple developments in HIV testing technologies, effective HIV treatments, and HIV prevention approaches have changed the HIV testing landscape. When rapid HIV diagnostic tests became available, clients no longer had to return to the clinic weeks after a blood draw to learn their results. Instead clients could receive their results on the same day during the same visit, thus increasing the number of people learning their test result, especially among marginalized populations (Pottie et al. 2014). The development of effective ART in the mid-1990s also expanded the value of HIV testing and counseling from a preventive measure with psychological benefits, to a necessary step to entering care and accessing treatment. In response to such developments, new models of HTC emerged.

These models include provider-initiated HTC and, more recently, based on advances in testing technology, self-testing for HIV. These evolving approaches have also allowed HIV testing and counseling to be offered beyond clinic settings and could then be expanded to community settings. Furthermore, the importance of knowing one's HIV status has only intensified as a critical step for engaging in newer prevention tools that offer increased protection from the virus, including medical male circumcision (see “► [Circumcision and AIDS](#)”) and preexposure prophylaxis (PrEP) (see “► [Preexposure Prophylaxis \(PrEP\)](#)”).

Evolving Models of HIV Testing and Counseling

HIV Voluntary Counseling and Testing (VCT)

One of the first models of HIV testing and counseling that emerged was VCT. VCT is client-initiated, meaning that the person who wants to know his or her HIV status actively seeks the test. VCT also includes both pre- and posttest counseling. Pretest counseling is an opportunity for the counselor and client to review the test process, discuss the client's risk behaviors, review prevention options, reaffirm the decision to take an HIV test, and assess coping strategies if found to be living with HIV (Divine et al. 2001; UNAIDS 2000). During posttest counseling clients receive their HIV test results and discuss personalized risk-reduction and disclosure strategies as well as receive referrals for care and support. VCT was first promoted as a way for individuals to learn their HIV status and then expanded to include couples counseling and testing. Couples VCT involves two people undergoing VCT together and learning each other's status through mutual disclosure (WHO 2012b). VCT is often offered in a stand-alone location, separate from other health-care services, although VCT was being integrated into some existing services such as antenatal care (ANC) and sexually transmitted infections (STI) clinics even prior to the

shift toward provider-initiated testing and counseling.

Provider-Initiated Testing and Counseling (PITC)

In the PITC model, HIV testing is offered as part of routine clinical care with an emphasis on identifying and linking people living with HIV with care and treatment. Unlike VCT, which relies on individuals and couples to actively seek an HIV test, PITC emphasizes the health-care provider offering HIV testing to their clients as part of routine care. PITC guidelines require less counseling than VCT, with a focus on the provision of pretest information, which can be didactic, as compared to pretest counseling, which is client-centered and interactive (Branson et al. 2006; WHO and UNAIDS 2007). Pretest information may be provided using an opt-in or opt-out approach. In the opt-in approach, the patient must explicitly agree to have an HIV test when offered by the provider. Conversely, in an opt-out approach, the HIV test is routinely performed with the patient's awareness unless the patient explicitly declines to take the test (UNAIDS 2007). WHO/UNAIDS recommends opt-out testing except when working with vulnerable populations who may have limited freedom to refuse a test (e.g., prison populations) (WHO and UNAIDS 2007). Following the test, all PITC clients should receive posttest counseling tailored to their HIV test result. Such counseling focuses on HIV prevention for people who test either negative or positive. For those who test positive, HIV status acceptance and linkages to HIV care and treatment are also emphasized (WHO and UNAIDS 2007). WHO/UNAIDS provides further guidance to recommend PITC for all patients who exhibit signs and symptoms of HIV infection in any setting and as a standard part of medical care for all patients in settings where HIV prevalence is greater than 1%. In epidemics where the HIV prevalence is high among defined subpopulations but less than 1% among the population at large, WHO/UNAIDS recommends more selective implementation of PITC only in facilities reaching

groups at higher risk (e.g., tuberculosis (TB) and STI clinics).

HIV Self-Testing

Self-testing for HIV occurs when an individual collects a saliva or blood specimen themselves, performs an HIV rapid diagnostic test, and interprets the results in private (WHO 2014a). Self-testing differs from home specimen collection, which was approved by the US Food and Drug Administration (FDA) in 1996, and allows individuals to collect a sample of blood that is sent to a laboratory for testing. A self-testing kit developed by OraSure Technologies was approved for use by the FDA in 2012 (FDA 2012). US-based studies have demonstrated the acceptability and feasibility of self-testing among key populations, including men who have sex with men, and studies from low- and middle-income countries show promising results regarding the uptake, feasibility, and acceptability of self-testing (Krause et al. 2013).

Potential benefits of self-testing include expanded access to HIV testing, especially for high-risk populations, increased convenience and privacy, user empowerment, and the potential for mutual partner testing prior to sex (Wood et al. 2014). Concerns regarding self-testing include inequitable access due to its relatively high cost, the potential for user error and failure to seek confirmatory testing, a lack of counseling and linkage to care for infected individuals, and the potential for forced or coerced testing (Wood et al. 2014). HIV self-testing is a promising new approach to HTC, but questions remain about the logistical, regulatory, and policy implications of its implementation.

Service Delivery Approaches to HIV Testing and Counseling

The World Health Organization outlines different service delivery approaches for providing HTC in both facility and community settings (WHO 2012a).

Facility-Based

Facility-based HTC approaches consist of HTC conducted in a health-care facility. Most facility-based HTC consists of PITC in specialized clinics, including ANC, TB, STI clinics, and in general medical practice clinics. Facility-based HTC also encompasses stand-alone VCT centers serving the general population, as well as drop-in centers targeting key groups at heightened risk of HIV such as sex workers, people who inject drugs, and men who have sex with men.

Community-Based

Community-based HTC approaches include home-based testing, mobile and outreach testing, and testing that takes place in institutional settings such as schools and workplaces. Self-testing is also included as a community-based approach. Home-based testing consists of both HTC being offered door-to-door in a community and the targeted provision of HTC in the homes of index cases who are already identified as living with the virus. Mobile HTC involves the use of mobile vans or tents and may be offered in connection with specific public gatherings or events. Finally, HTC can be conducted in institutional settings such as workplaces and schools, either as a one-time event or a regular component of institutional health and wellness services.

Effectiveness

HTC has multiple goals, including people learning their HIV status to facilitate uptake of appropriate care and treatment as well as to reduce HIV-related risk behaviors. Several review articles and meta-analyses have examined whether HTC is successful at achieving these goals. Meta-analyses use statistical techniques to combine results across different studies in order to quantitatively assess the impact of the intervention. Below are brief summaries of review articles and meta-analyses to date that examined the preventive benefits of HTC – as a way to change sexual risk behaviors or as a way to increase HIV serostatus awareness and linkages to HIV care and treatment.

Changing Sexual Risk Behaviors

The earliest review articles on HTC focused on the effects of VCT on sexual risk behaviors among populations, mainly in high-income settings (Higgins et al. 1991; Wolitski et al. 1997). These reviews found that results varied by the populations studied, such as men who have sex with men and people who inject drugs, with the most consistent findings showing increased condom use and safer sexual practices among couples where at least one partner was HIV positive. In 1999, the first meta-analysis of the impact of HTC on sexual risk behavior was published (Weinhardt et al. 1999). Analyzing data from 27 published studies, the findings supported HTC as a secondary prevention method with the greatest changes in behavior occurring among individuals or couples living with HIV.

In 2008, the first meta-analysis focusing on studies from low- and middle-income settings was published. Data from seven studies showed that people who received VCT were significantly less likely to engage in unprotected sex when compared to their behaviors before receiving VCT, or as compared to people who have not received VCT [OR 1.69; 95% CI 1.25–2.31]. VCT was not found to have a significant effect on the number of sex partners [OR 1.22 95% CI 0.89–1.67] (Denison et al. 2008). A 2012 Cochrane Review updating these data found 19 eligible studies (Fonner et al. 2012). This meta-analysis found that study participants who received VCT, when compared to those who did not, were less likely to report an increased number of sexual partners [OR = 0.69 95% CI 0.53–0.90]. The odds of protected sex/condom use as a result of HIV testing was also significant when data across studies were pooled, but only among people who were living with HIV [OR 3.24 95% CI 2.29–4.58].

Only one review article to date has looked at behavioral outcomes following provider-initiated testing and counseling in low- and middle-income countries (Kennedy et al. 2013). This article provides a qualitative review of existing data as heterogeneity among intervention approaches and outcomes precluded meta-analyses. The majority of articles (12 out of 19) focused on PITC offered

in ANC, family planning, and postpartum/child health clinics. The rest of the studies took place in TB, outpatient, STI, or methadone maintenance clinics. Trends from these studies indicate an increase in condom use after PITC.

These review articles and meta-analyses highlight several trends, including growing evidence over time for desirable behavior change among people who test positive for HIV compared to their HIV-negative counterparts. Most reviews have focused on VCT, with newer reviews considering the impact of newer models of HTC on risk behavior outcomes, reflecting the history in the evolution of HTC.

Increasing Knowledge of HIV Status and Access to Care

Globally, more than half of all people living with HIV are unaware of their HIV infection status (UNAIDS 2014). Multiple reviews and meta-analyses document that HIV testing using rapid tests, conducted in clinical settings with a PITC model, and HIV testing offered in community settings all have been shown to increase the uptake of HIV testing and the number of people who learn their HIV status (Hensen et al. 2012; Sabapathy et al. 2012; Kennedy et al. 2013; Suthar et al. 2013; Pottie et al. 2014). In particular, community-based HIV testing strategies can reach groups who do not typically access routine medical care (Pottie et al. 2014). For example, a meta-analysis of home-based voluntary HIV testing found that men, who in many settings test for HIV much less frequently than women, were just as likely as women to accept the test with home-based testing (Sabapathy et al. 2012). This review of home-based HIV testing also presented a pooled estimate that 83.3% of participants (95% CI 80.4–86.1%) accepted to undergo home-based testing and 76.7% (95% CI 73.4–80.0%) received their HIV test results. Less is known, however, about how people identified as living with HIV through the different approaches to HTC link with HIV care and treatment. A review of community-based testing approaches found that 80% (95% CI 75–85%) obtained a CD4 cell count test result after learning their HIV status and 73% (95% CI 61–85%) eligible participants initiated ART

(Suthar et al. 2013). Another review of self-testing for HIV found that 96% of participants who tested positive reported they would seek posttest counseling, although none of the studies in the review published data on linking to care after the test (Pai et al. 2013). Overall, while the different models of HTC are increasing people's awareness of their HIV status, a better understanding of how these HTC strategies can connect people newly diagnosed with HIV to care and treatment is needed.

Continuing Challenges and Emerging Issues

Quality Assurance/Quality Control

Many HTC programs, particularly in low- and middle-income settings, rely on testing algorithms composed of several rapid diagnostic tests. Rapid diagnostic tests approved by the World Health Organization have shown high levels of sensitivity and specificity based on field studies conducted in low-income settings (WHO 2014b). Despite the very good quality of these tests, errors could result in the misclassification of HIV status. Inaccuracies during rapid testing can result from operator error and improper testing procedures, faulty test kits (e.g., use past expiration), using an improper test sequence, and potential cross-reactivity due to certain population characteristics (Klarkowski 2014). WHO has created guidelines to help countries determine which specific rapid diagnostic tests and algorithms are most appropriate to use (WHO 2012a). To cope with the rapidly expanding number of HTC programs and facilities, having adequate quality assurance and opportunities for confirmatory testing is critical in ensuring HTC clients are provided with accurate HIV test results.

Task Shifting

Countries have different regulations for who can conduct HTC. Only licensed nurses or doctors are allowed to conduct HTC in some countries, while in other countries, trained lay people (lay health providers) are allowed to perform HTC. With many countries experiencing health workforce

shortages, task shifting – or the rational redistribution of tasks from higher-trained providers to less well-trained providers – may help expand the reach of HTC services. Existing research, while limited, suggests that lay providers can conduct high-quality HTC and may even be better than more highly trained providers. For example, a US-based randomized trial comparing HTC provision in an emergency department setting by trained HIV counselors versus regular emergency department health-care providers found higher rates of HTC uptake in the counselor arm (57%) compared with the provider arm (27%; $p < .001$) (Walensky et al. 2011). Studies from South Africa (Jackson et al. 2013), Malawi (Molesworth et al. 2010), and Cambodia (Kanal et al. 2005) have also shown that lay providers can conduct HTC with high rates of sensitivity and specificity (over 98%). Lay providers conducting HTC may be particularly important for reaching key populations and may provide an important source of support and information to these populations as self-testing becomes more common.

Role of Risk-Reduction Counseling

The importance, nature, and extent of counseling as part of HIV testing have long been a source of debate. Extensive counseling requirements may pose human resource and financial limitations to the scale-up of HTC, and the impact of prevention counseling on individuals who test HIV negative in particular remains unclear. One reason that the independent effect on risk behaviors of counseling linked to HTC is unclear is because it has been considered unethical to randomize subjects to HIV testing without counseling. Thus, no studies that directly compare the differential effects of the testing versus counseling with HTC have been conducted. In 2006, the US Centers for Disease Control and Prevention (CDC) recommended not requiring prevention counseling during provider-initiated or diagnostic testing in health-care settings. These guidelines emphasize that HIV diagnostic testing in health care – to identify people living with HIV in order to link them with HIV care – is distinct and separate from HIV testing and counseling which is conducted mainly for prevention purposes among uninfected persons

at increased risk of HIV (Branson et al. 2006). Several trials have assessed the impact of patient-centered risk-reduction counseling as part of HTC. In 1998, Project RESPECT reported that brief, patient-centered counseling in the context of HIV testing reduced subsequent STI risk (Kamb et al. 1998), while in 2013, the AWARE trial reported that such counseling did not result in any reduction in STI risk (Metsch et al. 2013). The discrepant findings of these two trials may reflect changes over the past two decades, including the development of rapid HIV testing (and consequently a single visit for HTC) and the changing epidemiological, clinical, and social context of HIV.

Types of HIV Tests

Many types of tests exist to identify HIV. Rapid diagnostic tests use a blood or oral fluid specimen to screen for HIV-1/2 antibodies and/or antigens (WHO 2013). Results from these tests are available 10–20 min after performing the test. Enzyme immunoassays (EIAs) are laboratory-based tests that require a blood specimen to screen for HIV antibodies and/or antigens. Western blotting and line immunoassays, often used for confirmatory testing, are also laboratory-based assays that detect certain HIV-specific proteins. Molecular-based technologies, such as polymerase chain reaction (PCR), can detect HIV viral nucleic acid. These types of tests, although expensive, can help monitor the progression of HIV infection and are particularly useful in diagnosing HIV-exposed infants who carry HIV antibodies from their mothers during their first months of life which eventually disappear if infants remain HIV uninfected. New technologies, known as fourth generation assays, are currently being developed that detect both HIV antibodies and antigens and can identify HIV infection earlier than previously possible, including during the window period (WHO 2013).

Conclusion

While models of HTC have expanded and changed over time, HTC remains a critical

approach to helping people learn their HIV serostatus, access HIV care and treatment services if positive, access HIV prevention services if negative, and consider behavior change. The importance of HIV testing as a critical element in the HIV care and treatment armamentarium has grown as the epidemic has grown and as prevention and care options have increased. Virtually all of the most highly effective HIV control, prevention, and mitigation efforts rely heavily on HIV testing. These include HIV testing as a key strategy for primary prevention through risk reduction, screening and diagnosing clients to assure that they receive appropriate care and treatment, prevention of mother-to-child transmission programs, medical male circumcision, pre- and post-exposure prophylaxis, and many others. Perhaps one of the most salient, and maybe less recognized, benefits of the wide-scale availability of HIV testing is that because large number of people now know that they are infected with HIV, there has been the emergence of constituencies of people living with HIV who now advocate for rights and services. As new generations develop in a world where HIV is well established and the personal threat of HIV has been lessened with effective treatments, new challenges in the prevention, care, and treatment of HIV are arising. With half of the people living with HIV worldwide unaware of their status, combined with the positive advances being made in treatment and prevention options, it remains vitally important that innovative ways are devised to sustain and scale-up HTC service provision.

Cross-References

- ▶ [Healthcare Workers, Shortage and Task Shifting of](#)
- ▶ [HIV Testing and Counseling](#)

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HIV Infection, Immune-Based Interventions for

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Definition

The overwhelming and continuous success of combination antiretroviral therapy (ART) is undermined by the inability to completely eradicate the HIV virus, requiring lifelong treatment, which is associated not only with side effects for the patients but also with substantial costs for the society. Immune-based interventions (IBTs) were developed during the last 15 years along two distinct paradigms. The first paradigm proposed during the late 1990s and early 2000s suggested either (1) using IBTs to help restore CD4 counts, in cases of failed immune reconstitution, or (2) using therapeutic vaccines to allow for prolonged treatment interruptions (TIs) and prevent disease progression by better controlling virus replication in the absence of treatment (Autran et al. 2004; Carcelain and Autran 2013; Pantaleo and Levy 2013). However, the SMART study, which demonstrated deleterious consequences of treatment interruptions, halted these TI-based strategies. The greater efficacy of new antiretroviral drugs, together with the “Berlin patient” demonstrating the first case of successful HIV eradication, suggested that a cure for HIV is an increasingly realistic objective (Richman et al. 2009; Trono et al. 2010). The current paradigm for IBTs, termed the “shock and kill”

approach, is to develop strategies in which therapeutic vaccines would be administered in combination with latency-reversing agents, including cytokines, that reactivate virus production from latently infected cells (International AIDS Society Scientific Working Group on HIV Cure et al. 2012; Katlama et al. 2013; Richman et al. 2009; Trono et al. 2010). This review will focus on these two paradigms and discuss IBTs in the context of strategies aiming at HIV eradication and cure.

Cytokine-Based Immunotherapy

Immune Restoration with Cytokine-Based Therapies: The 1990s Paradigm

A primary goal of cytokine-based IBT has been to restore, or maintain, CD4 T-cell counts above the critical threshold of 200 cells/ μ l, a major prognostic factor for clinical progression and opportunistic infections. The major clinical benefits of ARTs are mediated by the sustained quantitative and qualitative restoration of CD4 T-cell numbers and immune function in the context of durable viral suppression (Autran et al. 1997; Guihot et al. 2011). However, some patients, particularly elderly individuals or those with advanced disease, display delays in immune reconstitution despite an appropriate virological response. These patients have been termed “immunological nonresponders” (Massanella et al. 2013). In these situations, it was suggested that cytokines such as IL-2 or IL-7, which have known roles for supporting T lymphocyte development, proliferation, and homeostasis (Ma et al. 2006), might help to accelerate or enhance immune reconstitution during treatment with ART.

Interleukin-2-Based Immune Interventions

Interleukin-2 (IL-2) is a cytokine produced by activated T lymphocytes that induces proliferation of T cells, as well as B and NK cells. IL-2 also promotes cytokine secretion of Th1, Th2, and Th17 cells, promotes NK and CD8 cell cytotoxicity, and modulates programmed cell death (Ma et al. 2006). It is also essential for the development and peripheral expansion of regulatory

T cells involved in immune tolerance. Impairment of IL-2 production by CD4 T-helper cells is one of the first functional defects reported in HIV-positive patients and is predictive of the loss of CD4 T-cell counts and clinical disease progression. Altogether, these features led to the hypothesis that administration of recombinant IL-2 in conjunction with ART would boost the quantitative and/or qualitative immune restoration (Carcelain et al. 2013). Initial phase I and II trials were conducted in chronically infected, ART-treated patients with almost normal CD4 T-cell counts, to whom recombinant IL-2 was administered at a dose of 1.5–7.5 million IU per day by subcutaneous injection for five consecutive days every 6–8 weeks. These trials indicated the dose-dependent ability of IL-2 therapy to increase CD4 T-cell numbers.

In addition, IL-2 combined with ART also restored some qualitative CD4 cell parameters. For instance, there was not only an increase in T-cell proliferation, but there was a functional enhancement of thymopoiesis, suggested by increased proportions of naïve T cells and greater levels of T-cell receptor excision circles (TREC) and a limitation of CD4 T-cell activation with subsequent increase in their survival. It was also hypothesized from *in vitro* studies that IL-2 therapy would trigger active HIV replication from latently infected cells; however, no increases in HIV viremia were reported in the vast majority of IL-2-treated patients that were simultaneously treated with ART, despite transient blips of up to 1,000 copies/mL in some patients. Otherwise, the safety profile of combined treatment with IL-2 and ART was acceptable, despite flu-like symptoms associated with IL-2 infusions. These results raised questions regarding the possible clinical benefit of IL-2 therapy on the course of HIV disease in immunological nonresponders. In an attempt to answer this key question, clinical outcomes rather than CD4 counts had to be used as endpoints in clinical trials.

Two large phase III international clinical trials, SILCAAT (*Subcutaneous, Recombinant, Human IL-2 in HIV-Infected Patients with Low CD4 Counts under Active Antiretroviral Therapy*) and ESPRIT (*Evaluation of Subcutaneous Proleukin*

in a Randomized International Trial), both 7–8 years long, involved hundreds of HIV-infected patients and clearly illustrated that administration of recombinant IL-2 did not lead to long-term clinical improvement. Indeed, IL-2 failed to reduce the rate of opportunistic diseases or deaths, despite sustained increases in CD4⁺ T-cell counts, and was associated with a higher frequency of grade 4 adverse clinical events. Based on these findings, it was concluded that restoring CD4 T-cell counts with this growth factor does not provide clinical benefits, and the adjunct use of IL-2 for enhancing immune restoration was abandoned.

Interleukin-7-Based Immune Interventions

Another cytokine, IL-7, was subsequently proposed to promote or stimulate CD4⁺ T-cell recovery in patients failing to restore appropriate CD4 T-cell counts with ART alone. IL-7 is produced by bone marrow cells, thymus stromal cells, and dendritic cells. Indeed, it is essential for thymopoiesis, for T-cell differentiation and homeostasis, and for enhancing naïve and central-memory T-cell proliferation; moreover, it is a key antiapoptotic factor for lymphocytes (Ma et al. 2006). In addition, high plasma IL-7 levels coincide with CD4 T-cell lymphopenia in untreated HIV infection, while IL-7 levels gradually decline with CD4⁺ T-cell recovery during antiretroviral therapy. IL-7 was therefore suggested to drive CD4⁺ T-cell restoration once HIV replication is controlled.

Several clinical trials demonstrated that administering this cytokine to immunological nonresponders at doses ranging from 10 to 30 µg/kg sustainably increased both CD4 and CD8 T-cell counts over 3–6 months due to its pleiotropic effects on T-cell cycling, survival, and *de novo* generation (Carcelain et al. 2013). IL-7 infusions were associated with major increases in the long-lived naïve and central-memory CD4 T cells. In addition, IL-7 may also have helped to maintain or restore T-cell responses to pathogens. Finally, IL-7 tolerance was much better than that of IL-2. Therefore, IL-7 therapy appeared to have the potential to accelerate and/or enhance immune reconstitution in individuals who failed immune

reconstitution with cART alone. However, effects of IL-7 on clinical endpoints of HIV-1 infection have never been tested in randomized-controlled clinical trials.

Toward an HIV Cure: The New Challenge for Cytokine-Based Immune Interventions

The role of IBTs in the therapeutic arsenal has been completely revised with the emerging concept of HIV remission, exemplified by the “Berlin patient” and reports of small patient populations who were able to control HIV replication after stopping ART. This novel objective raises the possibility of finding strategies to purge the HIV reservoirs which cannot be eradicated with ART alone. Persistence of HIV reflects a composite mechanism of proviral latency in quiescent cells and of a low level of residual virus production. A “shock and kill” strategy has been proposed to reactivate HIV-1 production from latently infected cells, followed by a complementary strategy to kill the cells with pharmacologically induced HIV-1 production (International AIDS Society Scientific Working Group on HIV Cure et al. 2012; Katlama et al. 2013; Richman et al. 2009; Trono et al. 2010). Some IBTs have been proposed for the shock strategy.

Initial attempts using IL-2 showed that infusions of this cytokine increased the reservoir of HIV-infected cells. IL-7 was proposed as an alternative candidate (Richman et al. 2009; Carcelain et al. 2013; Pantaleo and Levy 2013) because of its ability to trigger signaling pathways that induce HIV production and increase and/or reactivate HIV replication in cultures of naturally infected primary CD4 T cells in vitro (Richman et al. 2009; Trono et al. 2010). IL-7 also mediates an increase in transcription and replication of the provirus integrated in Peripheral Blood Mononuclear Cells (PBMCs) of patients, particularly in central-memory CD4 T cells, which are preferentially infected by HIV. Moreover, IL-7 helps to maintain or induce a CTL response against viruses, due to its ability to enhance memory T-cell expansion, providing an additional rationale for proposing IL-7 as a candidate for a “shock and kill” strategy (Katlama et al. 2013).

However, the possible benefits of IL-7 remain controversial because IL-7 may also contribute to increasing the reservoir of HIV-1-infected cells by promoting homeostatic proliferation of latently HIV-infected central-memory CD4 T cells. The first two phase I clinical trials using infusions of exogenous IL-7 in immunological nonresponders treated with conventional ART showed, in addition to the major increase in peripheral blood, some “blips” of HIV replication and transient increases in the blood content of provirus returning to baseline at the end of the study.

In an effort to test whether IL-7 could play a role in the “shock and kill” therapeutic arsenal toward an HIV cure, a randomized proof of concept therapeutic trial recently tested the hypothesis that IL-7 could ultimately decrease the reservoir of HIV-1-infected cells when combined with a synergistic ART intensification to prevent infection of new cells by inhibiting virus entry and integration. Despite an acceptable safety profile, preliminary results indicate that the HIV reservoirs did not decrease 18 months after IL-7 infusions and ART intensification.

Therapeutic Vaccines Against HIV

Antiretroviral therapy induces an exponential decay of anti-HIV effector and effector-memory CD8 T cells that parallels HIV reduction. While this reduction of HIV-specific CD8 T cells occurs when ART is administered in late or chronic stages, there is a restoration of anti-HIV CD4 T cells. The clonal contraction of virus-specific CD8 T cells likely results from the treatment-related reduction of viral antigen below the threshold required to stimulate specific T cells. Immune memory to HIV persists nonetheless, as indicated by transient restoration of T-cell responses after virus relapses during treatment interruptions or by the slow restoration of memory CD4 T-cell responses to HIV after 10 years of fully suppressive ART (Guihot et al. 2011). In contrast to ART administered in late or chronic stages, early treatment of the acute HIV infection preserves the HIV-specific CD4 T-helper cells but delays and

limits the establishment of robust and diverse CD8 T-cell responses to HIV.

Therapeutic Immunization to Restore Immune Control of HIV-1: The 2000s Paradigm

In the early 2000s, “structured-treatment interruptions,” or STIs, were proposed to boost immunity against HIV. This method was proposed to combat the burden associated with daily, lifelong administration of ART as well as the toxicity and high costs associated with antiretroviral agents. An “autovaccination” strategy had been suggested to restimulate the patient’s HIV-specific T cells during STIs with the patient’s own virus in order to restore immune responses that could control HIV during prolonged interruptions of therapy. Although some transient HIV control during treatment interruption coincided with increases in HIV-specific immunity when treatment was initiated in acute infection, such control was not observed when STIs were performed after treatment initiation in chronic HIV infection, although HIV-specific CD4 and CD8 T-cell numbers were restored to some degree. In addition, the relapses of replication-competent virus appeared to be deleterious to the antigen-specific CD4 T cells, which rapidly disappeared after virus production was reestablished.

In order to prolong the control of virus for longer periods of time than with STIs alone, it was proposed to use anti-HIV vaccines to induce a protective immune barrier against HIV when patients were still on ART (Autran et al. 2004; Kinloch-de Loes and Autran 2002). However, several obstacles prevented the development of these strategies.

Firstly, immune parameters that accurately correlated with immune protection against HIV infection were poorly defined (Pantaleo and Koup 2004), although studies in long-term nonprogressors (LTNPs) and elite controllers (ECs) indicated that HIV-specific CTL and T-helper cells can contribute to controlling this retrovirus (Autran et al. 2011). In addition, in several studies, plasma viremia in untreated HIV patients was significantly associated with

the magnitude and HIV-1 *gag* specificity of CD8 T cells producing IFN- γ . Notably, plasma viremia or disease progression seemed to better correlate with the quality of HIV-specific T cells, specifically their multifunctionality, than with their magnitude. Similarly, preserved HIV-1-specific central-memory T cells strongly correlated with levels of both plasma virus and HIV reservoirs in PBMCs of LTNPs and ECs. High functional avidity of both HIV-specific CD8 and CD4 T cells also appeared as appropriate immune correlates of T-cell-mediated control of HIV. The levels of HIV reservoirs, identified by measuring cell-associated proviral DNA copies per PBMC in LTNP studies, were even more strongly correlated with the magnitude of the HIV-*gag*-specific CD8 or CD4 T cells than was the plasma viremia. Therefore, the goal of therapeutic vaccines was to adequately stimulate both central-memory HIV-specific T cells, involved in long-term survival and clonogenic potential, and short-lived effector-memory T cells, with an immediate efficacy to kill. In contrast, anti-HIV neutralizing antibodies – known to appear only during the third month after infection and less prominent in most LTNPs and ECs – did not appear during the early 2000s as decisive players in HIV control. Furthermore, even though passive administration of neutralizing monoclonal antibodies partially controlled HIV during STIs, the high antibody concentrations required in this method prevented further progress in antibody-based strategies.

Secondly, the HIV tropism for CD4⁺ T cells required restimulation of specific CD4 Th cells with noninfectious, nonpathogenic HIV antigens. However, the amplified HIV-specific CD4 Th cells activated by the vaccine would be reexposed to the virus after ART cessation and may then represent a preferential target for new rounds of viral infection.

Thirdly, re-immunizing already infected patients suffering from immune alterations caused by HIV could be futile or even harmful by amplifying these alterations in the face of continuous viral replication (Autran et al. 2004). The

presence of preexisting antigen-exposed T cells – and the anergy and immune exhaustion caused by the continuous exposure to HIV antigens in untreated patients – might limit the ability of a vaccine to stimulate HIV-specific T cells and to amplify new antiviral T-cell clones (Autran et al. 2004). By reducing antigen burden, however, immune restoration clearly had the capacity to reduce, albeit incompletely, such anergy and exhaustion while decreasing the numbers of preexisting differentiated T cells (Guihot et al. 2011). Therefore, it was proposed to wait several months after administration of fully suppressive ART to allow for immune restoration before initiating therapeutic re-immunizations. Fourthly, extensive HIV genomic variability in dominant epitopes could also limit the capacity of therapeutic vaccines to restimulate broad repertoires of specific T cells.

Therefore, therapeutic re-immunization against HIV required a synergistic combination of ART and vaccines: first initiating ART to reverse immunopathological consequences of HIV replication to restore effective immune competence and then boosting the rested immune responses to HIV with a vaccine before halting treatment (Autran et al. 2004; Guihot et al. 2011).

Modest Results of Therapeutic Vaccines Against HIV

The tentative correlates of protection described in the 2000s justified the development of T-cell-based vaccines (Autran et al. 2004; Guihot et al. 2011; Carcelain et al. 2013). Live-attenuated, non-replicative, genetically engineered recombinant viral vectors, such as poxviruses and adenoviruses, were most widely used in T-cell-based vaccines due to their ability to induce endogenous synthesis of antigens in CD8 T cells (McMichael 2006). Another strategy was to avoid vector-specific immunity by using inactivated viruses, pseudoparticles, proteins, peptides, polynucleotides, or RNA-based alphavirus vaccines, though such reagents are less immunogenic for CD8 T cells. These latter antigen preparations can be repetitively

administered and synergistically combined with viral vectors to induce stronger HIV-specific immune responses. Several clinical trials tested these various vaccine candidates in acutely and chronically infected ART-treated patients with a similar design consisting of an immunization added to ART followed by an analytical STI with strict guidelines for ART reintroduction (Carcelain et al. 2013).

Therapeutic Vaccines Using Live Vectors

Two major families of vectors investigated were based on poxviruses and adenoviruses.

Poxvirus Vectors The most widely used vectors were the non-replication-competent, recombinant canarypox vector-based (vCP) vaccines and the vaccinia-derived viruses, such as MVA or NYVAC, which were known to be safe and reasonably immunogenic in HIV-negative volunteers. Because vaccination against smallpox was arrested after the disease was eliminated, populations born after 1980 lacked preexisting immunity against these poxvirus vectors. As such, utilizing these particular vectors may be advantageous. In addition, these large viruses could be designed to express multiple HIV genes, including *env*, *gag*, *reverse transcriptase* (RT), and *nef*. Several generations of vCP-based vaccines, differing in the number of HIV genes inserted, were evaluated as therapeutic vaccines either alone or in combination with other candidate vaccines and both in acutely and chronically infected ART-treated patients. After a first pilot study of the HIV *env-gag-nef-pol* vCP1452 vaccine in conjunction with the gp160 soluble glycoprotein, modest though significant induction of HIV-specific T-cell responses was demonstrated. Subsequently, a large randomized, controlled study (QUEST) was conducted in patients who had initiated a four-drug combination of ARV before completion of seroconversion. Four injections of vCP1452, alone or associated with three injections of inactivated HIV particles (Remune), were efficient at boosting CD4 and CD8 HIV-specific IFN-gamma T cells. The dual vaccine combination illustrated no additional benefit (Goh et al. 2000). Despite vaccine

immunogenicity, the virus was not controlled after halting ART. Another controlled trial conducted in patients started on treatment in acute infection combined four injections of a closely related vCP (vCP1433) with HIV-lipopeptides followed by IL-2 injections. Despite induction of HIV-specific T cells, neither virus control nor clinical benefit was observed during the subsequent ATI, thus confirming the results of the QUEST trial.

Results obtained in chronically infected ART-treated patients were more encouraging. Overall, 800 patients were included in several clinical trials. All patients had undetectable plasma viremia and restoration of approximately normal CD4 T-cell counts. An exploratory study had shown a correlation between the CD4 T-cell responses induced by four vCP1433 injections, containing the same gene inserts as vCP1452, and the duration of time patients were able to stay off treatment during an analytical treatment interruption. In parallel, a controlled trial with the same compounds (vCP1433 plus HIV-lipopeptides and IL-2) and the same design as that of acutely infected patients also boosted HIV-specific T cells. After a series of ATIs, immune responses correlated with a lower viral set point. However, a separate randomized study that administered IL-2 simultaneously with the same vCP1433 was not followed by virus control, and vCP alone induced a modest but significant reduction in plasma viremia during subsequent ATI. To further explore these encouraging findings, another large randomized, controlled trial involved chronically infected patients and utilized regimens including two doses of the vCP1452 vaccine administered with a new schedule of 3 + 1 or 2 + 1 injections followed by ATI a month after the last immunization. Despite a significant booster effect on HIV-specific T cells, the vaccine did not only have no efficacy on virus relapses during the ATI, but the plasma viremia reached significantly higher levels in the immunized arm compared to the placebo arm, independently of the CD4 nadir. Two series of factors might have been responsible for this negative result. Firstly, only HIV-specific CD4 and not CD8 T cells had been significantly boosted by this vCP1452

vaccine. Secondly, the trial design might be responsible for this failure since the ATI was proposed at a time when activation of HIV-specific CD4 T cells might have been maximal, thereby exposing them to the virus in the absence of protective CD8 T cells. However, a similar schedule had been used in the QUEST trial without such negative effects. It was concluded that the HIV-specific CD4 T cells activated by the vaccine may have provided ideal conditions for infection by the virus due to the absence of CD8 T cells, as was previously reported during iterative STIs in the absence of therapeutic vaccine.

Altogether, results of this live-attenuated HIV-recombinant canarypox vector-based candidate vaccine showed a modest immunogenicity and poor efficacy in controlling HIV and preventing disease progression during the analytical treatment interruptions that followed immunizations. Despite some encouraging, though very modest, results obtained with the HIV-recombinant canarypox vector in some treated chronically infected patients, this candidate vaccine appeared to be immunogenic mainly for CD4 T cells. Discrepancies between results obtained in acutely and chronically infected patients might have reflected the differences in preexisting immunity to HIV in those two conditions. In acutely infected ART-treated patients, stronger HIV-specific CD4 T-helper cell responses were associated with lower HIV-specific CD8 T-cell levels compared to the chronically infected individuals, but they did not confer any clinically relevant benefit. These findings are important for developing designs toward an HIV cure.

Among other poxvirus vectors, several vaccinia-derived non-replicative live-attenuated poxvirus candidates were developed, including the MVA vector obtained after serial in vitro passages and the genetically modified NYVAC vector. MVA had been widely used previously in the smallpox eradication campaign and showed, as an HIV-recombinant vector, an acceptable safety profile and an encouraging immunogenicity, although mainly activating CD4 T cells, similar to the canarypox vector. Several HIV-recombinant MVA constructs expressing *nef* alone or multiple HIV

genes, including *gag*, have been tested in preliminary phase I and II clinical trials in chronically infected ART-treated patients. The NYVAC vector, recombined with the *env* and *gag* genes and part of the *RT* and *nef* genes, demonstrated good immunogenicity for CD8 T cells in a small trial of ART-treated chronically infected patients. Therefore, the immunogenicity of the MVA and NYVAC vaccinia derivatives appeared encouraging. However, their capacity to control the virus in the absence of treatment had not been evaluated due to the emerging reluctance against ART interruption after the SMART study demonstrated that even minimal HIV replication was deleterious via its pro-inflammatory effects and was, in fact, worse than most adverse effects of ART.

Adenovirus-Based Vectors and Other Vectors This vector family raised high expectations. The HIV-recombinant, genetically modified, replication-incompetent adenovirus-5 (rAd5)-based vectors appeared to confer a strong immunogenicity for CD8 T cells in both non-human primate models and HIV-negative human volunteers (McMichael 2006). However, major limitations arose – mainly due to the preexisting immunity against adenovirus-5 in almost half of the patients – requiring an increase in the doses to overcome the specific neutralizing antibodies. One HIV-recombinant Ad5 candidate vaccine was evaluated in a phase I therapeutic vaccine trial in Ad5-seronegative chronically infected HIV-infected patients. Despite the robust T-cell immunogenicity, limited benefit was observed in terms of virus control during the subsequent ATI. More recently, two trials were conducted using a prime-boost combination with naked DNA and an even higher immunogenicity in long-term ART-treated chronically infected patients – one of which was performed in the context of HIV cure as detailed below. Because of the consistent negative results of two large clinical trials, i.e., the STEP and the HVTN-505 clinical trials, which evaluated similar Ad5 constructs as preventative vaccines, development of Ad5 candidate vaccines progressively declined. Their appropriate safety profile was, nevertheless, confirmed in ART-treated HIV-infected patients.

Therapeutic Subunit Vaccine Approaches

Recombinant soluble HIV envelope, HIV viral-like particles, inactivated HIV particles, HIV peptides, and naked HIV DNA were evaluated as possible subunit vaccines. Each element was evaluated alone as therapeutic vaccines and then rapidly tested in combination with live vectors in order to synergistically boost immune responses to HIV. In most cases, these subunit candidate vaccines were tested in a prime-boost strategy in combination with vectors. It was thought that “re-priming” with these compounds might help stimulate new T-cell clones with high clonogenic potential, thereby helping to generate durable immunity against HIV in the context of chronic HIV infection. However, such a strategy might be disputed because infected individuals may already have preexisting immunity to HIV.

First, inactivated gp120-depleted HIV particles (Remune) were tested alone, which induced minimal CD4 T-cell responses and no CD8 T-cell responses to HIV. This was associated with some decrease in the risk of virologic failure in a phase II clinical trial. When combined with the canarypox vector, however, it did not augment the vCP immunogenicity or efficacy in the QUEST study and was finally abandoned.

Second, naked DNA vaccines, known to be cheap and to have a good safety profile, were also tested alone or in combination with live vectors. The advantage of DNA vaccines is the lack of vector-specific immunogenicity; however, the weak immunogenicity of naked DNA requires high dosages. Naked DNA plasmids, generally composed of the *gag* gene alone or with *envelope* or *rev*, *tat*, and *nef*/HIV genes, were tested in pilot trials in chronically infected ART-treated patients with encouraging immunogenicity for HIV-specific CD4 T cells. Higher dosages increased immunogenicity for CD8 T cells and resulted in fewer “viral blips” during post-immunization ATIs. The combination of a multiclade *env* DNA plasmid prime followed by the HIV-*gag* recombinant adeno-5 boost induced broad HIV-specific CD4⁺ and CD8⁺ T-cell repertoires, but its efficacy was not evaluated.

Dendritic Cell Approaches

In the absence of an ideal vaccine candidate, approaches using dendritic cells (DCs) from autologous patients loaded *in vitro* with HIV antigens or mRNAs have been evaluated. The rationale was based on the superior ability of DCs in eliciting specific CD8 T cells. A first uncontrolled study of ART-naïve, chronically infected patients had suggested that autologous monocyte-derived dendritic cells (mDDCs) loaded *in vitro* with autologous chemically inactivated HIV may decrease plasma viremia reduction. Different preparations of mDDCs were pulsed either by heat-inactivated HIV particles, peptides, HIV-recombinant canarypox vectors, or autologous HIV mRNAs. Small phase I and II clinical trials generally confirmed their ability to elicit significant HIV-specific CD4 and CD8 T cells. In addition, two controlled studies evaluated high doses of autologous mDDCs pulsed *in vitro* with autologous heat-inactivated HIV in untreated or ART-treated chronically infected patients. A significant transient and partial control of viremia correlated with the boost of HIV-specific CD8 T cells, especially in ART-treated patients with up to a tenfold decrease in pVL. These encouraging proof of concept results confirm that robust and protective HIV-specific CD8 T cells can be generated by a therapeutic vaccine approach even in chronically infected patients. Therefore, further research should be conducted to find good alternatives to these expensive and complex procedures which will not be affordable by the majority of the HIV-infected individuals.

Alternative Strategies to Pulse Dendritic Cells

In Vivo Several innovative strategies aim to load DCs directly *in vivo* with HIV antigens through the skin, which is rich in DCs that are scattered in the epidermis or gathered along the hair follicles. A pilot study in HIV-negative individuals confirmed the superiority of hair-follicle DC targeting, compared with the classical intramuscular route, to induce specific CD8 T cells with an inactivated flu vaccine. Another trial showed some induction of T-cell immunogenicity when DCs were loaded via skin in conjunction with plasmid HIV DNA preparations combined with

mannosylated particles after partial epidermis abrasion in HIV-positive patients. However, clinical efficacy could not be tested in the absence of ATI. Other approaches involving the electroporation of naked DNA directly into skin DCs without skin abrasion will soon undergo clinical trials. These various attempts pave the way toward new modalities of skin or mucosal routes of immunization, such as loading DCs *in vivo* in order to enhance CD8 T-cell immunogenicity.

Overall, these therapeutic vaccine studies performed during the 2000s aimed at alleviating the lifelong burden of antiretroviral therapies. However, these studies were stopped after the SMART results (SMART 2006). In parallel, the continuous progresses in the development of more efficient and less toxic antiretroviral drugs led to a pause in the development of therapeutic vaccines against HIV.

The 2010s Paradigm: Toward a Cure for HIV or Remission

The long-term persistence of latent HIV reservoirs, despite highly effective ART, is the major obstacle toward an HIV cure. These reservoirs predominate in long-lived memory CD4 T cells, mostly in tissues, and can be assessed in the peripheral blood of patients (Guihot et al. 2011). The multifactorial mechanisms responsible for such persistence are due to (1) HIV latency in quiescent CD4⁺ T cells harboring the untranscribed provirus that is invisible to immune defenses and (2) low level residual virus production from activated CD4⁺ T cells that are not efficaciously targeted by ART. To try and purge these reservoirs, it has been proposed to combine a “shock and kill” strategy with agents that can reactivate HIV production from latently infected cells. Therapeutic vaccines can then be utilized to kill the reactivated infected cells producing HIV antigens (International AIDS Society Scientific Working Group on HIV Cure et al. 2012; Katlama et al. 2013; Trono et al. 2010; Autran et al. 2013). The rationale for a therapeutic vaccine is supported by the strong immune control of HIV reservoirs observed in elite controllers (ECs) in

whom a sustained quasi-equilibrium between the virus and its host mimics a functional cure for HIV (Autran et al. 2011). This immune control of HIV reservoirs is particularly obvious in ECs bearing the MHC class I HLA-B*57 gene in whom efficient anti-HIV CD8 T cells help limit the size and imprint the distribution of the HIV reservoirs in long-lived CD4⁺ TCM. The use of therapeutic vaccines was recently supported by in vitro findings which showed that HIV-specific CD8 T cells stimulated with HIV peptides from treated patients could eliminate in vitro autologous CD4 T cells exposed in vitro to an HDAC inhibitor disrupting latency in cells that were transcribing HIV mRNA but could not activate HIV-specific CD8⁺ CTLs. These findings provide a rationale for introducing anti-HIV therapeutic vaccines to HIV cure strategies.

A preliminary trial performed in long-term-treated HIV-infected adolescents born from infected mothers received two poxvirus vectors recombined with the *env*, *gag*, *tat*, *rev*, *nef*, and *reverse transcriptase* genes. Fowl-pox administration, followed by an MVA, illustrated significant but transient reduction in the numbers of infected cells producing replication-competent viruses. This contrasted with previous HIV-recombinant canarypox clinical trials which had failed to reduce the reservoirs. A more recent clinical trial also aimed to decrease the HIV reservoirs of residual virus-producing CD4 T cells using the VRC vaccines in combination with HIV-enveloped naked DNA plus the HIV-gag recombinant rAd5-based vector.

Will a T-cell-based therapeutic vaccine approach still be considered at a time when passive transfers of new generations of broadly neutralizing antibodies (bNABs) can already control SIV in the absence of treatment in SIV-infected macaques? Such passive transfers of bNABs represent another option that needs to be explored in future studies.

Conclusion

The rationale for developing immune-based therapeutic interventions in HIV infection has

recently been supported by the quest for an HIV cure. This new orientation should benefit from lessons drawn from past experiences in IBTs, although none of the multiple strategies and compounds tested offered any significant progress for the clinical management of HIV infection. We learned that cytokine administration or therapeutic immunization is safe, but both methods expose patients to the risk of enhanced virus production, a property of interest in the “shock and kill” strategies. We also learned that IL-2 and IL-7 enhance immune reconstitution, but the clinical benefits of IL-7 are yet to be demonstrated, and IL-2 demonstrated no significant benefit. Therapeutic immunizations have evaluated T-cell-based vaccine candidates almost exclusively, allowing marginal control of HIV; thus, efforts should incorporate broadly neutralizing monoclonal antibodies in the near future. Future cure strategies will benefit from these 15 years of experience in IBT.

Cross-References

- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [Immunological Responses to Antiretroviral Therapy](#)
- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [Overview: Immunopathogenesis](#)
- ▶ [T-Cell Homeostasis](#)

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HIV Life Cycle: Overview

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Definition

The HIV life cycle defines the steps and changes the virus undergoes from its first contact with a target cell to the production of new infectious viral particles that can initiate the next round of replication. The combination of reverse transcription of viral RNA into DNA and integration of the latter into the host cell genome is a key feature of the retroviral replication cycle.

Introduction

The goal of this chapter is to provide a brief overview on the “life” cycle of HIV, which should perhaps be better referred to as the viral “replication” cycle, since viruses do not have their own metabolism and are thus usually not considered living organisms. Viruses can be considered as intracellular parasites that are strictly dependent on living host cells for reproduction. Understanding how HIV interacts with its target cells in order to replicate is of great interest because it may provide important clues for the generation of improved antiretroviral drugs and the development of novel strategies to control or even eliminate the virus.

The main targets of HIV are CD4+ helper T cells, which are key regulators of the humoral and cellular immune responses. Thus, their destruction and depletion by mechanisms that are not fully understood render the body unable to defend itself against opportunistic pathogens. When HIV infects an activated CD4+ T cell, it hijacks and manipulates its transcriptional and translational machinery to reproduce itself. As briefly outlined below and specified in the following chapters, HIV has to utilize a multitude of

cellular factors and to counteract the antiretroviral activity of others in order to complete its replication cycle. Theoretically, each interaction with cellular factors that are essential for virus replication (termed “virus-dependency” factors) or strengthening of antiretroviral (or “host restriction”) factors provides a potential means to interfere with the HIV life cycle.

Although activated CD4+ helper T cells represent the main target cells for HIV replication, the virus can also infect other cell types, such as macrophages, immature dendritic cells, and more resting T cell subsets. The latter cell types are not important for the bulk of virus replication but most likely play important roles in innate and adaptive antiviral immune responses. Furthermore, they may harbor the virus in a silent integrated proviral form and thus contribute to the establishment and maintenance of viral reservoirs that prevent the eradication of HIV from the human body, even under optimized antiretroviral therapy (ART). Finding ways to activate these latent proviruses to eliminate the infected cells and to cure HIV infection is a main challenge in AIDS research. A better understanding of the steps in the viral life cycle, especially the regulation of proviral transcription, may help to achieve this.

HIV Structure

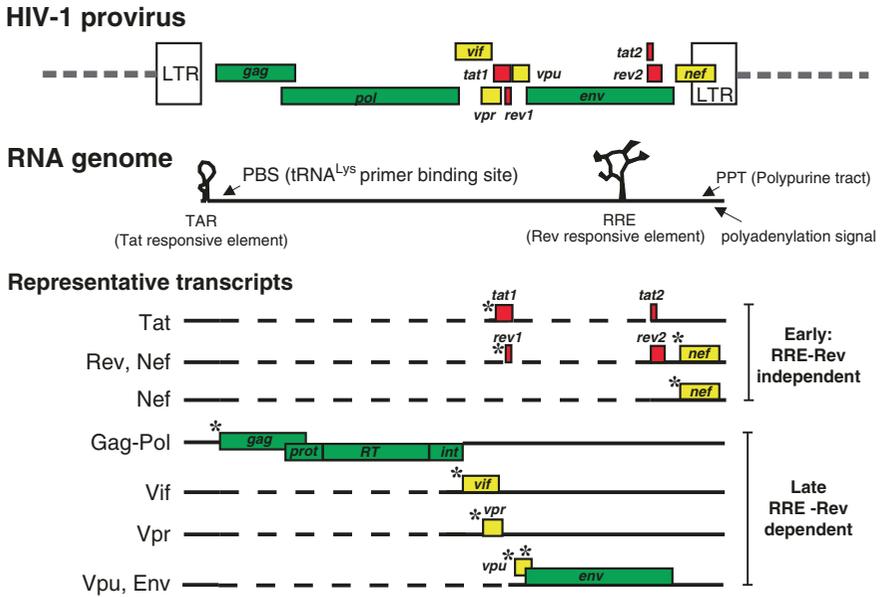
The HIV genome contains the typical retroviral genes *gag*, *pol*, and *env* flanked by long terminal repeats (LTRs), which contain the viral promoter (Fig. 1, upper). *Gag* codes for the structural proteins capsid (CA), matrix (MA), and nucleocapsid (NC); *pol* encodes the enzymes reverse transcriptase (RT), protease (PR), and integrase (IN); and *env* encodes the glycoproteins gp120 and gp41 (Swanson and Malim 2008). In addition, HIV has six regulatory genes (*tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu*) and is thus considered a “complex” retrovirus. Tat enhances proviral transcription, and Rev is essential for the export of incompletely or unspliced viral mRNAs into the cytoplasm. The remaining genes were named “accessory” because they are not absolutely required for replication in

some cell culture systems (Kirchhoff 2010). However, they perform a multitude of activities facilitating viral immune evasion and antagonize a variety of specific antiretroviral cellular (host restriction) factors and are thus essential for viral spread in vivo (Viral Auxiliary Proteins).

HIV-1 belongs to the *Lentivirus* genus (*lentis* = slow) of the *Retroviridae* family. The virion has a spherical shape and a diameter of ~100–130 nm (or 1/10,000 mm) (► [HIV-1 Virion Structure](#)). The viral envelope is composed of a lipid membrane, which is derived from the host cell and contains cellular proteins, as well as about 7–12 trimeric complexes of viral envelope (Env) protein (Fig. 2). Env consists of the external glycoprotein 120 (gp120) that mediates viral attachment and the transmembrane glycoprotein 41 (gp41) that is critical for viral fusion. Gp41 is associated with the viral p17 matrix protein and encompasses a conical capsid that consists of the viral Gag protein, p24. The capsid contains two single strands of viral RNA with positive polarity and a length of close to 10,000 nucleotides. The RNA is associated with the nucleocapsid proteins, as well as the RT and IN. The virions also contain some copies of the viral protease and the accessory Vif, Vpr, and Nef proteins, as well as some cellular factors, such as tRNA^{lys3} which is used as primer for reverse transcription.

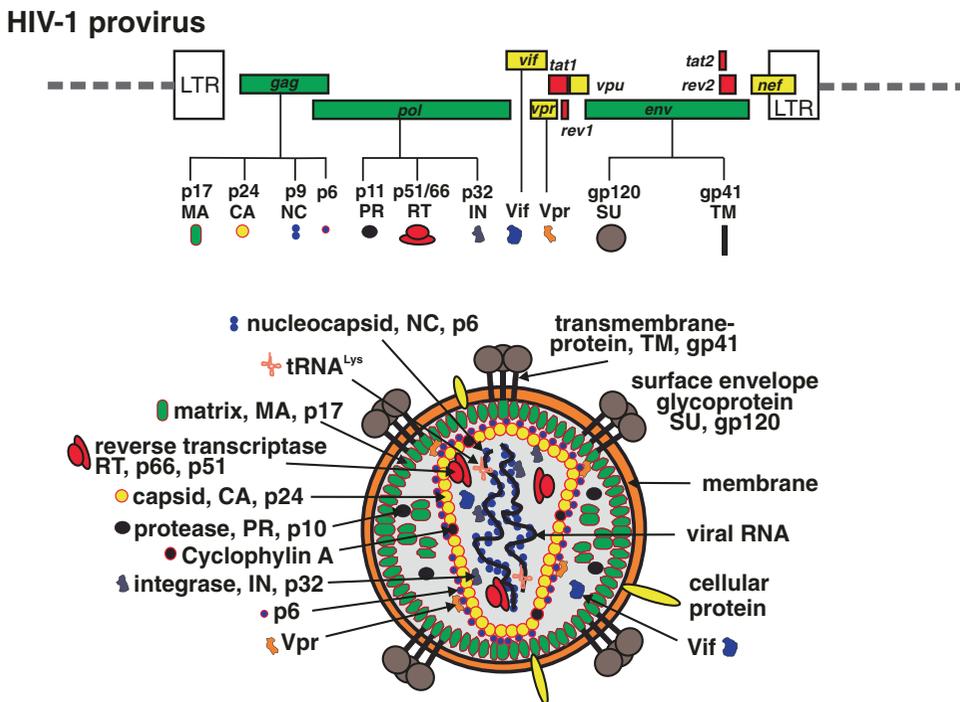
Overview on the HIV Replication Cycle

The HIV-1 life cycle is complex and can roughly be divided in an early and a late phase of replication. The early phase begins with the attachment of the virion at the cell surface and ends with the integration of the proviral DNA into the host genome (Fig. 3). The late phase of replication starts with the initiation of proviral transcription and ends with the release of fully infectious progeny virions. In highly activated CD4+ T cells, the HIV life cycle lasts just one to two days and is associated with the programmed death of both virally infected cells and uninfected bystander cells. The viral life cycle illustrates some of the challenges associated with HIV infection. The viral RT has a very high mutation rate (~1 error



HIV Life Cycle: Overview, Fig. 1 Overview of the organization and expression of the HIV-1 genome. Some *cis*-regulatory elements in the viral RNA genome and

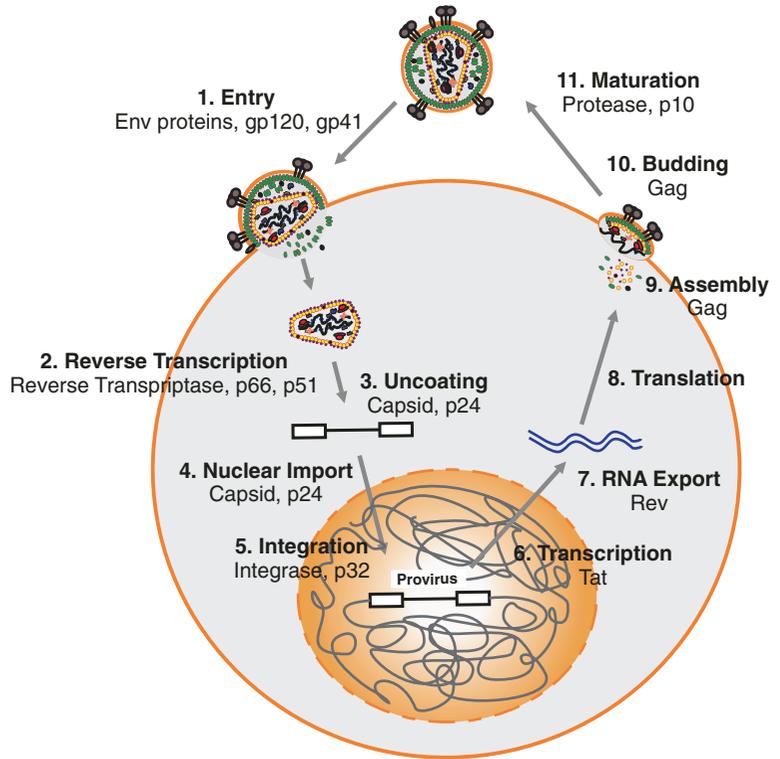
representative early and late RNAs are presented. Stars indicate splice sites



HIV Life Cycle: Overview, Fig. 2 Schematic presentation of the expression of viral proteins that are found in the viral particle (*upper*) and of the mature HIV virion (*lower*)

HIV Life Cycle: Overview,

Fig. 3 Overview of the viral replication cycle



H

per 10,000 nucleotides), and the viral populations in an infected individual are not uniform but rather a collection of the so-called quasispecies. Together with the short generation time and massive virus production (up to 2×10^9 virions per day), this allows HIV to rapidly adapt to its host environment and to develop resistance against antiretroviral drugs or immune responses. To generate a higher barrier for the evolution of resistances, combination therapies are currently used to treat HIV. Furthermore, proviral integration into the host genome allows the virus to hide in long-living cells. Finally, HIV infects and kills CD4+ helper T cells that are crucial for the maintenance of a functional immune system. It is important to consider, however, that other cell types can also be infected and that the time frame of viral replication and the fate of the infected cells vary. Macrophages, for example, may produce HIV over several weeks and store infectious virions intracellularly (► [Macrophage-Specific Aspects of HIV-1 Infection](#)). Although a lot of exciting progress has been made, we are still

just beginning to understand the multitude of interactions of HIV with its host cell.

Viral Attachment

Cell-free HIV virions usually have a half-life of just about 20–30 min in infected individuals. Thus, the virus must find and infect a new target cell within a very short time frame. As described below, CD4 is the primary receptor, and the chemokine receptors CCR5 and CXCR4 are the main co-receptors of HIV entry (► [Fusion](#)). These receptors are sufficient to render cells susceptible to HIV entry and thus determine the viral cell tropism. However, the densities of the Env trimers on the virions and of the CD4 receptor on the target cells are frequently low. Thus, viral attachment is often inefficient and a limiting step for HIV infection. Several receptors, such as polyanions, lectins, and others, can bind HIV virions in a more unspecific manner and may thereby greatly increase viral infection rates

(► [Attachment/Binding](#)). On the one hand, they concentrate virions at the cell surface and facilitate their interaction with CD4 and a co-receptor to allow virion fusion. Furthermore, they may trap viral particles at the cell surface to stabilize them and to mediate trans infection of susceptible T cells. For example, it has been suggested that dendritic cells (DCs) bind HIV virions at the site of genital exposure and transport them to the lymph nodes where they mediate both *trans* infection and stimulation of T cells that results in massive virus production (van Kooyk and Geijtenbeek 2003).

Binding and Fusion

Viral entry is a complex multi-step process that offers multiple possibilities for therapeutic intervention (Didigu and Doms 2012). Either directly or following unspecific binding of HIV to its target cell, the infection process is initiated by the interaction of the external viral glycoprotein gp120 with the cellular CD4 receptor (► [Fusion](#)). CD4 binding induces conformational changes in the Env trimer that allow the interaction of gp120 with either the CXCR4 (X4) or CCR5 (R5) co-receptor. Usually, only R5 viruses are sexually transmitted and found during chronic infection. In comparison, X4 HIV strains emerge late during the course of infection and are associated with rapid progression to AIDS in the absence of antiretroviral therapy. Co-receptor interaction induces additional conformational changes that allow the gp41 transmembrane protein, which is usually hidden by the gp120, to insert its hydrophobic fusion peptide into the cell membrane to make the first direct contact between the virus and its target cell. Thereafter, the trimeric gp41 complex forms a helical bundle structure which pulls the cellular and viral membranes together, thus allowing virion fusion and the release of the contents of the virus particle into the cell. Drugs that block the CCR5 co-receptor or prevent viral fusion are already used to treat HIV infection in the clinic, and other HIV entry inhibitors are in preclinical development.

Reverse Transcription

Once fusion is completed, the genetic information of the virus can enter into the cell. The HIV genome consists of two plus-stranded RNAs that are protected by the nucleocapsid. After fusion, the single-stranded viral RNAs are transcribed into linear double-stranded DNAs by a process called reverse transcription (► [RT](#)). It is called “reverse” transcription because it reverses the order of events that take place during the regular transcription process, i.e., generation of messenger RNA from nuclear DNA followed by export into the cytoplasm and protein synthesis. It is performed by an enzyme called reverse transcriptase that is characteristic – although not unique – to retroviruses and involves a very complex series of events that are outlined in chapter ► [Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis](#). The very first drugs against HIV inhibited reverse transcription. Currently, there are two different classes: nucleoside and nucleotide analog reverse transcriptase inhibitors (NRTIs), which prevent further elongation of the DNA chain, and non-nucleoside reverse transcriptase inhibitors (NNRTIs) that bind to the enzyme and render it inactive (*cross-link to ART section*).

Uncoating and Nuclear Entry

Uncoating refers to the disassembly of the viral capsid before import of the viral genome into the nucleus. Untimely uncoating due to point mutations in the capsid protein or interactions with the tripartite motif 5-alpha protein (TRIM5a) impairs viral infectivity (► [Uncoating and Nuclear Entry](#)). Early studies suggested that uncoating may occur immediately after viral entry. More recent data, however, suggest that capsids may remain intact for several hours and that this stability is critical for HIV-1 infection. Furthermore, it seems that uncoating is tightly associated with reverse transcription and accompanies the transition of the reverse transcription complex (RTCs) to the pre-integration complex (PIC) that is competent for integration into the host cell genome (Arhel 2010). However, the exact timing of uncoating is

still poorly understood and subject to intense research. Lentiviruses have the unique ability to infect nondividing terminally differentiated cells, such as macrophages; thus, the viral genome must be transported through an intact nuclear membrane. HIV-1 PICs must be actively transported through the nuclear pore since they are too large to cross them by passive diffusion. Originally, the viral matrix protein, Vpr, and integrase have been implicated in nuclear entry. However, recent studies suggest that none of them are essential for infection of nondividing cells and that the HIV-1 capsid protein may play a key role in this process, although the underlying mechanisms remain to be fully elucidated.

Integration

After successful generation of linear ds DNA and its transport across the nuclear membrane, HIV must insert its genome into that of the host cell for gene expression and productive infection (Fig. 3). Once the viral DNA is inserted into the cellular DNA by an enzyme called integrase (► [Integration](#)), the cell is usually infected for the remainder of its life span. As part of the host cell chromosome, the proviral DNA is replicated along with the host DNA. Thus, spread of infection can be achieved either by infection of new cells or by multiplication of cells already containing proviral DNA. Notably, several compounds that inhibit viral integration are now successfully used in the clinic (*cross-link to ART section*). In some long-living cells such as memory T cells, the integrated viral genome, which is referred to as a provirus, may remain silent for many years. This constitutes a main problem for viral eradication because as long as the provirus remains inactive, these cells are not recognized by the immune system and thus not eliminated. Sometimes the proviruses may become activated and produce infectious HIV that damages the immune system if ART is discontinued after many years. Whether or not a cell becomes latently or productively infected depends on the type and state of activation of the infected target cells during infection as well as subsequent

exogenous stimulation (Colin and Van Lint 2009). The viral reservoirs are not fully defined, but quiescent CD4⁺ T cells seem to constitute an important part of them.

Transcription

In productively infected cells, the integrated HIV provirus serves as a template for the transcription of both viral messengers and genomic RNA by the cellular Pol II polymerase (► [Transcription \(Initiation, Regulation, Elongation\)](#)). Proviral transcription is initiated by the viral promoter, which is located in the U3 region of the 5' LTR and active in many cell types. Viral gene expression is strictly dependent on cellular transcription factors, such as NF- κ B and NFAT. Initially, the transcriptional output is low because elongation of viral transcripts is very inefficient and the viral transactivator protein Tat is required for effective viral gene expression (► [Tat Expression and Function](#)). Tat binds to a specific sequence in the R region of the 5' LTR, named the trans-acting response (TAR) element, to increase transcriptional processivity (Fig. 1). This effect is dependent on the cellular factor pTEFb. Tat allows the efficient synthesis of full-length HIV transcripts, and more than 25 different mRNAs in three size classes are generated by alternative splicing: (i) unspliced RNA (9 kb) serving as genomic RNA or to produce the Gag and Gag-Pol precursors; (ii) singly spliced (4 kb) RNA encoding Vif, Vpr, Vpu, and Env; or (iii) fully spliced (2 kb) RNA expressing Tat, Rev, and Nef (Fig. 1). Transport of unspliced and partially spliced mRNAs from the nucleus to the cytoplasm is mediated by the viral Rev protein which interacts with the rev responsive element (RRE) in the viral RNA and the cellular export factor Crm1 to connect these viral RNAs to the export machinery (► [Rev Expression and Function](#)).

Translation and Assembly

As mentioned above, the fully spliced viral RNAs that are initially generated encode for Tat, Rev,

and Nef. Tat boosts viral transcription and RNA elongation, and Rev mediates the transport of unspliced and partially spliced viral RNAs to the cytoplasm. Nef performs a large number of functions and basically seems to make the infected cell less visible to the immune system by down-modulation of several surface receptors, such as CD4 and class I MHC, and manipulates the cells in a way that they become more effective producers of fully infectious viral particles. Synthesis of Tat and Rev allows the generation of full-length unspliced mRNA that expresses the Gag and Gag-Pol precursors which are then processed to major structural and enzymatic proteins. In parallel, the Vif, Vpr, Vpu, and Env proteins are synthesized from single spliced viral RNAs (Fig. 1).

Viral assembly is a complex and highly ordered process (► [Virus Assembly](#)). In brief, the Gag and Gag-Pol precursors multimerize via interactions between Gag proteins. Furthermore, both precursors are N-terminally myristoylated in the matrix domain and thus concentrated in lipid rafts at the inner leaflet of the plasma membrane (Ganser-Pornillos et al. 2008). The viral Env glycoproteins are recruited to these building platforms through interactions with the matrix protein. Finally, two copies of genomic viral RNA are recruited to this complex through interactions of their stem loops packaging signal with the zinc fingers present in the NC domain of Gag (Fig. 3). Furthermore, the viral Vif protein, which antagonizes the restriction factor APOBEC3G, as well as some cellular factors, are also recruited to the sites of virion assembly and incorporated into viral particles. The accumulation of viral proteins and RNA at the plasma membrane induces first its curvature and subsequently the formation of a membrane-coated spherical particle.

Budding

The release of progeny virions from the infected cells is called budding. The late domain of the p6 part of Gag and the cellular Tsg101 protein are involved in this step which allows newly formed HIV to pinch off and enter into the circulation (► [Budding](#)). Notably, also this late step of the

viral replication cycle is targeted by a restriction factor: tetherin (BST-2) tethers mature and infectious viruses to the cell surface and is counteracted by the HIV-1 Vpu and the Nef or Env proteins of other primate lentiviruses (Martin-Serrano and Neil 2011).

Maturation

The HIV particles are released in an immature and noninfectious form that is morphologically characterized by a thick layer of radially arranged Gag and Gag-Pol precursors (► [Maturation](#)). During or shortly after budding, the viral protease becomes activated and cleaves the Gag and Gag-Pol precursors into their mature final components. As a consequence, the configuration of the proteins is reorganized to generate the characteristic electron-dense conical inner core and to render the virus infectious (Briggs and Kräusslich 2011). Drugs that block this last essential step of the viral life cycle by inhibiting the viral protease are a main component of effective ART.

Viral Dependency and Host Restriction Factors

It has long been known that viruses are strictly dependent on live host cells in order to replicate. Only recently, however, it has become clear that these interactions may be far more complex than previously appreciated. Several studies have used genome-wide knockout strategies in order to better assess the cellular factors that may be critical for effective replication of HIV. All of them found that HIV may utilize hundreds of cellular proteins in order to complete its life cycle. Similarly, a recent study performed elegant broad-based screens to clarify how many cellular proteins interact with viral proteins and identified about 500 of them (Jäger et al. 2011). Altogether, these studies provide an exciting first glimpse at the enormous complexity of interactions between HIV and the host cell. However, there are some caveats. For example, the overlap between the potential viral dependency factors in the

genome-wide knockout studies was minimal (Bushman et al. 2009) most likely because of variations in the cell types, viral strains, and experimental conditions used. Furthermore, it is difficult to assess how many other cellular proteins may be affected by each individual knock-down. Similarly, most interaction studies cannot distinguish between direct interactions between viral and host cell proteins or interactions with larger protein complexes. Nonetheless, these studies have provided exciting first insights into the enormous complexity of interactions between the virus and the host cell and may help to identify additional key factors involved in HIV replication.

Although HIV hijacks the host cell and takes advantage of many cellular proteins and pathways, it has become clear that the cell is not a very friendly environment for the virus, because it encodes specific antiviral factors that may inhibit HIV at various steps of its life cycle and are counteracted by the viral accessory proteins (► [Cellular Restriction Factors](#)). In brief, the best characterized antiviral or host restriction factors are APOBEC3G which induces lethal hypermutation of the retroviral genome and affects reverse transcription, TRIM5 α protein that induces untimely uncoating of incoming retroviral capsids, and tetherin that inhibits virion release. Unfortunately, HIV-1 evolved effective strategies to evade or counteract these antiviral host factors (Kirchhoff 2010).

Target Cell Dependency

The main target cells for viral replication *in vivo* are activated CD4⁺ T cells, and the viral life cycle illustrated in Fig. 3 provides a rough overview of the events in this cell type. Similarly, almost all studies on the HIV replication cycle have been performed using immortalized cell lines or fully activated T cells. *In vivo*, however, many T cells are minimally activated. This may not be very important for the bulk of virus replication but most likely highly relevant for the establishment of latent viral reservoirs and for the pathogenesis of AIDS. For example, some long-living memory T cells may return to a quiescent stage upon HIV

infection and not show any gene expression for several years before they finally become activated and start to produce infectious HIV particles. Cells that carry the provirus but do not produce viral proteins cannot be recognized and eliminated by the immune system and constitute a major obstacle to virus elimination. Furthermore, it is important to consider that HIV does not only infect T cells and that the viral replication cycle may be somewhat different in other cell types. Macrophages, for example, are also productively infected by HIV and may contribute to the viral reservoirs and facilitate viral evasion of the blood-brain barrier (► [Macrophage-Specific Aspects of HIV-1 Infection](#)). Furthermore, HIV may also infect immature dendritic cells, microglial cells, and (possibly) stem cells although the relevance of these and other potential target cells of HIV for viral spread and pathogenesis *in vivo* is currently largely unclear.

Conclusions and Perspectives

Although enormous progress has been made in understanding the complex events and interactions that are critical for the life cycle of HIV, a lot remains to be learned. It will be essential to further clarify which host dependency factors are really critical for viral replication in the human host. Currently, only a single cellular factor (CCR5) is targeted for antiviral treatment. Preventing the use of other host cell proteins that are obligatory for viral replication may lead to the development of novel antiretroviral strategies that make it more difficult for HIV to develop resistance. Furthermore, it seems likely that additional restriction factors will be discovered and strengthening them or weakening the viral antagonists may also allow to impair the viral replication cycle at different steps. It will also be important to further clarify why some T cells get stuck after the early stage of infection and become latently infected and how to stimulate them in order to eliminate the viral reservoirs. Furthermore, recent studies suggest that the protection of specific T cell subsets may play a major role in the lack of disease progression in some monkey

species that are naturally infected with simian immunodeficiency viruses. Since the fate and type of the viral target cell determine the damage for the individual, it seems important to further study target cell-dependent differences in the viral replication cycle. Finally, it is important to consider that in vivo HIV is mainly replicating in lymphoid organs that are densely packed with different cell types and may spread through direct cell-cell contact.

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HIV Neurocognitive Diagnosis, Natural History, and Treatment

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HAND Definition

When carefully assessed, human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) affect approximately half of persons living with HIV (Heaton et al. 2010). Three subcategories of HAND can be distinguished using a combination of neuropsychological testing, neurological and medical examination, and ancillary testing (Antinori et al. 2007): asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). Regardless of the severity of the HAND diagnosis, neuropsychological impairment is often associated with negative daily functioning outcomes such as unemployment and poor medication adherence (e.g., Hinkin et al. 2002). It is important to note, however, that impairment in daily functioning is a key criterion that distinguishes MND and HAD from ANI. One significant challenge in assigning HAND diagnoses is differentiating HAND from other illnesses that can have neurocognitive effects (e.g., substance use, head injury, other chronic medical illnesses). Additional research is needed to clarify the role of risk factors in the development of HAND and to improve treatment of HIV brain disease without causing neurotoxicity. HAND prevention relies on early identification of those at risk to enable mitigation.

It is not yet clear whether the subcategories of HAND (ANI, MND, HAD) represent degrees of cognitive dysfunction on a spectrum or distinct disorders with different mechanisms and treatments. Each of the subtypes of HAND can exist in an active state or enter remission. Thus, unlike some other neurodegenerative disorders such as Alzheimer's disease, HAND-related impairment is not relentlessly progressive. Identification and classification is achieved through neuropsychological (neurocognitive) testing in at least five cognitive domains (attention-information processing, abstraction-execution, complex perceptual motor skills, memory with learning and recall testing, language, simple motor skills or sensory perceptual skills). To allow meaningful comparisons across different tests, results are presented on normalized scale such as a Z score or t score, adjusted for age, education, and other demographic factors as appropriate. Also critical to the current diagnostic schema is an evaluation of daily functioning capabilities, which can be assessed via self-report or other approaches such as performance-based measures of daily functioning.

Research Versus Clinical Diagnostic Approaches

Researchers and clinicians differ in their approach to the diagnosis of neurocognitive disorders in HIV. This fact has caused some confusion. In the research approach, all individuals are assessed with a detailed neuropsychological battery, and then each is classified based on the severity of neuropsychological and daily functioning impairment. By contrast, the clinical approach begins with patient symptoms. Patients (or in some cases family members) reporting difficulties with memory, attention, or concentration are screened for cognitive impairment and depression. In the absence of symptoms, no screening is done. Screening results are then used to decide on subsequent referral for more comprehensive evaluations. Individuals with ANI will of course be missed by such an approach, since by definition they have few if any cognitive symptoms. Additionally, many screening batteries are insufficiently sensitive to detect the predominantly mild neurocognitive impairments that are present

among HIV-infected persons. Thus, there remains a need for instrument that is both sensitive and specific, yet brief, to detect HAND.

ANI

Patients with ANI must be at minimum one standard deviation below the mean in at least two of the aforementioned cognitive domains, and the impairment cannot be explained by non-HIV-related comorbidities such as head trauma with permanent neurological sequelae. An important characteristic of ANI is that there is no evidence that the neurocognitive impairment disrupts the patient's daily functioning.

MND

Mild neurocognitive disorder meets the same neurocognitive testing criteria for ANI but in addition causes mild impairment in performance of activities of daily living. There must be either self-report of reduced mental acuity, difficulty with social functioning, and inefficiency in work or homemaking or first-hand report from a knowledgeable source that the individual has exhibited at least a mild decline in mental acuity that causes difficulty in managing work, homemaking, or social functioning. As with ANI, MND is not due to other conditions.

HAD

The most severe form of HAND is HIV-associated dementia (HAD). Individuals with HAD display marked acquired impairment in cognitive functioning in multiple domains and are at least two standard deviations below the mean. Verbal and nonverbal learning, information processing, and attention are particularly affected. The neurocognitive dysfunction causes significant impairment in daily functioning. HAD is not due to delirium, and there is no alternative diagnosis that would better explain the disorder, such as CNS infection, neoplasm, substance abuse, stroke or cerebrovascular disease, or other neurological disease.

Comprehensive Neuropsychological Testing

Comprehensive neuropsychological testing is the "gold standard" to characterize HAND and is

necessary to differentiate cognitive impairment due to HIV from other dementias and pseudo-dementias. Using valid and reliable assessments chosen and administered by a trained psychologist, a comprehensive neuropsychological testing evaluates at least following seven domains: attention/working memory, verbal fluency, learning and recall, nonverbal memory, abstraction/executive function, motor coordination and praxis, and information processing speed. The raw performance data must be adjusted for demographic and socioeconomic norms before being used for diagnosis. The whole process takes a few hours, thus is not practical for every HIV patient, and is usually reserved for patients who have cognitive deficits that impair their activities of daily living, have progression of HAND, and have an unclear diagnosis or patients with significant risk for HAND due to comorbid conditions.

Epidemiology

The epidemiological findings discussed here apply to individuals with HIV clade B virus, prevalent in North America, Europe, and Australia. Despite early entry of the virus into the central nervous system (CNS), HAND is relatively rare during acute and early infection (the first year after infection), becoming somewhat more common as immunity declines. Thus, incidence increases as the disease progresses. The prevalence of HAND and the demographics of those with HIV/AIDS have undergone a transformation since the introduction of combination antiretroviral therapy (cART). Prior to cART, 15–30% of all HIV-infected individuals developed HAD prior to death. Typically these individuals had advanced immunosuppression (CD4+ T-lymphocyte counts under 200/uL), and their mortality was high. Since cART became widely available in the United States, the incidence of HAD has declined markedly, but the prevalence of HAND, including the less severe forms, remains high, with 52% of patients demonstrating neurocognitive impairment (Heaton et al. 2010). Of those with HAND, most (approximately 60–70%) have ANI, a smaller fraction have MND (30%), and HAD is rare.

Potential explanations for these changes in prevalence and severity of HAND are several. Mortality has declined with cART, but those living longer with HAND do not necessarily return to premorbid cognition. Some cases previously classified as HAD experienced improvement, warranting reclassification to milder forms of HAND. Aging, an inevitable consequence of increased survival due to cART, is itself a risk factor for HAND. This observation will be explored in greater depth below. HIV may continue to cause CNS damage regardless of viral suppression, possibly due to sustained inflammation and immune activation. Finally, antiretrovirals themselves may cause CNS toxicities that contribute to HAND.

The demographics of patients diagnosed with HAND in the United States are reflective of the shifts in the HIV/AIDS epidemic. That is, the current population living with HIV/AIDS has a higher proportion of females and ethnic minority groups while having a lower level of education. Those with HAND in the cART era are more likely to report heterosexual-only contact and injection drug use. In large pre-cART studies, 65% of patients with cognitive impairment were Caucasian, 87% were male, 52% were employed, 70% were men who have sex with men only, and the median duration of HIV infection was 3.4 years. In the cART era, similar large studies have found that of those with neurocognitive impairment, 44% are Caucasian, 81% are male, 29% report being employed, 37% are men who have sex with men, and the median duration of infection is 9.3 years. This increase in duration of infection and the comorbidity linked to intravenous substance use may contribute to the pathophysiology of the disease. Moreover, as persons live longer with HIV infection, it may get increasingly difficult to distinguish HAND from typical age-related cognitive changes or dementias that can develop later in life.

HAND Risk Factors

Risk factors for HAND can be conceptualized in four broad categories: HIV disease severity indicators, nonmodifiable demographic factors, potentially modifiable comorbidities, and other

comorbidities. Whereas in the pre-cART era markers of HIV disease severity such as lower current CD4 counts and higher plasma viral loads conferred an increased risk of dementia and neurocognitive impairment, cART changed this. In the cART era, only nadir CD4 has retained an association with HAND risk. Thus, lower CD4 nadirs are linked to a “legacy” effect of increased HAND likelihood, despite subsequent immune recovery on cART (Ellis et al. 2011). Non-modifiable demographic risk factors include age (older individuals at greater risk than younger) and family history of dementia (patients with positive family history of dementia being more likely to develop HAND). In some studies, women are at greater risk of HAND than men. Additionally, low education, a surrogate for low cognitive reserve, confers greater risk. Among the potentially modifiable risks, recent evidence strongly points toward metabolic syndrome and vascular risk factors. The metabolic syndrome is quite common in HIV (Krishnan et al. 2012), and its underlying pathogenesis is likely to involve visceral adiposity with persistent inflammation (McCutchan et al. 2012). Metabolic syndrome components such as insulin resistance, lipid abnormalities, and hypertension, in conjunction with inflammation and immune activation, may contribute to CNS endothelial dysfunction, leading to neurodegeneration and cognitive impairment. Cerebral small vessel disease is common in patients dying with HIV infection in the cART era (Soontornniyomkij et al. 2014). Whether aggressive management of metabolic syndrome and vascular risk factors reduces HAND risk remains to be tested.

Screening for HAND

Effective screening to identify individuals with neurocognitive impairment is a critical step toward protecting and preserving brain function in individuals with HIV disease. For patients with acute infection, current guidelines recommend neurocognitive screening within 6 months of diagnosis of HIV infection, as HIV invades the CNS early in the disease course and a slow

progression to HAND may commence at this point. For long-term patients, the frequency of screening has not been well defined, and there is still much debate about whether asymptomatic patients should be screened. The current guidelines are that higher-risk patients receive screening more frequently (every 6–12 months), while lower-risk patients can be screened every 12–24 months. In addition, screening should be conducted at the time of antiretroviral changes, when comorbid conditions affecting HAND are identified and if the patient deteriorates. It is also important to differentiate HAND from other neurodegenerative processes such as AD, particularly as HIV-infected individuals age as a result of the HIV epidemic maturation in developed countries.

Since HAND affects a number of different cognitive domains (see above) at different points during the disease course, a useful screening tool should therefore evaluate all potentially affected domains. While there are a number of rapid cognitive assessments available for the clinical setting, each of these has its own limitations, and unfortunately there is no one clinical screening tool that has been found to be appropriately sensitive or specific for HAND yet. The HIV Dementia Scale (HDS) and its variation, the International HIV Dementia Scale, were specifically designed to examine neurocognitive dysfunction due to HIV infection and have been widely used to screen HAND. However, while HDS identifies HAD well, its application in the cART era has been limited due to its insensitivity to the more prevalent milder forms of HAND (Valcour et al. 2011). The Folstein Mini-Mental State Exam (MMSE), a general cognitive function tool that is designed to screen for acute confusional state (delirium) as well as cortical dementias (e.g., AD), might be ineffective to screen HAND as many of its test items are preserved in HAND (Valcour et al. 2011). Montreal Cognitive Assessment (MoCA), a more recently developed tool to assess general cognitive function, might be more useful to screen HAND than MMSE. MoCA examines executive functions, visuospatial abilities, working memory, language, naming, attention, and abstraction, but not psychomotor slowing, a prominent feature of HAND. The

scoring system is similar to that of the MMSE, with which many clinicians are familiar, with a score greater than or equal to 26 treated as normal. Overton and colleagues demonstrated a sensitivity of 59% and specificity of 81% for identifying cognitive impairment in HIV with MoCA using 26 as the cutoff score (Overton et al. 2011). In addition to these three screening tools, there are numerous other screening batteries available, including the Computer Assessment of Mild Cognitive Impairment, Medical Outcomes Study HIV Health Survey, Prospective and Retrospective Memory Questionnaire, and the Rey-Osterrieth Complex Figure Copy and Memory Tests. However, these batteries have been deemed insensitive for diagnosing HAND.

Clinical Features and Differential Diagnosis

HAND affects cognition, behavior, and the motor system. Motor symptoms are typically mild and include slowed repetitive movements and non-specific gait abnormalities. Cognitive deficits may decrease adherence to antiretroviral therapy, and patients may also experience difficulty with employment (Woods et al. 2011).

HAND differs from Alzheimer's disease (AD) in that AD is a cortical, predominantly amnesic syndrome with impaired episodic memory, naming, and visuospatial functions being present early in the disease course. By contrast, HAND is an apathetic-executive syndrome affecting subcortical white matter early in the disease course. HAND should not be confused with frontal-temporal dementia (FTD), in which behavioral disturbances such as disinhibition are common. Patients with cognitive impairment, visual hallucinations, and akinetic-rigid syndrome should be evaluated for dementia with Lewy bodies or idiopathic Parkinson disease depending on the time course and the neurological findings. Vascular cognitive impairment is an important consideration as HIV increases the risk of ischemic cerebrovascular disease. History and radiologic evidence of cerebral infarction assist with diagnosis.

Medical Evaluation

Aside from neurocognitive testing, HIV-infected patients with suspected or confirmed cognitive impairment require additional medical evaluation. The most important part of this evaluation is comprehensive history taking, which includes symptom evolution which may point to another disorder, the presence of confounding comorbid conditions such as depressive mood disorder, head trauma or substance abuse, nadir and current CD4 T-lymphocyte counts, and antiretroviral regimen. In addition, a thorough physical examination including a complete neurological examination should be done to exclude alternate diagnoses. Laboratory evaluation assists the clinician in excluding reversible causes of cognitive impairment including subacute combined degeneration from hypovitaminosis B12, hypothyroidism, and neurosyphilis. Routine laboratory evaluation for reversible causes of dementia includes: complete blood count, metabolic panel, syphilis serology (either rapid plasma reagin or Venereal Disease Research Laboratory test), vitamin B12 and red blood cell folate levels, and thyroid function tests. In addition, CD4 T-lymphocyte count and plasma HIV RNA should be obtained.

Neuroimaging is required to exclude CNS opportunistic disease or other pathologies in cases with focal neurological deficits or low CD4+ T-cell counts. Cranial magnetic resonance imaging (MRI) is preferred over computed tomography (CT), unless the patient is not a candidate for MRI. In this case, contrasted CT should be performed. Intravenous contrast administration is particularly useful in individuals whose CD4 count is less than 200 cells/mm³ to evaluate for CNS malignancies and opportunistic infections. The MRI is an invaluable tool in evaluating for structural lesions from cerebrovascular disease, opportunistic infections, neoplasm, or inflammatory conditions that can affect cognition. Though, unfortunately, pathognomonic MRI findings for HAND have not been defined, abnormal white matter T2 signal prolongation and global or basal ganglia atrophy have been observed in HAND. Magnetic resonance spectroscopy, diffusion tensor imaging, functional MRI, PET, and

SPECT have not demonstrated clinical utility at this time and are therefore reserved for research purposes.

Evaluation of cerebrospinal fluid (CSF) is useful in seeking alternative potentially reversible diagnoses such as infection and neoplasm. CSF cell count, glucose, and cultures are helpful for excluding infection in individuals with CD4 T-lymphocyte counts less than 200 cells/mm³. CSF cytology can be performed as clinically indicated. CSF protein is often elevated in patients with HAND, but this is a nonspecific finding. CSF HIV RNA levels may be useful in patients who have not been treated with cART or who have failed therapy. However, the association between HAND and CSF HIV RNA in patients on cART is less clear. Individuals with detectable CSF HIV RNA in the presence of undetectable plasma HIV RNA may have compartmental drug resistance leading to CSF viral escape. Alternatively this may relate to poor CNS penetration of antiretroviral medications. The clinician should be mindful that CSF HIV RNA does not necessarily reflect parenchymal viral burden.

Biomarkers

The HAND diagnostic process is likely to continue to evolve as additional details are uncovered regarding the pathophysiology of HAND. For instance, recent guidelines in the context of mild cognitive impairment and Alzheimer's disease use a combined approach of neuropsychological testing, biomarkers, and imaging in assigning diagnoses (e.g., McKhann et al. 2011). While neuropsychological impairment and its associated impact on daily functioning are likely to remain the core diagnostic components of HAND, there may be added incremental value to both peripheral (e.g., blood and CSF) biomarkers and imaging for both diagnostic and prognostic purposes.

Plasma and CSF Biomarkers

Given the easy access and low cost of blood sampling, there has been considerable interest in finding blood-based biomarkers that correlate with neurocognitive impairments in HIV.

However, isolating blood biomarkers sensitive to neurocognitive impairments in the cART era has proven to be difficult and is limited by low accuracy. For instance, CD4 cell counts and HIV viral load in peripheral blood do not correlate well with neurocognitive function in patients on cART. In contrast, recent studies have suggested that plasma-soluble biomarkers like CD14 (Lyons et al. 2011) and CD163 (Burdo et al. 2013) might be better predictors of neurocognitive impairment. These plasma-soluble biomarkers can help to monitor monocyte activation, which is related to chronic inflammation.

Compared to plasma biomarkers, CSF-based biomarkers might represent neural injury more closely due to their direct contact with the central nervous system. However, obtaining CSF is generally considered to be more invasive. In some studies CSF, tau levels are elevated in individuals with symptomatic neurocognitive impairment. However, such findings need verification.

Hcorr as a Possible In Vivo Noninvasive MRI Biomarker of Neural Injury in HIV

Advances in neuroimaging techniques like MRI have provided a noninvasive way to examine HIV-related pathological changes. For instance, structural MRI has been used to examine brain lesions and/or volume reductions that can be linked to cognitive impairments in individuals with HIV disease (Ances et al. 2012; Chang et al. 2011; Towgood et al. 2011). However, brain atrophy usually happens at rather late stages of disease with significant and usually irreversible brain damage. Given that HIV infection is hypothesized to lead to changes in neuronal function long before changes in anatomy can be detected (possibly due to early synaptodendritic damages (Ellis et al. 2007)), functional MRI (fMRI), with its ability to "directly" image brain function, has the potential to serve as a biomarker of HIV to identify neural injury at early stages of disease, including those with asymptomatic HAND. However, the effectiveness of recent efforts to use fMRI to study HIV has been limited by the absence of a clear model of how HIV affects the neuronal processing that give rise to behavioral

deficits, and how these changes in neuronal processing could be detected with fMRI at high sensitivity. For instance, recent fMRI studies of HIV have reported “conflicting” findings: both decreased and increased neural activity have been reported in HIV+ patients. These findings might reflect the technical limitations of conventional fMRI techniques: due to the limited spatial resolution, relating fMRI response amplitude to underlying neuronal function is rather ambiguous and difficult (Grill-Spector et al. 2006).

In contrast, a novel fMRI data analysis technique, local regional heterogeneity analysis, or Hcorr, which estimates neuronal sparseness as an indirect measure of neuronal function, has proven to be able to measure neuronal function more directly than conventional techniques (Jiang et al. 2013). This technique was motivated by findings from electrophysiological studies, which suggest the sparseness of neuronal activation pattern correlates with neuronal function and synaptic integrity. Using this technique, a recent study (Liu et al. 2015) provided evidence supporting the existence of neuronal dysfunction in asymptomatic middle-aged HIV+ women, who were on cART with suppressed viral load and normal CD4 cell counts. In contrast, both conventional fMRI techniques and behavioral test failed to detect these early neuronal dysfunctions. While future studies with different and large cohorts are necessary to validate and further improve this novel technique, Hcorr holds a strong promise to serve as a noninvasive biomarker of HAND; compared to more conventional structural and functional MRI techniques, Hcorr offers several distinct advantages: Hcorr is more sensitive to early and subtle neuronal dysfunction. While conventional fMRI techniques are limited to examine neuronal function at the brain regions related to studied cognitive function(s), Hcorr can be calculated for any functionally or anatomically defined brain region using a single data set, making it an ideal tool to examine widespread pathological changes in diseases like HAND. Hcorr can be calculated from resting state data, which avoids the performance-related confounds with the heterogeneous population. Compared to most fMRI techniques that require length scanning time,

Hcorr can reliably assess neuronal function with a few minutes of scan (which is compatible to the time required for a typical structural MRI scan), making it a more feasible tool in typical clinical settings. In addition, we have obtained preliminary data suggesting that Hcorr is significantly more sensitive to detect and evaluate therapeutic effects than traditionally used behavioral assays, suggesting it might be capable of serving as a surrogate measure to assess clinical outcome or to assist developing drugs that target specific brain regions.

Natural History and Treatment

HIV CNS Entry During Acute Infection

HIV enters the nervous system within days after initial infection. Although most cases of acute HIV infection go undiagnosed due to the unspecific nature of symptoms (a flu-like illness, in many cases), rarely acute HIV infection can present with a fulminant encephalopathy. In one case of iatrogenic HIV infection in which the patient died 15 days postinoculation, autopsy already revealed proviral HIV DNA and HIV proteins within the cerebral cortex, even though virus could not be detected in several other organs (Davis et al. 1992). An absence of specific neutralizing immune responses in the hyperacute stage might permit such early and extensive CNS invasion.

Neurocognitive impairment is relatively uncommon during the first year after infection, but brain changes nevertheless may be detected by imaging. One study (Moore et al. 2011) evaluated neurocognitive functioning using a comprehensive battery in individuals with early infection (less than 1 year) using Fiebig criteria and directly compared their performance to that of individuals with chronic HIV infection (median duration of infection 4.9 years) and to a control group of HIV seronegatives (HIV-). The HIV- group showed the best neurocognitive functioning, and although statistically indistinguishable from the acute/early HIV group, the latter showed performance intermediate between that of the HIV- and the chronically HIV-infected subjects.

While clinically evident neurocognitive disorders are uncommon early in infection, brain changes can nevertheless be seen using imaging techniques. For example, proton magnetic resonance imaging spectroscopy (1H-MRS) shows abnormal levels of several cerebral metabolites associated with brain inflammation and neuronal injury – glutamate (Glu), *N*-acetylaspartate (NAA), myoinositol (MI), and choline-containing metabolites (Cho) (Young et al. 2014). Early initiation of combination antiretroviral therapy reduced worsening of these inflammatory cerebral markers, but did not restore them to normal levels as seen in HIV-negative controls.

Impact of cART

Before the introduction of cART in 1996, HIV-associated dementia (HAD) occurred with some regularity and was only temporarily ameliorated by therapy with zidovudine and other antiretroviral medications available at that time. HAD was much more likely to occur in patients with advanced immunosuppression (CD4+ T-lymphocyte counts less than 100 cells/mm³). Untreated, HAD was rapidly progressive, with death occurring often within 6 months of diagnosis. With cART, HAD has become strikingly less common. Nevertheless, ANI and MND frequently persist.

In addition to its benefit on HAD, cART has also lengthened survival, permitting many HIV+ individuals to survive into middle and old age. Indeed, recent UNAIDS data show that the over-age-50 HIV+ subset is growing particularly rapidly in Western Europe and the United States, reaching over 800,000 in 2014 (Mahy et al. 2014). Other parts of the world are not far behind. This is important because older HIV+ individuals disproportionately bear the burden of brain injury and cognitive impairment. This may be partly explained by the high prevalence of abdominal obesity and metabolic syndrome in cART-treated HIV+ individuals, as previously noted.

ANI Progression

While asymptomatic neurocognitive impairment (ANI) – the most common form of HAND nowadays – is mild relative to HAD, it is not a

benign condition. Although not meeting formal criteria for MND, those with ANI on average have more difficulties in activities of daily living, are less likely to be employed, and show deficits on tasks simulating self-management of medications. Furthermore, their risk of symptomatic progression – that is, to MND – is quite high. In one recently published report, over a median follow-up of approximately 4 years, 50% of 121 individuals with ANI at study entry progressed to symptomatic HAND (MND or HAD) during follow-up (Grant et al. 2014). The relative risk for progression of ANI as compared to neuropsychologically normal was 5.8. This relationship held true even for individuals who were virally suppressed on ART at study entry and remained significant after statistically adjusting for important covariates.

Management of HAND

After confirming that HAND is present using neurocognitive testing and the diagnostic scheme described previously, patients should be screened for reversible causes of cognitive impairment such as thyroid deficiency and neurosyphilis. Although major depressive disorder alone rarely explains neurocognitive impairment, when present, depression is disabling in its own right and should be managed with antidepressants and psychotherapy. Additional details of laboratory evaluations and other may be found in a published consensus document (Mind-Exchange-Working-Group 2013).

Antiretroviral Treatment for HAND

All individuals with HAND should be on virally suppressive cART, regardless of CD4 count. In the United States and other developed-world settings, cART comprises combinations of three or more drugs from a least two classes: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), early entry/fusion inhibitors, and integrase inhibitors. Individuals with successful virologic suppression on cART have better preserved synaptodendritic complexity and are less likely to be neurocognitively impaired than non-suppressed individuals on cART or those

who have not received cART. The extent and time course of neurocognitive improvement for patients with HAND initiating cART varies widely, but improvement can be detected in 12–24 weeks in 15–30% of individuals and plateaus at 40% after 36 weeks (Cysique et al. 2009). Individuals with HAD and those who are naïve to antiretroviral therapy show the largest benefits (Tozzi et al. 1999; Letendre et al. 2004).

Because antiretrovirals penetrate differentially across the blood-brain barrier into CNS tissues, there has been great interest in the potential to select drugs so as to maximize their effectiveness in treating brain infection. Letendre et al. devised a scheme for ranking individual drugs according to their ability to penetrate the blood-brain barrier and reduce HIV RNA levels in cerebrospinal fluid (Letendre et al. 2009). This scheme has been updated as new medications have been introduced (Letendre et al. 2010). A recently published clinical trial evaluated the potential effectiveness of a CNS-targeted drug selection scheme for HAND (Ellis et al. 2014). The trial failed to fully accrue because of unexpectedly low referrals. It found no evidence of neurocognitive benefit for the CNS-targeted drug selection strategy as compared to a non-CNS-targeted, standard-of-care drug selection approach. Because of incomplete accrual, a benefit for a subgroup or small overall benefits could not be excluded.

Numerous alternative treatment strategies have been tested for HAND. These include memantine, transdermal selegiline, and nimodipine. Unfortunately, as has been the case for neuroprotective strategies in a number of other disorders, benefits have not met prespecified criteria for statistical significance, and these drugs are clinically indicated. Central cholinesterase inhibitors such as donepezil have not been rigorously evaluated in HAND.

Conclusion

HIV-associated neurocognitive disorders (HAND) are defined based on a combination of neuropsychological testing, neurological and medical examination, and ancillary testing into

three subcategories: asymptomatic neurocognitive impairment (ANI) will be missed in typical clinical practice, where individuals are evaluated only if they report cognitive difficulties, since by definition, symptoms in ANI are minimal or absent. Comprehensive neuropsychological testing is the “gold standard” to characterize HAND and is necessary to differentiate cognitive impairment due to HIV from other dementias and pseudodementias. HIV-infected individuals with suspected or confirmed cognitive impairment require additional medical evaluation, since some may have contributing causes other than HIV itself. The risk of symptomatic progression in ANI – that is, worsening to MND – is quite high: 50% over 4 years in one study. Although all individuals with HAND should be on virally suppressive combination antiretroviral therapy (cART), regardless of CD4 count, symptomatic progression may occur even in those with who are virally suppressed on cART.

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HIV Prevention and African Americans

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Definition

African Americans are the largest racial minority in the United States. They comprise approximately 13% of the total US population and are disproportionately affected by HIV/AIDS.

Introduction

HIV affects African Americans at a disproportionately higher rate than other races. Compared to their White counterparts, rates of new HIV infections are over six and a half times higher for African

American men and over fifteen times higher for African American women (CDC 2012). Just under half of the population living with HIV in the United States (US) is African American. While HIV risk for African American men remains highest among MSM, African American women are over twice as likely to be infected with HIV through heterosexual activity. Possibly most alarming is the difference in mortality rates for individuals with HIV. The mortality rate for African Americans with HIV is almost 9 times higher than the mortality rate for White individuals with HIV (CDC 2010). Taken together, these data demonstrate the significant impact of HIV among African Americans and highlight the need to identify factors contributing to these elevated rates to acquire a better understanding of HIV among African Americans and guide prevention and intervention efforts.

In an effort to explain racial disparities in HIV prevalence in the United States, several hundred studies have provided data on the risk factors associated with HIV. However, when these studies are examined individually, the results are often inconsistent. These inconsistencies are likely attributable to factors such as differences in sample size, methodology, and populations. Furthermore, it becomes difficult to separate out individual and environmental risk factors that are solely accountable for higher rates of HIV among African Americans, since they are often interrelated and involve the interplay of several situational and environmental factors. Identified risk factors among African Americans include poverty, risky sexual behaviors, substance use, incarceration, education level, access to health care, discrimination, and HIV/AIDS-related stigma. This entry provides an overview of risk factors related to HIV infection among African Americans and concludes with a discussion of promising interventions for reducing HIV among African Americans.

Factors Contributing to HIV in African Americans

Poverty

Individuals who live below the poverty line or who live in low-income areas are at an increased

risk for HIV such that poverty may be the single most powerful risk factor outside of sex between males and IDU. An analysis in 2010 of over 9,000 heterosexual adults (non-injection drug users) revealed a direct correlation between socioeconomic status (as indicated by income, education, and housing status) and contracting HIV (Denning and DiNenno 2010). The rates for poverty-stricken areas in the United States are similar to countries with widespread AIDS epidemics such as those in Africa. Furthermore, there are no significant differences in the prevalence of HIV by race within poverty areas, which bolster the evidence that economic factors, more than race, may drive the prevalence of this epidemic within the African American community (Denning and DiNenno 2010). Poverty is believed to increase vulnerability to HIV through its association with a myriad of other identified HIV risk factors including substance abuse, homelessness, high rates of sexually transmitted infections (STIs), limited access to health care high incarceration rates, and psychiatric problems.

African Americans face several obstacles which may increase exposure to HIV as well as impede treatment for HIV. Regardless of race, approximately 20% of individuals with HIV are unaware of their status. African Americans have a higher base rate of HIV compared to other races and are more likely to have sex with individuals from their own racial group. A lack of awareness of HIV status combined with a higher base rate of HIV and a tendency to have sex within one's own racial group likely lead to a higher rate of accidental exposure to HIV for African Americans compared to other racial groups. Stigma and discrimination also impede treatment. African Americans already face high levels of stigma. Added stigmas of engaging in high-risk sexual behaviors or injection drug use (IDU) may deter individuals from seeking help or confiding their difficulties in others.

Substance Use

Substance use has long been recognized as a leading risk factor for HIV and is one of several factors driving the HIV epidemic among African

Americans. Drug users are at risk for contracting HIV through two avenues: injection drug use and high-risk sexual behaviors associated with drug use, such as trading sex for drugs or having unprotected sex when under the influence of drugs or alcohol. According to a national survey conducted by the Substance Abuse and Mental Health Services Administration (SAMHSA 2007), past-month illicit drug use was slightly higher among African Americans (9.2%) relative to the Whites (8.1%). However, only half as many African Americans engage in IDU compared to Whites. Overall HIV prevalence as a result of IDU has declined in recent years due to prevention efforts and education; however, IDU continues to be a major risk factor for HIV among African Americans. Compared to Whites, African American injection drug users are at a higher risk for contracting HIV through IDU.

Injection drug users are also at risk for HIV transmission through engaging in high-risk sexual behavior, and this association may be more significant for women than for men (Strathdee et al. 2001). High-risk sexual behaviors have been found to be more predictive of HIV seroconversion among African American women who are injection drug users compared to their male counterparts. Specifically, sex with other injection drug users may be a route through which African American women contract HIV. This relationship is particularly strong for cocaine injection, which has been shown to be more predictive of high-risk sexual behaviors for African American women compared to opiate injection.

Non-injection drug use has also been associated with the sexual transmission of HIV. Drug intoxication often induces hypersexuality, disinhibition, and impaired judgment, all of which can influence risky sexual behaviors. While a number of substances have been associated with high-risk sexual behaviors, crack cocaine in particular has been associated with increased sexual activity, inconsistent condom use, sex with multiple partners, and exchanging sex for drugs or money (Campsmith et al. 2000). Because of its association with risky sexual behaviors, the contribution

of crack cocaine to the HIV transmission has been the focus of the majority of the drug-related research. Furthermore, crack cocaine use is more prevalent among low-income African American communities in which HIV prevalence is already high. This racial disparity in crack cocaine use has been found to be especially strong in HIV-positive individuals, and African Americans have been shown to be more likely to use cocaine both before and after HIV diagnosis in comparison with Whites (Campsmith et al. 2000).

Biological Factors

The prevalence of STIs other than HIV is important when examining factors related to HIV because STIs facilitate the transmission and acquisition of HIV by creating open sores and allowing the virus to enter the bloodstream (Nusbaum et al. 2004). African Americans have higher rates of other STIs than Whites. Gonorrhea rates are especially high among African Americans, though rates have declined from about 30 times higher to 20 times higher than Whites over the past 10 years. Chlamydia and syphilis rates are also higher among African Americans relative to Whites. While STI rates have generally been declining in response to better educational and media campaigns, syphilis rates have been on the rise in young African American men. This increase may be explained by the portion of African American men who have sex with men, as this group represents over half of all new syphilis cases.

Lack of Awareness of HIV Status

One major contributor to the transmission of HIV among African Americans, and certainly other races, is the lack of awareness of one's HIV status (see entry "[► HIV Counseling and Testing, Prevention of HIV](#)"). A meta-analysis found that individuals who are aware of their HIV-positive status are less likely to engage in risky sexual behavior (Marks et al. 2005). Furthermore, more recent evidence has found that individuals who are taking antiretroviral therapy (ART) are less likely to transmit HIV to unaffected sexual partners (Cohen et al. 2011). Increasing HIV testing

and providing treatment among African Americans are both essential components to HIV prevention. However, a number of variables have been identified which may impede African Americans' willingness and ability to be tested, including a lack of knowledge concerning HIV risk factors as well as not perceiving themselves to be at risk. African Americans generally have sought less medical care and may be more apprehensive to seek treatment due to prior bad experiences with the medical system or lack of health insurance. Additionally HIV-/AIDS-related stigma and fear of knowing one's HIV status have both been suggested as potential barriers to treatment.

Sexual Network Patterns

Sexual network patterns (see entry "[► Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#)") appear to be a major contributing factor to the HIV epidemic among African Americans, rather than engaging in unusually high-risk sexual acts (see entry "[► Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#)"). There is no evidence to suggest that African Americans engage in higher-risk sexual behaviors (e.g., anal sex, sex without condoms), yet they remain at higher risk for both HIV and STIs compared to Whites for a number of reasons. African Americans are more likely to engage in concurrent sexual partnerships (sexual partnerships that overlap in time) than other racial groups, which has been identified as a major risk factor for HIV transmission (Morris et al. 2009; Morris and Kretzschmar 1997). Furthermore, concurrent sexual partnerships increase the probability of sexual mixing between high- and low-risk individuals. Since African Americans, similar to other racial/ethnic groups, are more likely to engage in sexual relationships with members of the same race, the risk of HIV remains concentrated among African Americans. Further, the increased rates of the HIV infection in this population increase the likelihood of exposure from having sex with a high-risk partner that gets transmitted to a low-risk partner.

Interventions, Prevention, and Future Directions

Much of what applies to prevention efforts in general also applies to African Americans. Education, media campaigns (see entry “► [Mass Media and HIV Prevention](#)”), and more frequent testing all contribute to reducing HIV. However, tailoring these efforts may better address the specific HIV prevention and treatment needs of the African American community. Tailored interventions and those delivered to African Americans by African Americans have been shown to be more effective. The CDC and several of its collaborators have worked through advertising and funding campaigns to target high-risk groups in efforts to encourage testing in poor neighborhoods, African American women, and African American MSM. They have also put forth an initiative to encourage HIV testing at historically African American universities and colleges and worked to create educational and support groups (i.e., Many Men, Many Voices and Sister to Sister) to destigmatize HIV, provide education, and help groups such as MSM seek support and deal with discrimination. The CDC has also worked to support National Black HIV/AIDS Awareness Day (February 7.) Other organizations such as the Black AIDS Institute and the National Association of People with AIDS have made tremendous strides in publicizing HIV risk in the African American community, increasing testing, and promoting education about HIV testing and prevention. Many of these efforts have come from African American leaders and celebrities and received wide mass media publicity, extending their influence to a nationwide audience.

Conclusions

HIV affects African Americans at a disproportionately higher rate than other races. A number of sociodemographic factors place African Americans at a heightened risk for HIV such as elevated levels of poverty, patterns of substance use, and the stigma associated with the illness. Furthermore, sexual networks greatly exasperate the risk for contracting HIV in the African American

population. Tailored interventions and those delivered to African Americans by African Americans have been shown to be effective. Media campaigns designed to reduce stigma, raise awareness, and increase testing have shown promise as an effective means of intervening at the population level.

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HIV Prevention and Asians and Pacific Islanders

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Definition

The population category of Asians and Pacific Islanders (API) comprises individuals of more than 70 diverse nationalities and more than 100 languages and dialects. The incidence and prevalence of HIV and HIV risk behaviors vary across subgroups of API. Barriers to prevention include linguistic isolation, stigma and discrimination, immigration issues, and cultural norms. Support from social networks and the involvement of religious organizations may be critical to primary, secondary, and tertiary HIV prevention efforts among APIs.

HIV Among Asians and Pacific Islanders in the United States

The Asian and Hawaiian Native/Pacific Islander (API) population of the United States has grown rapidly, comprising 1.5% of the US population in 1980 and growing to 4.4% of the population by 2002 (Reeves and Bennett 2003). According to census data, almost two-thirds (61.4%) of the API population is foreign-born. Although APIs are often thought of as a homogenous group, "Asians and Pacific Islanders" actually encompasses individuals of more than 70 different nationalities, widely differing cultures, and more than 100 languages and dialects. According to the definition

utilized by the US Census Bureau, Asians are persons "having origins in any of the original peoples of the Far East, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam"; Native Hawaiians/Pacific Islanders include persons "having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands" (Humes et al. 2011). Although individuals of all such groups are encompassed under the same umbrella term, it is important to recognize that the incidence and prevalence of both risk behaviors and HIV/AIDS may vary greatly across the numerous subgroups and the development and implementation of effective prevention strategies necessitates consideration of the differences, as well as the similarities, that exist across the many subgroups.

Data from the 33 states that have long-term, confidential name-based HIV reporting indicate that as of 2005, Asians and Native Hawaiians/Pacific Islanders (API) accounted for approximately 1% of all HIV/AIDS cases in the United States. A recent HIV/AIDS surveillance report indicates that between 2006 and 2009, the rate of AIDS diagnosis decreased among Asians and remained stable among Native Hawaiians/Pacific Islanders (Centers for Disease Control and Prevention 2009).

These HIV/AIDS rates appear to be relatively low in comparison with the rates among other US racial subgroups, such as African Americans and Latinos/Hispanics. However, they belie the increasing rate of transmission among some subgroups of Asians and Pacific Islanders in the United States, particularly among men who have sex with men (MSM). The primary mode of HIV transmission among men is unprotected sexual contact between men, which accounts for 67% of all cases, followed by high-risk heterosexual contact, which accounts for an additional 16% of the cases. Several studies conducted in San Francisco indicate that HIV prevalence among some samples of Asian and Pacific Islander MSM may be as high as 3% and that API MSM communities may have a high prevalence of risky sexual behaviors. The use of crystal methamphetamine ("crystal"), methylenedioxymethamphetamine ("ecstasy"),

and inhalant nitrites (“poppers”) in the MSM community has been associated with engaging in high-risk behaviors, which include unprotected anal sex, commercial sex work, and low rates of HIV testing. Because more than one-half of API MSM infected with HIV/AIDS have been born outside the United States, it has been hypothesized that individuals’ experience of stigma and discrimination due to their status as members of a racial minority, as MSMs, and as immigrants may be associated with increased HIV risk. At least one study has found that experiences of anti-immigrant discrimination are associated with increased levels of unprotected anal intercourse with secondary partners (Yoshikawa et al. 2004).

Eighty percent of HIV/AIDS cases among API females are attributable to high-risk heterosexual contact; injection drug use accounts for an additional 16% of these cases (Centers for Disease Control and Prevention 2008). The proportion of young unmarried API women who report having engaged in sexual intercourse is much lower than that among their White, Black, and Hispanic/Latino counterparts; it has been hypothesized that this relative postponement of sexual activity may be related to cultural norms that mandate sexual modesty for women. However, once sexually active, the rates of engaging in unprotected sex appear to be comparable between API women and women of other racial/ethnic groups. Research findings also suggest that API women may be at risk for HIV due to their sexual partners’ risk behaviors, including unprotected sex with secondary partners when traveling overseas (Carrier et al. 1992), a refusal to utilize condoms, even when explicitly asked to do so, and the threat of partner violence (Jemmott et al. 1999).

HIV/AIDS Prevention Efforts

Individual-level prevention efforts may focus on primary prevention (preventing the transmission and acquisition of HIV), secondary prevention (preventing the progression of HIV to symptomatic disease among those who are HIV infected), and tertiary prevention (preventing death among those with symptomatic disease). In the absence

of a vaccine, primary prevention can only be accomplished by reducing risky behaviors. Secondary prevention can be accomplished through early HIV diagnosis and linkage to care, while tertiary prevention is effectuated through the provision of effective HIV treatment.

Primary Prevention Efforts

Individual-Level Prevention Efforts

The single controlled trial of an HIV prevention program for APIs utilized the social cognitive model as the basis for the intervention to reduce incidents of unprotected anal sex and number of sexual partners among API gay men (Choi et al. 1996). Currently, the health belief model appears to be the most frequently suggested framework for the development of effective HIV prevention interventions with API communities. The health belief model suggests that an individual will take preventive action to avoid a disease if (1) the individual believes that he or she is susceptible to the disease (perceived susceptibility); (2) the disease may have a moderately severe impact on a domain in the individual’s life (perceived severity); (3) specified behaviors may help to reduce the susceptibility or severity (perceived benefits); (4) the adoption of these behaviors is not impeded by perceived barriers such as cost, shame, or pain (perceived barriers); and (5) the person believes that he or she is able to perform a specified behavior (Rosenstock 1974; Rosenstock et al. 1988). Research with Taiwanese immigrants to the United States suggests that self-efficacy for maintaining a monogamous relationship and for condom use may be key in primary prevention efforts among some APIs (Lin et al. 2005). Individuals with low self-efficacy for maintaining a monogamous relationship may be more likely to have multiple sexual partners, thereby increasing their HIV risk, and individuals with low self-efficacy for condom use may be less likely to utilize condoms, again resulting in greater HIV risk.

API individuals may experience numerous barriers that not only inhibit their adoption of HIV prevention behaviors but also diminish their willingness to obtain HIV prevention information.

API individuals may be reluctant to discuss HIV risk or prevention or to present for HIV testing due to the relative unavailability of language-appropriate prevention interventions; racism and an accompanying distrust of physicians; cultural norms that shame sexual behavior, sexual orientation, substance use, and/or HIV status; cultural taboos prohibiting the open discussion of sex and sexuality; a belief that discussion of illness or death will lead to bad luck; and fear of potential immigration consequences among those who are not US citizens. API MSM in particular may experience strong feelings of guilt about their sexual behavior due to family expectations that they marry and produce children; the anxiety associated with discussions of sexual behavior may trigger identity conflicts that they wish to avoid (Kanuaa 2000).

Support from social networks may be important in reducing HIV risk behaviors and HIV transmission among API MSM. Research suggests that having conversations with family members and gay friends about experiences of stigma and discrimination is associated with lower levels of unprotected anal intercourse among API MSM (Yoshikawa et al. 2004).

A variety of delivery mechanisms have been identified that could be utilized effectively in Asian Pacific Islander communities. These include newspaper articles published in a variety of languages, mobile vans offering a variety of services including those related to HIV, take-home videos in appropriate languages, home-based prevention programs, and Internet-based strategies (Jemmott et al. 1999).

Community-Level Prevention Efforts

Although community-based initiatives may provide HIV prevention services, their efforts to do so may be hampered by a variety of factors. These include a lack of organizational stability and viability related to human and financial resources, service delivery, and external relations, all of which are critical to engender trust in the community to be served (Sheth et al. 2007). Organizations that lack adequate HIV knowledge or familiarity with and

sensitivity to cultural issues may be unable to develop or implement effective and acceptable HIV prevention services.

The national capacity building assistance (CBA) program established by the Asian & Pacific Islander Wellness Center in the San Francisco and the Asian & Pacific Islander American Health Forum illustrates one model that is available to address many of the barriers that impede prevention efforts (Sheth et al. 2007). The CBA program is premised on three core values: client centeredness, collaboration, and cultural competence. Through the development and provision of individual client-centered trainings, national and regional workshops, and information and technology transfer, the CBA works with community-based organizations, health departments, research scientists, and health and medical service providers to increase the number of health departments that collect and report data related specifically to APIs, increase community investment in HIV-related services for APIs, increase HIV leadership and sustainability in the Pacific Island jurisdictions, and, ultimately, increase the numbers of API individuals who are tested for and learn their HIV status and the acceptance of HIV in API families and communities (Sheth et al. 2007).

Religious organizations are quite important within many Asian immigrant communities (Min 2002) and can potentially aid in HIV prevention efforts (Loue et al. 1999). However, there may be significant divergence of opinion regarding the extent to which involvement of the religious institution in HIV prevention efforts would be considered to be acceptable by attendees at the religious institutions and by the institutions' respective communities (Chin et al. 2005). This may be a particular issue among adherents of Buddhism and Hinduism due to perceived conflicts between religious teachings and the focus of HIV prevention discussions.

Secondary and Tertiary Prevention Efforts

It is clear that many of the barriers that operate to impede primary prevention efforts, such as linguistic isolation, cultural taboos impacting the

willingness and ability to discuss sexuality and sexual behavior openly, and a fear of potential immigration consequences, also impact secondary and tertiary prevention efforts. As an example, researchers have found that HIV-positive APIs are less likely than their White counterparts to be aware of the CD4 count or of services that might be available to them (Wong et al. 2004). Individuals who are unaware of the status of their immune functioning or services that may be available to them for the treatment of their HIV infection will not be able to optimally manage their disease progression.

API individuals who do not disclose their HIV status to friends and/or family members may be deprived of the support that might otherwise be available to them. A desire to shield one's family members from shame, to protect family members from an obligation to provide assistance, and to avoid communication about highly personal matters, such as sexual behavior, has been found to inhibit the ability of HIV-positive API MSMs from disclosing their serostatus to their family members (Yoshioka and Schuztack 2001). API individuals may seek emotional support from gay friends and disclose their HIV seropositivity to family members only when his health deteriorates to a point where disclosure becomes absolutely necessary.

It has been suggested that not only is the provision of HIV/AIDS education to family members important for HIV-positive API individuals but that health-care professionals may need to be actively involved in this process. This can be accomplished through a variety of mechanisms, including addressing the family members with the assistance of an API physician and/or utilizing the services of a trained interpreter in situations in which the family members and health-care provider(s) do not speak the same language.

Conclusion

Asians and Pacific Islanders are highly diverse with respect to culture, religion, and language. Although the numbers of HIV-positive

individuals are low in comparison with other minority groups, specific subgroups are experiencing high rates of HIV transmission.

Significant barriers to both prevention of HIV transmission and treatment for those who are already HIV-positive exist at both the individual and community levels. At the individual level, these include language, fear of immigration consequences, lack of social support, cultural norms related to sexuality, and racism. Community-level barriers include organizational stability, lack of familiarity with Asian and Pacific Islander cultures, inadequate knowledge relating to HIV, and lack of language-appropriate resources. Potential approaches to reduce these barriers and enhance prevention efforts include the provision of support services to individuals and their family members, the development of HIV prevention programs through religious institutions, and increased reliance on home-based delivery mechanisms for the provision of HIV-related information and testing.

Cross-References

- ▶ [Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#)
- ▶ [Women, Epidemiology of HIV/AIDS](#)

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HIV Prevention and Hispanics

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Definition

HIV Prevention in Hispanics:

The study of the prevention of HIV among the Hispanic population that includes examination of etiology and intervention strategies at the individual, family, peer, school, community, and macro level.

Epidemiology of HIV/AIDS

Hispanics are disproportionately affected by HIV/AIDS, a public health concern driven by multiple factors including, but not limited to, individual, family, and macro-level variables. For example, while Hispanics accounted for approximately 17% of the total US population, they comprised almost 24% of new HIV infections (CDC 2016a). Young people are also disproportionately affected by HIV, representing 39% of new cases among 13–29-year olds-(CDC 2016b) (Children, epidemiology of HIV/AIDS). Among Hispanics, inequities also exist among young people and men who have sex with men (MSMs). For example, from 2005 to 2014 HIV diagnoses among gay and bisexual men aged 13 to 24 increased 87% (CDC 2016a). Hispanic MSMs accounted for 81% of new HIV infections among Hispanic men (CDC 2015a). Further, 45% of new HIV infections among Hispanic MSM were among people below the age of 30 (MSM, Epidemiology of HIV/AIDS)(risk groups). Since the epidemic disproportionately impacts Hispanic young people and MSMs, this chapter will focus on these subgroups of the Hispanic population.

Hispanics constitute a large and quickly growing segment of the US population with a growth rate faster than all other ethnic/racial groups, including non-Hispanic Whites (U.S. Census

2011). Further, Hispanics are a young population; approximately 34% of Hispanics are under the age of 18 compared to 24% for the rest of the total US population (U.S. Census Bureau 2009). Therefore, given the high percentage of new HIV cases among young people and that the Hispanic population is rapidly expanding, the need for prevention research and intervention is clear.

For HIV prevention to be effective, it is important to understand the social and contextual factors (social contextual factors in HIV prevention) associated with it (Prado et al. 2010). Prevention frameworks underscore the necessity for conceptualizations of problem behaviors among youth, including HIV risk behaviors, as embedded within a system of networks (e.g., Prado et al. 2010; Viruell-Fuentes et al. 2012). Researchers posit that risk and protection do not function independently and that, indeed, conceptualizing risk and protection as separate processes can overstate the impact of each (Prado et al. 2009). Rather, risk and protective factors exert their influence on HIV risk behaviors within the environment in which they are embedded. As such, this chapter will focus on individual, family, and macro-level factors of HIV among young Hispanics and Hispanic MSMs, along with a brief discussion on preventive interventions for Hispanic youth.

Individual Level Factors

Sexually risky behaviors and substance use are two individual-level factors that impact HIV transmission with high rates among Hispanics compared to non-Hispanic whites. Transmission of HIV among youth commonly occurs through risky sexual behaviors including sex without a condom and multiple sexual partners (sexual risk factors for HIV infection, personal). The proportion of Hispanic youth reporting condom use at last sexual intercourse is lower (55.6%) than that of non-Hispanic white (56.8%) and African-American (63.4%) youth (CDC 2015b). Additional individual-level precursors to HIV infection include substance use, which increases the risk for sexually risky behaviors in young people (Prado et al. 2006).

The dangers of drug use are exacerbated among Hispanic youth because they have higher use rates compared to other minorities, making illicit drug use a major risk factor for engagement in unsafe sexual behavior and consequently HIV infection (Prado et al. 2006). According to the CDC's Youth Risk Behavior Surveillance Survey, of those adolescents who reported having sex within 3 months prior to completing the survey, 23.3% reported drinking alcohol or using illicit drugs before their last sexual intercourse. Hispanic adolescents also reported drinking or using illicit drugs before last sexual intercourse (25.6%) more often than both non-Hispanic Whites (25.0%) and African-Americans (14.1%).

The relationship between substance use and unprotected sex is particularly strong among MSMs (Celentano et al. 2006; Dolezal et al. 2000) (sexual factors for HIV infection, personal). A study with mostly young Hispanic MSMs (76% under the age of 35) found higher frequencies of anal intercourse without a condom were correlated with increased alcohol and illicit drug use (Dolezal et al. 2000). Substance use has also been documented among Hispanic MSMs as a means of increasing desire and decreasing inhibition and has been associated with unprotected anal intercourse and multiple sex partners (Fernandez et al. 2007). Age also seems to be a factor in unprotected sex among MSMs. Previous studies have found rates of unprotected anal sex ranging from 40% to 50% among 18–30-year-olds, across studies, suggesting that preventive interventions among young MSM need to include substance use reduction strategies (Celentano et al. 2006).

Family-Level Factors (Family Structure)

Parents and family play a central role in youth development, particularly in Hispanic culture, and are therefore an important point of intervention for HIV prevention. Research demonstrates parents' role in molding youth sexual attitudes and contraceptive behavior and recommends the inclusion of parents in HIV prevention interventions for adolescents (NCLR 2011). Family functioning is a strong predictor of HIV risk behaviors

among Hispanic youth and has been shown to reduce the onset of risky sexual behaviors (Prado et al. 2010).

Communication is an important component of family functioning (communication). Parent-youth communication about sex allows for the transferring of values and beliefs regarding sex in addition to providing a reliable foundation related to sex education for youth (NCLR 2011). Youth who receive information about sex are less likely to participate in sexually risky behaviors, but, unfortunately, Hispanics do not communicate with their parents as much as non-Hispanic whites when it comes to topics related to sex (Baumeister et al. 1995). Lack of communication can lead to decreased familism, a family-level factor which is heavily emphasized in Hispanic families.

Familism refers to an emphasis on family relationships, loyalty, and reciprocity (Sabogal et al. 1987). Familism involves being responsive toward family obligations and placing family interests above self-interests. Higher levels of familism have been associated with better health profiles for Hispanics and with better adaptation to US society (Sabogal et al. 1987). Among MSMs, immediate family support has been identified as a protective factor associated with decreases in unprotected anal intercourse, social isolation, and psychological distress (Diaz and Ayala 2001) (social support). In an examination of protective factors for HIV infection among Mexican-American MSMs, investigators found respect for family as an important motivator and that participants honored their families by safeguarding their health through avoidance of sexually risk behaviors (Meyer and Champion 2010). Researchers have suggested that the value of familism can be used as a tool to prevent HIV infection (NCLR 2011). For example, familism can be called upon as a way of creating family unity in regard to engaging in positive health behaviors that benefit the family as a whole (Guilamo-Ramos et al. 2009).

Macro-Level Factors

Multiple researchers have highlighted the importance of moving away from behavioral strategies of HIV prevention and moving toward models

that account not only for the immediate social environment but that also account for broad social, economic, and cultural environmental factors (e.g., Organista et al. 2012) (social contextual factors in HIV in HIV Prevention). The heavy impact of HIV/AIDS on minorities, such as Hispanics and MSMs, indicate that individual level interventions, while important, are insufficient and that broader, macro-level factors unique to these populations should be considered in HIV prevention efforts (Wyatt et al. 2012). The context of HIV risk must be understood and targeted beyond a behavioral paradigm in order to obtain effective risk reduction (NCLR 2011). Some of the social and cultural drivers of HIV infection among Hispanics include cultural values, acculturation, and discrimination.

Cultural Values (Social Contextual Factors in HIV in HIV Prevention)

Understanding Hispanic cultural constructs are fundamental to the advancement of HIV prevention (NCLR 2011). Cultural beliefs around sex impact how relationships are defined, how and when condoms are used, and what sexual behaviors are considered acceptable (Wyatt et al. 2012). While there has been a focus on how cultural beliefs influence decision-making around sexuality, less is known regarding how cultural beliefs among Hispanics may contradict HIV prevention messages and therefore impact risk for infection and transmission. For example, collectivistic orientations which value interconnectedness and placing the needs of others over self-needs may interfere with consistent condom use (Wyatt et al. 2012). For HIV prevention to work among Hispanics, messages need to be delivered in culturally syntonous ways and must reflect Hispanic cultural values (Marin 2003). The risk for HIV is augmented when populations are not given the necessary skills that are acceptable and congruent with long-held beliefs (Wyatt et al. 2012).

Researchers hypothesize that certain Hispanic cultural values serve as protective and risk factors for HIV. As a group, discussion about sex and sexuality for Hispanics is more private and personal than in white non-Latino cultures (Marin 2003). Due to language and cultural barriers,

accessing information about HIV prevention, HIV testing, and securing timely medical attention can be difficult for individuals born outside the USA (Prosser et al. 2012). Changes associated with the immigration experience like the loss of stable romantic relationships and social networks may contribute to changes in behavior that lead to increased risk of HIV infection (NCLR 2011). Additionally, there are gender norms among the Hispanic population that put males and females at risk for HIV; in particular, gender roles have a significant impact on HIV infection (gender roles).

Hispanic cultural constructs such as machismo and marianismo have been associated with Hispanic sexual behaviors that can lead to or prevent HIV infection. Machismo usually involves beliefs around the meaning of manhood and encompasses hypermasculinity, sexual prowess, and bravado (Raffaelli and Iturbide 2009) which can lead to sexually risky behaviors. The traditional male machismo role supports the acceptability of multiple partners for males and places Hispanic women at risk for HIV and other sexually transmitted diseases (Teitelman et al. 2008). Among Hispanic MSMs, there is a relationship between higher levels of unprotected sex and machismo (among adult men) (Dolezal et al. 2000). In this particular study, machismo was related to unprotected anal intercourse for insertive sex but not receptive. Research also indicates that among Hispanic MSMs, machismo beliefs are associated with a greater number of sexual partners (Jarama et al. 2005). Further, beliefs around machismo uphold the notion that males cannot control their sexual desires and that these desires require release (Jarama et al. 2005). Perceptions of low self-control in regard to sex are frequently used to justify anal sex without a condom in research with Hispanic MSMs (Jarama et al. 2005).

Contrarily, machismo has also been studied in terms of its positive attributes. For example, a “macho” man is a protector of self and family, is monogamous, and sees women as equals (e.g., DeMente 1996). Some researchers have suggested that the construct of machismo among Hispanic youth can be channeled in a manner that highlights males as protectors and used as

motivation for youth to avoid endangerment of their families by taking precautions in their sexual behavior (Meyer and Champion 2010). Overall, while machismo may have positive aspects such as males being seen as the family protector and provider, machismo can disempower both men and women as male masculinity must constantly be proven through multiple sexual partners and demonstrations of fearlessness and lack of sadness (Marin 2003).

Alternatively, the Hispanic traditional value of marianismo suggests that women should be self-sacrificing and subordinate to male authority (Cauce and Domenech-Rodriguez 2002). This cultural value upholds female passivity in sexual relations and places females in a position of disempowerment when addressing sexual needs such as condom use. In many relationships, the suggestion of a condom is enough to call into question its monogamy status and prevent females from insisting on protection. Research with Hispanic females has cited male’s unwillingness to use condoms and inability to employ risk reduction behaviors with partners as barriers to HIV prevention (Amaro 1995). Further, gendered beliefs, such as endorsement of the belief that men should know more about sex than women, promote embarrassment and discomfort with sex and perpetuate sexual silence which results in a lack of access to preventive information (Marin 2003). Although marianismo upholds women’s silence, self-sacrifice, and reluctance to discuss sexual matters, it has also been conceptualized as protective in that it can lead to behaviors such as fewer sexual partners. In summary, HIV prevention and risk reduction strategies need to consider the contextual social factors that shape sexual behaviors and how these are impacted by gender norms (Amaro 1995) and incorporate deeper understandings of how gender roles (gender roles) impact behavior.

Acculturation (Social Contextual Factors in HIV in HIV Prevention)

Acculturation appears to be both a risk and protective factor for HIV infection across multiple studies (Rafaelli and Iturbide 2009). Acculturation involves cultural changes resulting from

interaction between two or more different cultural groups and their members (Berry 2007). Acculturation is important to consider given that some studies indicate 42% of HIV infections among people born outside the USA occurred among Hispanics (Prosser et al. 2012). While most studies regarding Hispanics and sexuality have been conducted with adults, it is likely that many of the findings are applicable to Hispanic youth (Raffaelli and Iturbide 2009). Although lower levels of acculturation have been linked to fewer sex partners in Hispanic women, it has also been linked to lower condom use (Marin 2003). Concomitantly, higher levels of acculturation have been shown to bring along an increase in female power in interpersonal relationships which is associated with more condom use (Raffaelli and Iturbide 2009). Among new immigrants, adolescents living in English-speaking homes were at less risk for sexual activity than their counterparts in Spanish-speaking homes. The opposite was true, however, for US-born Hispanic youth, who were at higher risk for early sexual activity if they were living in English-speaking homes (Guilamo-Ramos et al. 2009).

Among males, higher levels of acculturation have been associated with fewer sexual partners, while other studies have shown the opposite. Lower levels of acculturation have also been associated with coercive sexual practices among Hispanic men (Raffaelli and Iturbide 2009) and indicate that Hispanic males who adhere to traditional sex roles are more likely to endorse or participate in coercive sexual practices, therefore increasing the risk for unprotected sexual contact (Rafaelli and Iturbide 2009). Hence, some findings suggest that higher levels of acculturation lead to more risk behaviors, while others suggest that higher levels of acculturation are associated with less risk behaviors. These mixed findings are also present among youth. The differences in research findings mentioned above are likely related to how acculturation is measured and conceptualized (Schwartz et al. 2010) and to the specific behaviors being measured (Raffaelli and Iturbide 2009). For example, acculturation is usually measured through the use of proxy measures such as language use and amount of time in the

USA instead of directly assessing cultural values (Schwartz et al. 2010). Changes in cultural values resulting from the acculturation process also impact the family system.

Family changes, such as the ones brought on by the effects of acculturation between parents and youth, greatly affect the family system. Acculturation, the process of adapting to a new culture, can have an impact on family processes due to the conflict created by differing cultural values between youth and parent (Prado et al. 2012). As youth become more acculturated than their parents, parents begin to lose parental authority due to a reliance on youth for information, linguistic interpretation, and cultural navigation (Unger et al. 2009). The loss of parental authority can lead to family conflict and inconsistent parental discipline and monitoring, which can result in increased risk for problem behaviors (Unger et al. 2009), including risky sexual behaviors. Parents may become stricter as they attempt to control their youth's behavior, resulting in increased negative behaviors and eventual parental disinvestment (Pantin et al. 2003). Further, parents may continue to hold on to their culture of origin while youth become more Americanized, causing discrepancies in values and beliefs between parent and youth. These discrepancies can lead to family conflict and behavioral difficulties.

Discrimination (Discrimination)

While great strides have been made regarding discrimination and racism in the USA, they continue to be pervasive social problems, particularly in regard to HIV/AIDS and MSMs. Discrimination refers to behaviors from others that cause feelings of being unwanted, stereotyped, or demeaned (Todorova et al. 2010). Discrimination has been shown to have chronic negative health consequences for Hispanics including HIV. There is a growing body of research suggesting that a discriminatory environment is related to lower ratings of self-efficacy and decreased attempts at negotiating safe sex practices or engaging in preventive behaviors such as routine HIV testing (Adimora and Schoenbach 2005). Further, discrimination based on homosexuality has been

found to be related with a higher prevalence of risky anal intercourse (Jarama et al. 2005). Among Hispanics, Latin-American cultures tend to have strong anti-gay views which may prevent gay men from securing information about HIV or be tested for HIV. Further, expectations about male sexuality take place among a backdrop of stigma and discrimination against homosexuality which can decrease exposure to preventive interventions and denial of HIV vulnerability (Jarama et al. 2005)(stigma and stigmatization). Researchers have cited that minorities, including Hispanics, view homosexuality as harmful to core family values and to the continuation of their culture (e.g., Saddul 1996). Indeed, research with lesbian, gay, bisexual, and transgender adolescents indicates that discrimination contributes to HIV disease risk by lowering self-efficacy and reducing the benefits of social and cultural contexts such as peer connectedness and family relationships (Markham et al. 2010). While discrimination and its role in HIV infection is important to examine, researchers suggest investigations that also incorporate explanations for how structural racism affects immigrant health (Viruell-Fuentes et al. 2012). Structural racism such as those present in immigration policy has underlying ideologies which give rise to day-to-day discrimination. Immigration policies have well-documented effects in restricting access to health and social services and have a direct impact on the causes of disease by influencing access (or lack of) to life opportunities such as higher education and well-compensated employment opportunities (Viruell-Fuentes et al. 2012).

Preventive Interventions

Although there is a high need for prevention, few programs are available that target the needs of Hispanic youth and Hispanic MSMs and that incorporate the role of culture into prevention (Unger et al. 2009). The need for interventions is clear, and in order for these interventions to be effective and acceptable to Hispanics, cultural factors should be considered (Prado et al. 2006). Research suggests that culturally specific interventions demonstrate better outcomes than generic interventions. For example, a meta-

analysis across 76 studies found that culturally adapted interventions delivered to specific cultural groups were moderately strong and four times more effective than interventions delivered to clients of diverse backgrounds (Griner and Smith 2006).

Some interventions have been designed for Hispanic youth, including *Familias Unidas* (united families) (family interventions) and *Cuidate!* (take care of yourself!). *Familias Unidas* consists of parent group sessions and participatory dialogue to empower parents in helping adolescents engage in positive behaviors. It addresses the needs of Hispanic families by incorporating cultural components in the prevention of behavior health problems (Pantin et al. 2003). The *Cuidate!* program is a group intervention designed to prevent sexually risky behaviors. *Cuidate!* takes into account the cultural values inherent in Hispanic populations to build HIV knowledge, increase condom use self-efficacy and negotiation of safe sex practices, and identify attitudes and beliefs (NCLR 2011).

Similarly, several macro-level interventions have been developed for youth, but none specifically for Hispanic youth in general. For example, The ACCESS program utilized social marketing to promote HIV testing among adolescents, including Hispanics, in five urban cities across the USA. Findings showed increased HIV testing among youth and an increase in number of phone calls to information hotlines (Futterman et al. 2001). Finally, in 2009 the CDC launched a media campaign, *Act Against AIDS*, to promote HIV and AIDS awareness in the USA. The campaign has a focus on highly impacted groups such as African-Americans, Hispanics, and MSMs (CDC 2009).

Interventions with Hispanic MSM

Though not originally studied with Hispanic MSMs, the following interventions are currently being used to address HIV/AIDS risk among Hispanic MSMs living in the USA: Many Men, Many Voices (3MV), Popular Opinion Leader (POL), and Mpowerment/Mpoderoso (CDC 2011b). The efficacy studies of 3MV, a group-level intervention that addresses social and behavioral

determinants of HIV/STI risk and protective behaviors, were originally conducted exclusively with Black MSMs of varying ethnic backgrounds. POL is a community-level intervention designed to mobilize and empower opinion leaders to promote safer sexual norms within their social networks. It was originally studied for use with white MSM (mean age 29 years old) with low-education attainment and has also been evaluated for use with Black MSM (Kelly et al. 1997; CDC 2011b). Mpowerment/Mpoderoso was adapted for delivery to monolingual Hispanic MSM in at least one setting, among Hispanic migrant worker communities (CDC 2011). To summarize, while there is a need intervention, most interventions have been developed for non-Hispanic populations and later adapted for this population.

Conclusion

HIV continues to be a significant problem with wide health disparities among minority populations, including Hispanics and Hispanic MSMs. Despite great advances in the etiology of HIV/IDS, there are areas of opportunity in HIV prevention:

1. Few interventions have been evaluated for Hispanic subgroups who are most at risk, including Hispanic youth and Hispanic MSMs. Even fewer interventions developed specifically for Hispanics. Cultural factors that impact the acceptability of these interventions including family, gender norms, and discrimination need to be considered in prevention development.
2. Most interventions that have been evaluated for the Hispanic population are small group/behavioral interventions. A focus on targeting macro-level factors in this population, particularly those factors specific to Hispanics, is necessary.

Progress has been made in the HIV epidemic, and although we have seen some gains among Hispanics, there is more to be done. Perhaps it is time that researchers, public health professionals,

and policy makers look in a new direction, one that meets the specific needs of Hispanics and Hispanic MSMs.

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HIV Prevention and Labor Migration

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Definition

Migration refers to the movement of people from one place to another, temporarily or permanently, across borders or from one location to another within a defined territory. Individuals engage in labor migration voluntarily in search of better economic opportunities, or they may become victims of organized trafficking rings. Research has found that labor migrants are frequently at elevated risk of HIV due to individual and structural-environmental factors. Although a number of individual-level intervention strategies for HIV prevention among labor migrants have been evaluated, relatively few prevention interventions have attempted to address the structural-environmental factors that may predispose labor migrants to increased HIV risk.

Labor Migration and HIV Risk

Migration refers to the movement of people from one place to another, temporarily or permanently, across borders or from one location to another within a defined territory. Frequently, migration is driven by social, economic, and political factors; traumatic events such as hurricanes and tsunamis may also cause people to leave their homes in search of another. In some cases, entire families or communities may migrate in search of labor opportunities or as the result of man-made or natural disasters. In the latter case, migration may necessitate a search for labor opportunities even though that may not have been the motivation that prompted the migration. Individuals engaged in labor migration may do so voluntarily or involuntarily; in some instances, individuals

may migrate voluntarily in response to what appears to be a legitimate employment offer, only to find later that they have become, essentially, slaves (labor trafficking). Migration can increase an individual's HIV risk as the result of alterations in his or her risk or prevention behaviors and/or exposure to a differential HIV prevalence between the places of origin and destination.

Researchers have investigated the relationship between HIV risk and voluntary labor migration in numerous contexts. These include the temporary migration of agricultural workers from Mexico to the United States, of construction and bazaar workers from Tajikistan to (Moscow et al. 2008), of construction workers from one province of China to another (Zhuang et al. 2012), of factory workers in Zimbabwe and in Mexico (Magis-Rodriguez et al. 2009), of mine workers in South Africa (Crush et al. 2005), and of labor migrants from one region of Papua New Guinea to another or from one district in India to another (Wardlow 2007). Studies have consistently reported relatively high rates of HIV risk due to unprotected sex with multiple partners, unprotected sex with partners who inject drugs and of unknown HIV serostatus, and relatively low levels of HIV knowledge.

Numerous underlying factors have been implicated in this elevated risk (Soskolne and Shtarkshall 2002). Premigration factors include the prospect of adequately paying employment at the place of origin and the individual's marital or partner status, age, educational level, and attitude toward risk-taking. Emotional and physical trauma during the course of migration or after arrival at the destination may also impact individuals' sexual and substance-using behavior, leading to increased HIV risk. Post-migration factors that have been found to be associated with increased HIV risk among labor migrants include physical and linguistic isolation; loss of social support; limited social capital; anxiety due to migrants' absence from their homes for extended periods of time; poor access to health-related services, including HIV counseling and testing; increased exposure to high risk behaviors due to close living quarters with individuals participating in high risk behaviors; exposure to sexual norms

that are more permissive than those in their home localities; comorbidities such as sexually transmitted infections; and increased financial assets that facilitate the purchase of alcohol, drugs, and/or sexual services. Individuals may also be unwilling to seek out health-related services due to the loss of income associated with time away from their place of employment and/or the threat of loss of employment for failure to appear at a job site. The social and familial controls that may have moderated migrants' risk behaviors at their place of origin may no longer have any effect at their destination, and the migrants' anonymity at their destination may further encourage them to abandon their previous sexual norms.

Gender, Labor Migration, and HIV Risk

Research suggests that the factors that heighten labor migrants' HIV risk may differ between males and females. Among male migrant laborers, loneliness, isolation, increased financial assets, and ready access to sex workers, alcohol, and drugs may lead to a greater likelihood of participation in sexual relations with sex workers or the initiation of short- or longer-term relationships with male and/or female sexual partners (Organista 2007). A lack of transportation, stigma associated with HIV/AIDS, a fear of being discovered and deported, and ineligibility for health services may impede any efforts to obtain HIV testing and treatment. Significantly, researchers have reported that migrants who have resided for longer periods of time at the labor destination are less likely to use sex workers, experience less social isolation, and have increased opportunities to engage in social relations with women other than sex workers, potentially helping to decrease their HIV risk (Saggurti et al. 2009).

The sexual partners, including wives, of male labor migrants may also be at elevated risk of HIV. To some extent, this increase is attributable to the sexual- and drug-associated risk behaviors of their returning male partners and their own lack of HIV-related knowledge and/or inability to negotiate condom use upon his return home.

However, women who are left at home and have limited financial resources to support their families may engage in transactional sex or multiple partnerships to earn additional income.

In contrast to male labor migrants who often migrate in search of better economic opportunities due to the relatively poor employment prospects at their place of origin, the initial motivation for female migrants to search for new opportunities may be the breakdown of a significant relationship (Kendall and Pelcastre 2010). Female labor migrants may be at particularly high risk of HIV infection for several reasons. First, while away from home, female labor migrants may engage in sexual relations with multiple partners as a means of surviving economically. In some cases, they may receive monetary compensation, but in others they may exchange sex for food, safer housing, or other necessities. Second, female labor migrants may become victims of organized prostitution rings and/or physical violence, including rape. Third, women who find employment in entertainment establishments or personal services, such as bars, massage parlors, barber shops, and dancing halls, may be pressured by the establishment owners and/or clients to provide sexual services or face the possibility of losing their employment. Finally, female labor migrants who are engaged in sex work may need to migrate from one country to another in order to reduce the likelihood of being discovered by police and to maintain access to a larger client base in order to meet living expenses. The women's risk of contracting HIV and of transmitting the virus to others is heightened due to movement between higher and lower HIV prevalence areas and the large number of sexual partners (Surratt 2007). Like male labor migrants, female labor migrants may experience reduced access to HIV prevention information, testing, and care due to illegal immigration status (O'Conner 2009).

Prevention Strategies

Despite the large and growing literature indicating an elevated level of HIV risk among labor

migrants, relatively little research has evaluated possible prevention strategies for this population. Significant challenges are associated with the evaluation of HIV prevention strategies for this population, many of which are associated with the population's high level of mobility and their deportation in the case of illegal migration. These challenges include the establishment of trust, the development of a rigorous tracking system, the identification of qualified interviewers, the scheduling of interviews to accommodate the participants' work schedules, and staff safety concerns (de la Rosa et al. 2011).

Intervention studies with migrant laborers have focused on the enhancement of HIV knowledge and promotion of condom use among migrants, the promotion of condom use among female sex workers who were engaged by migrant farmworkers, the identification of problems and potential solutions by migrant farmworkers, and the training of rural health and educational professionals on HIV/AIDS prevention (Magis-Rodriguez et al. 2004). A three-pronged strategy of "Abstinence, Be Faithful, Use a Condom" (ABC) has been advocated as a means of reducing HIV risk among labor migrants. However, reliance on this approach may not be adequate due to fluctuations in the local supply and cost of condoms, economic pressures that reduce women's ability to negotiate male condom use, and the risk of physical violence that may accompany an attempt to negotiate safer sex (Dworkin and Ehrhardt 2007; Kiš 2010).

Intervention studies focusing on migrant laborers have not, in general, attended to the structural or environmental factors that appear to play a role in the increase in risk. In part, this omission may be due to the need to fashion interventions that are context-specific. Nevertheless, intervention strategies that fail to focus on both individual and structural factors may have limited success in reducing HIV risk among labor migrants. Although a number of multilevel and structural approaches have been suggested to reduce HIV risk among labor migrants and individuals to whom they may transmit the infection, such as wives, husbands, and temporary sexual partners, it appears that, to date, none of these

have been subjected to rigorous evaluation. These include:

- Syndromic treatment of sexually transmitted infections in origin and destination areas
- Increased availability of condoms at the sites where labor migrants congregate and live
- Integration of HIV testing, counseling, and treatment services into general health-related services for migrants
- Provision of HIV-related services, such as education, testing, counseling, and treatment, at nontraditional locations such as churches and shopping malls
- Development of mobile system of providing health-related services to migrants
- Increased economic opportunities for women
- Improved housing conditions for migrant laborers
- Integration of migrants' social support networks into HIV prevention services
- Initiatives to reduce the social isolation of migrant laborers
- Provisions for family members to accompany and be housed with migrant laborer
- Coordinated HIV prevention programs between the sending and receiving countries, including HIV testing and treatment
- Family based interventions
- Implementation of interventions through popular opinion leaders

Conclusion

Migrant laborers appear to be at elevated risk of HIV infection due to various individual-level and structural-level factors that may exist before, during, or subsequent to the actual process of migration. Female labor migrants may be at particularly high risk of HIV infection.

Despite the growing literature indicating an increased risk of HIV, relatively little empirical research has been conducted to identify effective HIV prevention programs. Intervention strategies for this population may be most effective if they focus on both individual and structural factors.

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HIV Prevention and Women

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Definition

HIV prevention among women involves understanding social risk factors (e.g., relationship power, gender-based violence), cultural risk factors (e.g., gender norms and expectations), and biological risk factors (e.g., anatomy) that are unique to women. It is paramount to also consider contextual issues, such as the social and structural determinants that vary within and across populations and settings and can either support or impede HIV prevention strategies. Biomedical, behavioral, and structural interventions have been developed and implemented to prevent acquisition of HIV infection among women.

Disproportionate Burden of HIV Among Women

An estimated 16 million women aged 15 or older are living with HIV around the world (Joint United

Nations Programme on HIV/AIDS 2014). Globally, women represent approximately half of the global adult population living with HIV. While HIV remains a global epidemic, approximately 80% of the cases among women occur in sub-Saharan Africa, where young women between the ages of 15 and 24 have twice the prevalence (a count or percentage of the number of people with the disease in a given population at a specific point in time) of HIV as young men in this same age group, and they acquire HIV infection 5–7 years earlier than their male counterparts (Joint United Nations Programme on HIV/AIDS 2014). Further, women of childbearing age are disproportionately affected, and heterosexual sex is the primary mode of HIV transmission in this region. In other regions, such as Eastern Europe and Central Asia, injection drug use is a common mode of HIV transmission disproportionately affecting women, and women who inject drugs have higher HIV prevalence compared with their male counterparts (Burns 2009; Pinkham et al. 2012). Because of various social, biological, and cultural factors, women living in low- and middle-income countries, in particular, often have additional contextual issues related to their HIV risk (Joint United Nations Programme on HIV/AIDS 2014). Consequently, it is essential that HIV prevention strategies address individual and interacting environmental factors.

Contextualizing HIV Prevention for Women

HIV infection among women and associated risk behaviors do not occur in a vacuum. HIV risk involves a complex intersection of multiple biological, social, and cultural determinants (El-Bassel et al. 2012; Wechsberg et al. 2015).

Women are biologically more susceptible to HIV infection through heterosexual sex (World Health Organization 2009b), and this vulnerability is compounded by social and cultural factors, particularly for women most at risk for infection. In many societies, gender norms perpetuate stigma and discrimination against women who may engage in risk behaviors such as transactional

sex (the exchange of sex for money or goods) or alcohol and other injection or noninjection drug use (Pinkham et al. 2012). Women at highest risk of HIV infection commonly face gender inequality and experience the resulting disempowerment that often places them in vulnerable circumstances without agency to negotiate safer sex practices and to protect their well-being (Wechsberg et al. 2015). Gendered stigmatization, inequality, and disempowerment can also restrict women from accessing necessary HIV prevention services, which is often compounded for key populations by restrictive legal environments penalizing or criminalizing high-risk behaviors such as injection drug use and sex work (Pinkham et al. 2012).

While these issues are important for HIV prevention, it is also imperative to consider how men are vulnerable as a result of gender norms and other factors, particularly in places where the HIV epidemic is generalized (Higgins et al. 2010). Consequently, effective HIV risk-reduction interventions need to address these multilevel social, cultural, and biological factors.

How HIV Prevention Can Address Women's Vulnerabilities

Biomedical Prevention

Studies have shown that women are biologically more susceptible to HIV infection through heterosexual transmission compared with their male counterparts (World Health Organization 2009b). Factors such as the relatively large surface area of the vagina exposed to semen, high viral load (the amount of HIV) in the semen, and susceptibility of tissue tear during dry vaginal or anal sex contribute to a heightened risk of infection (Canadian AIDS Society). Consequently, biomedical advances in HIV prevention must address the anatomical and biological susceptibility of women to HIV and provide women-controlled methods of protection.

Oral Preexposure Prophylaxis (PrEP)

Preexposure prophylaxis (or PrEP) is when people at very high risk for HIV take HIV medicines daily to lower their chances of acquiring HIV.

Truvada [tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC)] is a combination antiretroviral (ARV) medication for PrEP that was approved for use in adults at high risk for HIV by the US Food and Drug Administration (FDA) in 2012 (Centers for Disease Control and Prevention 2012). Individuals at high risk for HIV, such as women who are HIV negative whose partners are HIV positive, can take PrEP daily to reduce the chances of acquiring HIV infection. Currently, the United States is the only country that has approved TDF/FTC as oral PrEP.

The US Centers for Disease Control and Prevention (CDC) released guidelines for the use of PrEP to prevent HIV infection and recommended it to individuals at high risk of acquiring HIV infection, including women whose partners are HIV positive, women who have had recent bacterial sexually transmitted infections (STIs), and women who engage in high-risk behaviors such as having multiple sex partners and inconsistent condom use (Centers for Disease Control and Prevention 2014). Twenty-two other countries, including the United Kingdom, South Africa, Brazil, and India, are exploring the provision of PrEP through pilot projects (AVAC). Although the United States is the only country to approve the use of PrEP, the World Health Organization (WHO) recommended the use of PrEP for people with substantial risk of HIV infection (World Health Organization 2015). As an intervention that can be woman-initiated and woman-controlled, PrEP can empower women to take control of their sexual health (Kofman and Adashi 2014).

Treatment as Prevention (TasP)

Recently, research has shown that when individuals living with HIV are initiated on antiretroviral therapy (ART) early on and they are adherent to treatment, they are significantly less likely to transmit HIV. This approach, known as treatment as prevention (TasP), has been shown to prevent HIV transmission by up to 96% in the HPTN 052 (Cohen et al. 2011). TasP has essentially bridged the divide between treatment and prevention by demonstrating that treatment “is” prevention. Consequently, promotion of early initiated and sustained ART for people living with HIV, particularly for men when

prevention among women is concerned, has significant potential to curb the number of new HIV infections among women and men.

Additionally, TasP strengthens the earlier termed “test and treat” strategy, which was based initially on the idea of treating people for HIV and then subsequently linking those who test positive to appropriate medical treatment while also engaging them in other interventions to reduce their risk behavior (Kurth et al. 2011). The science now demonstrates that testing and linking persons who are HIV positive to treatment not only reduces risk behavior but also suppresses their virus level, thereby reducing the risk of transmission.

Microbicides

Microbicides are chemical compounds that can be applied inside the vagina or rectum prior to sexual intercourse to protect against STIs, including HIV. Microbicides are most commonly produced in a gel form but can also be creams, films, suppositories, or vaginal rings (Microbicide Trials Network 2012; World Health Organization n.d.). Although microbicides have shown promise in preventing HIV transmission, testing is ongoing and microbicides are not currently on the market for HIV prevention.

Research is currently focused on ARV-based microbicides, specifically tenofovir gel. The initial groundbreaking study, CAPRISA-004, was conducted in South Africa and demonstrated a 39% reduction in new HIV infections when the tenofovir gel was applied vaginally prior to sex (2015; Kofman and Adashi 2014; Mastro et al. 2014). Subsequent studies using tenofovir gel microbicides have not demonstrated the same level of efficacy, although this could be due to inconsistency in use or other factors. Currently, there is an ongoing study, CAPRISA-008, which is reexamining the efficacy of vaginal tenofovir gel (Kofman and Adashi 2014; Mastro et al. 2014).

Behavioral Prevention

The Importance of Male and Female Condoms in Combination with Behavioral Prevention

The promotion of barrier methods, such as the male latex condom and female condom, has been an integral part of behavioral interventions

for women (Centers for Disease Control and Prevention 2015a). A common issue raised about the male condom is that if a woman has a male partner and is trying to get him to use a male condom, it involves negotiation of condom use with the partner, whereas the female condom is a woman-controlled HIV prevention strategy.

The female condom can be inserted vaginally hours before sex, and in certain cases it can be used without the knowledge of a sex partner. The original female condom (FC1), manufactured by the Female Health Company, was introduced in the mid-1990s, but failed to gain the same popularity as the male condom because of issues of high cost and limited acceptability (World Health Organization, Department of Reproductive Health and Research 2007a). A less expensive, second-generation female condom (FC2) was introduced by the Female Health Company in 2005, and it was approved by the US FDA in 2009. Other companies also have developed new designs for the female condom to address issues of cost and acceptability (World Health Organization, Department of Reproductive Health and Research 2007b). To assist with cost and accessibility issues, some health departments provide female condoms free of charge.

HIV prevention behavioral interventions have been developed for women around the world. Rigorous review and evaluation of US-based behavioral interventions have categorized some interventions as “best” or “good” evidence interventions (Centers for Disease Control and Prevention 2015b). Such evidence-based interventions (EBIs) have been efficacious in reducing the incidence (the number of new cases in a given period of time, such as a year) of STIs and/or reducing HIV risk behaviors among women, such as unprotected sex, number of sex partners, and injection drug use (Lyles et al. 2007).

Typically, these behavioral interventions are grounded in a health behavior theory or framework, occur over multiple sessions, and give women the knowledge and skills to take control of their sexual health by reducing risk behavior. While EBIs for women vary in program content and how they are administered, they often use multiple methods and activities for intervention delivery. Common methods and activities include

discussions, role-play, practice, goal setting, and lecture. These components allow for program participants to apply the information to their own lives by building essential skills and strategies for HIV prevention, such as male and female condom use skills and negotiation. Some interventions, known as peer-based interventions, are delivered in group settings and encourage a peer support system. Other interventions use individual sessions to focus on personalized risk-reduction methods.

Behavioral interventions that have reduced HIV-related risk behavior among women are tailored to the cultural and social context of the women being served and take into account the intersection of various HIV risk behaviors, such as alcohol or other drug use, intimate partner violence, age-disparate relationships, inconsistent and incorrect condom use, and concurrent sexual partnerships. Many interventions work to eliminate gender-based inequalities that render women vulnerable to HIV infection. Such interventions may assist women in finding employment, give them skills to maintain healthy relationships, or work to improve their economic status (Centers for Disease Control and Prevention 2015a). Some of these EBIs have been packaged for further testing, dissemination, and sustainability or have been adapted to other key populations of women in the United States and other countries (Saleh-Onoya et al. 2009; Wechsberg et al. 2010).

In addition to the EBIs for women that are administered individually or in single-gender groups (i.e., only with women), some EBIs include women’s sex partners so that HIV prevention can be addressed among couples. Such interventions provide a unique opportunity to address not only sex risk behaviors but also other factors related to HIV risk such as alcohol and other drug use, intimate partner violence, concurrent partnerships, and gender roles (El-Bassel et al. 2012). These interventions are geared toward changing social and cultural norms within a partnership and fostering an equal balance of sexual responsibility in couples. Interventions involving men are also helpful in changing gender norms related to masculinity, fatherhood, and sexual responsibility in a way that is conducive to female empowerment (World Health Organization 2009b).

One general criticism of some behavioral interventions is that outcomes have shown reductions in only proxies for HIV infection, such as acquisition of STIs and/or sex risk behaviors, but not reductions in the incidence of HIV infection. However, when studies measure HIV incidence, it can be difficult to observe significant changes, particularly in areas with low HIV prevalence.

Structural Prevention

Human behaviors, including those that put individuals at risk for acquiring HIV infection, are embedded in a larger context encompassing social, cultural, economic, legal, and political environments (Hardee et al. 2014; Parkhurst 2013). Consequently, structural HIV prevention interventions address these realms in an effort to create enabling environments so that individuals are able to fully benefit from other biological or behavioral HIV prevention efforts. Structural methods of HIV prevention are essential because they provide the basis for long-term change in society.

When considering HIV prevention among women, it is important to address the unique challenges faced globally by many women who are at risk for acquiring HIV infection. Globally, gender inequality and related gender norms, violence against women, and legal norms unfavorable to women have been identified as significantly contributing to the risk of women acquiring HIV infection (Hardee et al. 2014). Increased educational opportunities, decreased violence against women, increased employment and income-generation activities, and the promotion of gender equality have demonstrated a positive impact on decreasing HIV risk (Edwards and Collins 2014; Fieno and Leclerc-Madlala 2014; Hardee et al. 2014).

Many times, cultural expectations prescribe gender roles that encourage male superiority and female subservience. Such gender-based discrimination prevents women from being afforded the same rights as men and can increase their vulnerability to acquiring HIV infection (Hardee et al. 2014). Women in societies with greater gender inequality are more likely to be subject to gender-based violence. This can make it difficult to negotiate safer sex. Also, violence or fear of violence can be an impediment to HIV testing

(Hardee et al. 2014). Inequality in educational attainment and income-generation activities in particular are attributed to higher HIV risk among women. Economic dependence on men can motivate women to engage in alternative risky income-generation activities such as transactional sex or intergenerational sex.

Many countries have laws that criminalize high-risk behaviors such as prostitution or injection drug use. However, such restrictive laws make it difficult for women who are most in need of HIV prevention services to access these services (Hardee et al. 2014; Pinkham et al. 2012). Other laws and policies that limit women's subordinate role can also have a negative impact on prevention efforts. For example, bride and inheritance rules (including those that prohibit women from filing for divorce), laws denying women the right to own property, and policies restricting women from equal access to services (such as opening a bank account) disempower women and can make it difficult to take control of their own health and well-being or to negotiate safer situations (Edwards and Collins 2014; Hardee et al. 2014).

Structural prevention interventions address the abovementioned issues through both broad macro-level and individualized microlevel factors that perpetuate HIV risk among women (Parkhurst 2013). Structural prevention efforts focused on macro-level factors work to tackle national level policies and laws as well as economic and other structures that at the national or subnational level prevent full uptake of HIV prevention efforts (Parkhurst 2013). International institutions such as WHO, World Bank, US Government's Global Health Initiative, and UNAIDS have dedicated much time and effort to HIV issues. Recently, these institutions have dedicated specific support to gender-based or woman-focused HIV prevention. Generally, they view barriers to HIV prevention for women through human rights lens and work to promote gender equality, to decrease gender-based violence, and to increase women's access to medical, social, and legal services (Women Won't Wait Campaign 2010).

For example, WHO has developed a tool to assist the health sector in integrating gender into HIV/AIDS programs. UNAIDS has drafted an

“Agenda for Accelerated Country Action for Women, Girls, and Gender Equality for HIV” that works to empower women at the national level. The US Government’s Global Health Initiative has invested in interventions that respond to gender-based violence. Additionally, the World Bank has supported initiatives to increase women’s employment opportunities and decrease poverty (Women Won’t Wait Campaign 2010; World Health Organization 2009a).

Despite these efforts, specific macro-level structural barriers to HIV prevention for women remain and need to be addressed. Consequently, countries are now including women in their national HIV strategies. For instance, of 170 countries reporting in 2012, 81% included women in their HIV strategies. However, only 41% of these 170 countries budgeted specifically for woman-focused HIV programs (UNAIDS 2012).

Structural prevention addressing microlevel factors affecting women, such as limited education or economic dependence, also has potential for influencing reductions in HIV risk behaviors and incidence (Fieno and Leclerc-Madlala 2014; Parkhurst 2013). Two prevalent types of structural prevention interventions addressing microlevel factors are microcredit financing programs and conditional or unconditional cash transfer programs. Microfinancing interventions that provide loans for women to establish businesses have demonstrated potential to economically empower women, making them less likely to engage in risky alternatives to generate income (Edwards and Collins 2014; Hardee et al. 2014).

Several countries have implemented pilot conditional and unconditional cash transfer interventions in efforts to reduce HIV vulnerability, particularly among girls and young women (Fieno and Leclerc-Madlala 2014). Unconditional cash transfer interventions are aimed at decreasing financial burden and improving women’s economic independence, thereby mitigating structural barriers to care and treatment, such as food insecurity or inability to attain education. Pilot interventions have demonstrated the potential of unconditional cash transfers in reducing sex risk behaviors that are perpetuated by economic dependence, such as transactional and

intergenerational sex (Fieno and Leclerc-Madlala 2014). Conditional cash transfers are financial incentives provided to young women and their families for fulfilling certain behaviors, such as young women staying in school (Doetinchem et al. 2008). For example, by providing incentives for school attendance, these interventions aim to increase educational attainment, thereby empowering women to make positive decisions about their health and life, such as decreasing the likelihood of early marriage, sexual activity, and pregnancy (Baird et al. 2010).

Although there have been some successes, issues of scalability for broad impact and cost for value – for example, are there other interventions that involve the same cost but have greater impact – need to be assessed further.

Conclusion

Research on women’s increased social, cultural, and biological susceptibility to HIV has identified gender-based violence, limited social mobility, economic inequality, intersecting risk behaviors such as alcohol or other drug abuse, and limited education as contributors to HIV risk. Despite the fact that strong research findings have moved HIV prevention for women onto the agendas of some nongovernmental organizations, health departments, national governments, and international health organizations, greater action is needed to incorporate a gender focus into all HIV risk-reduction policy and practice to effectively reduce HIV infection in women globally.

To capitalize on the strengths of biomedical, behavioral, and structural HIV prevention efforts, recent interventions are integrating these approaches in what is termed “combination HIV prevention” (Parkhurst 2013). In order for HIV prevention efforts to have lasting impact, it is crucial that they are multipronged and address the individual, social, and structural barriers to HIV prevention. Additionally, such efforts should take into account women across the HIV care continuum – from early prevention with key populations to treatment for women living with HIV. Taking the biomedical, behavioral, and

structural aspects into consideration can help to facilitate effective HIV prevention among vulnerable women.

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HIV Prevention Efforts Within Substance Use Disorder Treatment Settings

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Definition

Substance use disorders treatment agencies serve individuals known to be at high risk for engaging

in HIV risk behaviors. To target this group, these facilities are ideal settings in which to administer HIV prevention interventions.

Substance use disorder (SUD) treatment facilities represent a promising venue in which to implement behavioral HIV prevention interventions. Gathered within these clinics is an appropriate target audience for HIV prevention given that individuals struggling with substance use disorders often engage in risky drug use and sexual behaviors. Their status as treatment clients theoretically implies that they are working on substance use issues (Substance Use) and ideally making substantial life changes. Reflection on current behaviors can be an important part of recovery and therefore an appropriate time to introduce HIV prevention interventions. The following discussion will further flesh out the reasons why substance use disorder treatment agencies are an ideal setting for HIV prevention, review existing evidence-based HIV prevention interventions targeting substance use disorder treatment clients, and discuss the HIV prevention services typically offered in such settings.

Treatment Settings as a Platform for HIV Prevention Interventions

Substance use disorder treatment facilities by their nature capture a particular group of individuals at higher risk for engaging in behaviors that put them at increased risk for HIV. Out-of-treatment injection drug users (IDUs) have been shown to engage in high levels of risky drug use behavior (e.g., using contaminated injection paraphernalia; Strathdee et al. 2001) (► [Harm Reduction for Injection Drug Users](#)). Individuals struggling with addiction have been shown to engage in risky sexual behavior (Raj et al. 2007). In particular, the stimulant users in this sample had substantial rates of reporting multiple partners in the past 6 months, inconsistent or no condom use; this group also reported high rates of exchanging sex for drugs or money. While trading sex for drugs or money may not be inherently risky, coupling this behavior with other sexual risk factors raises the risk profile (Sex Work and Sex Workers). Once

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admitted to treatment, risk behavior may subside for a proportion of clients (Drug Treatment as HIV Prevention). That said, the most compelling changes made in the context of treatment are typically in substance use behavior and injection practices, whereas sexual risk behavior may or may not decrease as a result of drug treatment (Sorensen and Copeland 2000). In a focus group of SUD counselors, continuation of risky sexual behavior in the context of recovery was understood as an impediment to successful recovery; the perspective of these counselors was treatment seekers are at high risk for engaging in sexual risk behavior and that these behaviors are most assuredly a relevant treatment issue. Research studies show that treatment-seeking samples exhibit widespread sexual risk behavior in men (Calsyn et al. 2010) and women (► [HIV Prevention and Women](#)) (Engstrom et al. 2011; Tross et al. 2008). These studies, all of which use SUD treatment-seeking samples, take place in an array of settings (i.e., opioid treatment programs, outpatient, and residential) and demonstrate the need for HIV prevention efforts across a variety of treatment modalities.

SUD treatment facilities may be a place where people seek help specifically for alcohol and drug issues; however, the format is such that clients can easily be provided with or exposed to HIV prevention material. Clients typically meet regularly with counselors, who are in an ideal position to provide HIV prevention intervention, since they can do so in an ongoing way and there is already a clinical relationship established between counselor and client. Counselors may be in an ideal position to understand a client's current HIV risk behaviors and help their clients contemplate and facilitate change in HIV risk behaviors in the context of the client's history and current circumstances and ongoing patterns (Brown 2000).

SUD treatment seekers not only represent a captive audience to which HIV prevention interventions can be targeted, they are also active participants in the treatment process, many of whom are already reflecting on problematic behaviors in multiple domains of their lives. Engaging people as they enter treatment may be the ideal time to motivate individuals to adopt

safer sex practices. As SUD treatment clients begin recovery, they may begin to consider changing aspects of their lives that put their recovery at risk, including unsafe sexual behavior. Evidence indicates that HIV sexual risk behavior is something women in particular may want to change (Brown et al. 2005). Specifically, researchers found that readiness to change sexual risk behavior was significantly related to initial readiness to seek help for substance use problems among women in a community-based outreach program focused on preparing women for entry into substance use disorder treatment. Findings suggest that a woman's readiness to seek treatment may provide an opportunity to capitalize on readiness to change sexual risk behavior as well. It should be noted that the same conclusions cannot be made about men since the above mentioned study was conducted with women. Nonetheless, both treatment-involved men and women appear to be open to HIV prevention interventions based on changes in sexual risk behavior exemplified within these settings (Metzger and Navaline 2003a, b). Given that treatment settings capture an at-risk population that may exemplify readiness to change HIV risk behaviors, it makes sense to leverage existing SUD treatment agencies to increase the reach of HIV prevention interventions.

Evidence-Based HIV Prevention Interventions Targeting SUD Treatment Clients

Embedding HIV risk prevention interventions within existing SUD treatment programs has garnered attention by researchers and clinicians leading to development of a growing and robust evidence base. Three meta-analyses examining behavioral HIV prevention interventions among drug users have indicated that such interventions are effective at reducing injection and drug use behaviors, and to a lesser extent sexual risk behaviors (Prendergast et al. 2001; Semaan et al. 2002; Copenhaver et al. 2006). The interventions cited are often based on one of several behavioral change theories applied to HIV prevention interventions (Behavior Change). Prendergast

et al. (2001) included 18 studies of HIV prevention interventions conducted within SUD treatment settings; they found such interventions are effective in overall risk reduction and for sexual behavior risk reduction. Intervention effectiveness was associated with intervention intensity, number of therapeutic techniques utilized, and, to a lesser extent, total number of hours. Sexual risk behavior reduction was associated with having separate sessions for men and women, including coping skills training and peer group discussions. The Semaan et al. (2002) meta-analysis focused on effectiveness of HIV preventions targeting drug users at reducing sexual risk behavior (decrease in number of unprotected sexual events or increase in condom use). They included 33 studies with most targeting injection drug users (94%) or crack users (21%). Only 15% of the studies reported conducting the intervention within a SUD treatment setting. These researchers also determined that interventions can lead to reductions in sexual risk behavior among drug users. The meta-analysis conducted by Copenhaver et al. (2006) included 37 randomized controlled trials examining 49 interventions and focused on injection drug users. Participants were recruited from SUD treatment settings in 53% of the studies. Most interventions included the following components: HIV/AIDS education (90% of interventions), condom use skills (69%), self-management skills (e.g., coping with drug cravings; 57%), and both drug-related and sex-related risk reduction (70%). They found that compared to controls, intervention participants reduced injection drug use (IDU) and non-IDU, increased drug treatment entry, increased condom use, and decreased trading sex for drugs. Interventions were more successful at reducing IDU when participants were non-Caucasians, when content focused equivalently on drug-related and sex-related risks, and when content included interpersonal skills training specific for safer needle use. Overall, these meta-analyses validate the appropriate use of HIV prevention interventions with treatment-seeking individuals with substance use disorders.

Subsequent to the three meta-analyses the National Institutes on Drug Abuse (NIDA)

Clinical Trials Network (CTN) conducted two randomized clinical trials of HIV prevention within SUD treatment settings building on the knowledge gained from the prior work cited in these meta-analyses. The Real Men Are Safe (Calsyn et al. 2009) and the Safe Sex Skill Building for Women (Tross et al. 2008) were five-session gender-specific interventions targeting sexual risk behaviors. The interventions employed multiple techniques, didactic lectures, peer group brainstorming, male and female condom skill practice (► [Female Condoms](#) and ► [Male Condoms](#)), communication/negotiation skills role plays, and motivational exercises. The interventions were conducted by two counselors employed at the treatment programs as cofacilitators. Both “Real Men Are Safe” and “Safer Sex Skills Building for Women” were shown to be effective in reducing the number of unprotected sexual occasions (USO; vaginal or anal intercourse without a male or female condom) compared to a standardized HIV prevention educational intervention for both women and men, although the pattern of risk reduction differed by gender (Calsyn et al. 2009; Tross et al. 2008). Women randomized to the “Safer Sex Skill Building” intervention and women in the control HIV intervention had a similar significant decrease in the number of USO at the 3-month follow-up compared to baseline. For men, however, only those randomized to the “Real Men are Safe” intervention significantly decreased their USO at the 3-month follow-up. Both women and men who were randomized to “Real Men are Safe” or “Safer Sex Skill Building,” respectively, reported significantly greater reductions in the number of USO at 6-month follow-up compared to participants in the control HIV educational interventions.

Although the interventions for “Real Men Are Safe” and “Safer Sex Skills Building for Women” have many similarities, such as providing basic information about HIV and behaviors to prevent transmission as well as development of communication skills related to sexual situations, they are gender-specific HIV prevention interventions. In “Real Men Are Safe” there is a focus on the interplay between sex and drugs, a focus on

recognizing partner needs and the role played by society's "gender roles," the importance of accepting responsibility for one's own behavior is stressed, and the use of assertive communication skills and "I" statements in safe sex negotiations. In "Safer Sex Skills Building for Women," there is a focus on increasing self-efficacy, decision-making skills, negotiation and refusal skills, and skills for recognizing risk of partner abuse and preemptive safety planning in the context of sexual relationships with substance-using men. Consistent with the emphasis on reducing sex under the influence, men assigned to the Real Men Are Safe intervention were less likely to have engaged in sex under the influence of drugs or alcohol during their most recent sexual event prior to the 3-month follow-up assessment than men assigned to the control intervention (Calsyn et al. 2010). "Real Men Are Safe" and "Safer Sex Skills Building for Women" have been placed on the Centers for Disease Control and Prevention (CDC) HIV/AIDS Prevention Research Synthesis (PRS) Project website as evidence-based practices (CDC 2012). Initiated by the CDC in 1996, the purpose of PRS is to systematically review and summarize HIV behavioral prevention research literature with the goal of translating scientific evidence into practical information that can be used by prevention providers, state and local health departments throughout the United States, and HIV prevention researchers. To gain acceptance within the compendium, interventions must be formulated from established psychological and sociological theories and tested in rigorous, randomized controlled trials. It is clear that clinical researchers appreciate SUD treatment settings as an ideal platform for HIV intervention delivery, and they have generated a multitude of interventions, some of which have demonstrated a strong evidence base.

HIV Prevention in SUD Treatment Settings: Standard Practice

State and federal agencies recognize the value in promoting HIV prevention in SUD treatment settings. As one of its principles of effective

treatment, the National Institutes on Drug Abuse (NIDA) suggests that SUD treatment programs should assess clients for the presence of HIV/AIDS, as well as "provide targeted risk-reduction counseling to help patients modify or change behaviors that place them at risk of contracting or spreading infectious diseases" (NIDA 2009, p. 5). Guidelines regarding the implementation of HIV prevention in SUD treatment agencies appear to be – for the most part – observed, according to administrators and directors of relevant agencies. Brown et al. (2007) surveyed 269 agencies across the United States examining available services and related state policies and funding associated with HIV and other infections in SUD treatment settings. They found that most agencies (88–90%) offer HIV/AIDS education and risk assessment, even those without state guidelines, policies, regulations, or reimbursement.

While agencies report offering HIV prevention services, it is less clear what these services actually look like. One specific area emphasized within the literature is on HIV counseling and testing (C&T) (► [HIV Testing and Counseling](#)) in outpatient SUD treatment (Pollack et al. 2010). These investigators have found that the rate of agencies conducting HIV C&T increased significantly in the 1990s and more modestly in recent years to a total of 28.8% of agencies in a national sample. Other researchers have attempted to gauge the kinds of HIV prevention services occurring in SUD treatment settings more generally. Researchers in the NIDA Clinical Trials Network (CTN) conducted a "snapshot" survey of 65 agencies participating in the CTN to determine more details about the HIV services provided (Shoptaw 2001). Most agencies (73.8%) reported that formal HIV/AIDS assessment was conducted upon admittance to the agency, ranging from HIV counseling and testing to referral to outside community testing sites for these services. A much smaller percentage of programs (28.3%) reported conducting outreach to drug-using groups at high risk for HIV transmission. Finally, the snapshot indicates that 82.8% of agencies reported that AIDS education is provided to clients on site and upon admission. For most programs (85.4%) the

amount of education provided ranged from 30 to 90 min delivered in a single group or individual session. The bulk of the education delivered was limited to providing basic information about HIV and risk behaviors associated with its transmission. Skill training interventions, using tools such as role-plays and practice of putting condoms on models, were infrequent. Overall, the results from the “snapshot study” go against the findings from the three meta-analyses cited above that indicated that more effective HIV prevention interventions with individuals struggling with substance use disorders consist of multiple sessions, employ a wide variety of techniques, and have separate sessions for men and women. Instead, agencies involved in the snapshot study provide only a single session and use information-focused interventions. Furthermore, the HIV/AIDS educational and prevention programs provided are not standardized, resulting in an array of session format, content, and timing. This lack of standardization in services offered, along with other evidence demonstrating that treatment programs do not always offer the specialized services they claim to (Cochran et al. 2007), suggests the need to examine barriers to implementing HIV prevention services in SUD treatment settings.

Implementation of HIV Prevention Interventions in SUD Treatment Settings

SUD treatment clinics have been slow to adopt evidenced-based HIV prevention interventions despite empirical support, and implementation appears to be hindered both at the organizational and individual clinician levels. Campbell et al. (2011) and Calsyn et al. (2012, CTP representative survey – REMAS-CA, Unpublished) demonstrated a clear disconnect between the availability of evidence-based HIV prevention interventions and adoption of these interventions by SUD treatment facilities in their surveys of clinics participating in the CTN. Specifically, these researchers examined the extent to which these evidence-based interventions were adopted by clinics in the “Real Men Are Safe” and “Safer Sex

Skills Building for Women” original studies, as well as clinics involved in the CTN, but did not participate in the original studies. Both surveys found that only one program had fully adopted “Real Men Are Safe,” while three continued to use materials from the trial, mostly the information/educational materials rather than the motivational or skill-building materials. The main reasons given for not adopting “Real Men Are Safe,” were: lack of staff time (54%), competing treatment priorities (52%), need for additional funding (44%), or a mechanism for reimbursement (42%) (Calsyn DA, Hatch-Maillette MA, Burlew AK, 2012, CTP representative survey – REMAS-CA, Unpublished). Similarly, Bini et al. (2011) surveyed SUD treatment program administrators and counselors to determine barriers in providing HIV and STI health services (including prevention efforts). In addition to funding issues, the authors identified client acceptance and staff training as being important barriers to delivering these services.

At the individual level, counselor discomfort with the material may prohibit adoption of HIV prevention interventions. For example, Tracy et al. (2009) surveyed SUD treatment clinicians regarding opinions and the delivery of services for STIs and found that clinicians were more likely to provide more of these services if they were more comfortable discussing sexual and intimate relationship issues. SUD treatment patients may respond better to HIV prevention interventions administered by counselors more comfortable with the material, in particular if counselors take a nonjudgmental stance (Brems and Dewane 2007).

Conclusions

SUD treatment facilities are an ideal venue in which to administer HIV prevention interventions, not only because the people served in such settings exemplify high risk behavior but also because they receive ongoing care and interface regularly with counselors. Clinical researchers have responded to this need by generating and testing interventions, which are in accordance with well-known models of behavior change.

The wealth of research in this area has resulted in the identification of several evidence-based behavioral interventions. Of those examined in the context of existing SUD treatment programs, “Real Men Are Safe” and “Safer Sex Skill Building for Women” are among those that enjoy the strongest degree of empirical support. While these have been shown to be effective, implementation of evidence-based HIV prevention interventions has been hindered by a number of barriers, including cost to clinics, motivation, counselor desire, and skill. To ameliorate some of these barriers more resources should be allocated to clinics attempting to implement these interventions. Furthermore, interventions could be shortened, and counselors may be encouraged to talk with their patients in an ongoing way about risk reduction, instead of having intervention be a circumscribed event. Integrating HIV prevention interventions into SUD treatment should be a priority in order to create a safe and accepting environment in which clients can engage in conversations about sex and sexual risk reduction in the context of recovery.

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HIV Prevention for MSM

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Definition

While globally the number of newly HIV-positive people continues to fall, this is not the case for men who have sex with men (MSM) (Beyrer et al. 2012; UNAIDS 2012), who continue to be severely affected. HIV prevalence estimates among MSM range from 3% in the Middle East to over 30% in the Caribbean (UNAIDS Caribbean Regional Support Team 2010). In nearly

every country for which information is available, the odds of contracting HIV are higher among MSM than among men in general, sometimes by more than 30 times. This is the case even in most countries in sub-Saharan Africa – a region that was long believed to have an exclusively heterosexual epidemic. Studies suggest that HIV prevalence continues to rise among MSM for specific subgroups or in some geographical areas. In the USA, for instance, it is estimated that new HIV infections in this population have increased by 8% per year since 2001. This dire situation can be understood as a consequence of a variety of factors, including the high HIV transmission rate per sex act for anal intercourse – estimated to be about 18 times higher than for vaginal intercourse. In addition, the global response to the epidemic among MSM thus far has been insufficient, largely as a result of antigay stigma and, in some countries, the criminalization of homosexuality.

MSM: Diverse Category

The use of the label “MSM” to identify this population has become common practice since the late 1980s. The label was introduced in the context of public health to acknowledge that not all men who had sex with men are identified as “gay.” The label categorizes a very diverse group of persons who, as a single feature, have in common that they were identified at birth as biological male and have sex with other male-bodied persons. MSM is in itself a label that men do not usually identify with. It also leaves open how men understand and organize their sexual lives and whether they sexually identify in a specific way and, if so, how they identify. The label encompasses gay-identified men, Moffies in South Africa, Hijras in India, Kuchus in Eastern Africa, but also straight-identified men who sell sex to other men, men who have sex with men “on the down low,” men who have sex with men in prison, men who exclusively have sex with other men, and men who have sex with both men and women. Also included in this category are biologically born males who no longer identify as such, male-to-female transgender persons. The MSM

label has been criticized because it ignores cultural differences between the various groups, deflecting attention away from the “social dimensions of sexuality that are critical in understanding sexual health. . . [and obscuring] elements of sexual behavior that are important for public health research and intervention” (Young and Meyer 2005, p. 1144). Nonetheless, the term continues to be used by major stakeholders in the global response to HIV. In order to be effective, HIV prevention efforts should, however, acknowledge the diversity of men captured by the label.

Various Kinds of Risk

Not all MSM are at the same risk of acquiring HIV. This is clearly illustrated by the dramatic disparity between Black MSM and MSM of other ethnicities in the USA, where Black MSM are three times more likely to be HIV positive (Millett et al. 2012). Research aimed at understanding risk has focused mainly on engagement in unprotected anal intercourse (UAI) – the primary mode of transmission among MSM. Factors for risk of HIV infection can be distinguished on various levels, such as individual and structural, and do not necessarily have universal relevance. It is also important to note, for the purpose of developing prevention strategies, that some of these factors are more amenable to change than others.

At the individual level, knowledge, attitudes, beliefs, skills, and self-efficacy – factors derived from health behavior models – have been shown to be related to sexual risk as well as to protective behavior (e.g., condom use for anal sex). Other individual-level factors associated with HIV risk behavior include mood states, personal history, and various personality characteristics. For example, depression has been found to be related to sexual risk practices, although findings are inconsistent. In terms of personal history, risk factors include traumatic experiences such as sexual abuse and intimate partner violence. Personality characteristics related to sexual risk include internalized homophobia (which can also be understood as a structural factor which is as a

consequence of antigay stigma in one’s society) and sexual compulsivity.

Individual connectedness to a gay community has been thought to be a protective factor, because it increases exposure to HIV prevention messages and services and offers social support. The evidence for this is, however, inconclusive. This might be because being connected to a community also increases access to sexual partners and exposure to factors that might increase HIV risk, such as permissive norms around drug use.

Other microlevel factors associated with HIV risk behavior include peer influence, romantic relationships, family acceptance, and support. Identifying a sexual partner as primary (as opposed to casual) has consistently been found to predict higher rates of UAI, suggesting that trust and love as primary qualities of intimate relationships impede condom use.

Other risk factors are related to sexual interactions themselves, with alcohol and drug use most consistently shown to be associated with UAI. While the exact nature of the association still needs to be clarified, evidence suggests that it is moderated by other situational variables such as partner type (the association between drug use and UAI is stronger with casual partners versus primary partners) and sexual role (some drugs are more strongly associated with receptive UAI than insertive UAI and vice versa). The context of sexual interactions also structures HIV risk among MSM: public sex venues such as sex clubs or bathhouses are strongly associated with risky behaviors.

HIV risk is also dependent upon with whom MSM have sex. For instance, the ethnic disparity in HIV infection in the USA is not explained by higher levels of risky practices in Black MSM. Rather, it is explained by the higher prevalence of HIV among Black MSM and the fact that Black MSM are more likely to have sexual partners that are Black (Millett et al. 2012). More generally, the importance of the role of social and sexual networks in risk behavior and the spread of HIV is being increasingly acknowledged and understood.

The most crucial among various structural factors associated with HIV risk include societal

rejection and criminalization of homosexuality. Other structural factors are low education, unemployment, and poverty. These factors are thought to work in a variety of ways, though their effects are difficult to measure. That structural factors play a role in the risk of HIV infection is, for instance, suggested by the higher HIV prevalence among MSM in Guyana and Jamaica, where homosexuality is criminalized, compared to the Dominican Republic and Suriname, where no such laws exist (UNAIDS Caribbean Regional Support Team 2010). Stigma and discrimination also affect ways that sexuality is expressed, e.g., they promote adoption of heterosexual marriage to hide oneself, increase the risk of blackmail, and promote transactional sex. As such, stigma and discrimination might contribute to risk or create barriers to access prevention and care.

The various risk factors do not necessarily work independently but could reinforce each other, as formulated in syndemic theory (Stall et al. 2008). Syndemic theory is concerned with culturally produced epidemics that reinforce one another in a particular population. It has been used to explain the high rates of concurrent psychosocial problems among MSM including substance use, sexual abuse, violence, and mental health conditions, which together increase HIV risk.

Responses to Risk Among MSM

Since the beginning of the epidemic, MSM have adapted their behavior by using condoms and reducing number of partners. Without this response, the epidemic would likely have had an even more disastrous impact. Informed by medical evidence, MSM further invented alternative strategies to reduce their risk of contracting HIV. The introduction of HIV testing facilitated serosorting: a practice by which MSM only have unprotected sex with partners with the same HIV status in order to avoid contracting HIV or transmitting HIV to a negative partner. Because serosorting is not always based on accurate information about one's own HIV status or that of sexual partners, the term "seroguessing" has been coined to illustrate the pitfalls of strategy

(Zablotska et al. 2009). Other strategies include withdrawal before ejaculation and strategic positioning, in which the HIV-positive partner takes the receptive position and the HIV-negative partner takes the insertive position, based on the understanding that insertive partners are less likely to contract HIV than receptive partners (Parsons et al. 2005). Although these strategies plausibly reduce HIV risk, they do not always eliminate it and might lead to exposure to other risks, such as HIV superinfection or infection with other STIs (Vallabhaneni et al. 2012).

Another risk reduction strategy employed by MSM is negotiated safety (Kippax 2002). This strategy, adopted in intimate relationships, involves abandoning condoms after both partners have tested HIV negative and an agreement to avoid UAI with partners outside the relationship (and maintaining that agreement). While theoretically protective, in practice, the strategy does not always work, with some men simultaneously making negotiated safety agreements and engaging in UAI with outside sexual partners.

Prevention Approaches and Strategies

There is a wide diversity of HIV prevention interventions, informed by various theoretical approaches (Glanz et al. 2008). These interventions can be broadly divided into two categories: individual-level and structural-level interventions (Cohen and Scribner 2000). Individual-level interventions primarily have effects on the individual level. They include behavioral interventions such as education and social influence strategies and biomedical interventions such as condom use, postexposure prophylaxis (PEP), and preexposure prophylaxis (PrEP). Individual-level interventions can be implemented on an individual as well as a group level, but are expected to have their effect primarily on the individual; these interventions can have a population-level impact if individual changes lead to decreased HIV incidence and a reduced viral load in the community.

Structural-level interventions target conditions outside the control of individuals. Such

interventions primarily have an impact on the population level but may also affect individual persons. For MSM, structural interventions include ensuring that condoms and lubricants are adequately available, influencing social norms via mass media, implementing formal or informal policies that require provision of condoms in sex establishments, and decriminalizing same-sex sexuality.

Numerous studies have demonstrated that HIV prevention programs effectively contribute to the promotion of safer sex behavior among MSM (Herbst et al. 2007; Johnson et al. 2008; Lorimera et al. 2013). A review of systematic reviews of behavioral interventions among MSM (Lorimera et al. 2013) has shown that the effectiveness of interventions varies. There is strong and consistent evidence that group- and community-level interventions are associated with substantial reductions in UAI and increases in condom use. The evidence regarding interventions addressing individuals is, however, less consistent. In general, interventions based on theory have been shown to be more effective than those that are not, specifically interventions that are based on diffusion of innovation theory or the model of relapse prevention. Group-level interventions that incorporate skills-building activities have also been shown to be particularly effective. Interventions can be strengthened by addressing co-occurring psychosocial risk factors. Interventions that ignore structural barriers to safer sex, such as homosexual stigma, are likely to be less effective.

Most interventions have been tested with samples predominantly composed of white MSM from high-income countries. If other MSM groups were involved, the measured effect of interventions has been found to be stronger among white MSM. It is not clear whether and how these interventions can be adapted to understudied subgroups, such as MSM of color, MSM who do not identify as gay, and MSM who use drugs, nor is it clear how they can be adapted to MSM diverse cultural contexts and in lower-income countries.

While many interventions have been designed and implemented for MSM, program coverage

continues to be low. Although governments increasingly recognize the needs of MSM and funding for HIV programs for MSM is increasing (in low- and middle-income countries primarily from international donors), programs that target MSM have not enjoyed high priority, and epidemiological surveillance among MSM has been inadequate (amfAR and Johns Hopkins Bloomberg School of Public Health 2012). Community organizations, supported by international agencies, bear the burden of implementing HIV programs for MSM, jeopardizing sustainability.

Conclusion

Promotion of safer sex practices will continue to be a major aim of future HIV prevention among MSM. The prevention landscape however changes continuously. First, the sexual behavior of MSM will continue to change. This happened, for instance, in response to the availability of antiretroviral treatment (ART), when MSM who believed that ART reduced HIV transmission became more likely to engage in unprotected sexual behavior. In addition, the Internet has increased opportunities for MSM to find sexual partners and is changing the ways in which men connect. The worldwide development of gay communities, partly stimulated by HIV prevention efforts, will have consequences for how MSM in low- and middle-income countries express their same-sex sexuality.

Also affecting the prevention landscape is the increasing number of strategies that become available to address the epidemic. PrEP, daily oral antiretroviral preexposure prophylaxis, approved in the USA by the FDA, is widely seen as most promising. There is evidence that PrEP is safe and has potential for substantial efficacy. However, with its strong reliance on adherence and need for medical follow ups, PrEP will be challenging to implement on a large scale. Also, several unanswered questions still remain, including the effect of pill taking on risk behavior, long-term safety of ongoing use, and how to best make PrEP available. Furthermore, PrEP, as most other biomedical approaches, includes a behavioral component that

needs preventive attention. When the effectiveness of less challenging PrEP regimens is established and other modes of delivery (e.g., by injection) prove to be effective, PrEP likely will be a major ingredient in future HIV prevention. Ethical issues, resulting from the fact that there is no universal access to HIV medication for those who are HIV positive, will need to be resolved.

The Internet and the growing popularity of social media have not only changed MSMs dating patterns but also greatly expanded opportunities to deliver evidence-based HIV prevention (Chiasson et al. 2010). While gay consumers seem to be among the earliest adaptors of new technology, the “digital divide” continues to shrink. Major advantages of the Internet and other digital media, such as cell phones, include the ability to tailor prevention efforts to the needs of individual men and the relatively low costs. One of the major barriers of these new strategies is attracting users.

In addition to the promotion of safer sex, early treatment of HIV has become a major way to prevent further spread of HIV. There is increasing evidence that early treatment improves health outcomes for the individual and also curbs the epidemic by reducing infectiousness. This strategy requires that MSM test regularly for HIV, are linked to care if HIV positive, and stay in care, resulting in suppressed viral load. Interventions are needed to promote frequent HIV testing and to ensure that barriers to accessing HIV care are reduced for MSM. A negative effect of such campaigns could be that HIV testing replaces safer sex behavior, a negative test result lowering perceptions of HIV risk.

There is a growing awareness that there is not one intervention strategy that will end the epidemic among MSM (Coates et al. 2008). A combination of approaches is needed not just to change MSM sexual practices but also to sustain accomplished changes. Combination approaches work best when employed synergistically. For instance, the protective effect of PrEP can be enhanced by combining it with interventions to promote adherence and prevent risk compensation. Approaches that integrate behavioral strategies with biomedical and structural

approaches are likely to be most effective. Current efforts to address the epidemic among MSM remain insufficient. Innovative science and political commitment are needed to effectively address the HIV epidemic among MSM.

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HIV Prevention for Serodiscordant Couples

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Definition

Introduction

HIV transmission in serodiscordant couples continues to fuel the spread of HIV worldwide, particularly in Africa, which has the largest share of HIV cases (UNAIDS 2010). Globally, 80% of new HIV cases among women occur through heterosexual transmission, most often from steady partners (UNAIDS 2010). Moreover, men who have sex with men (MSM) bear a disproportionately heavy burden of the HIV pandemic in low- and middle-income countries, and the majority of

sexually transmitted HIV cases among MSM also stem from primary partners (UNAIDS 2010).

Despite high rates of HIV transmission in heterosexual and MSM couples worldwide, research suggests that both sexual and drug use risks occur largely within a dyadic context between intimate partners, and few couple-based HIV prevention interventions have been tested and disseminated. In this entry, the authors define couple-based HIV prevention and describe couple-based HIV prevention approaches for serodiscordant couples including couple HIV counseling and testing (CHCT), HIV/STI risk reduction beyond CHCT, and adherence to ART and biomedical prevention strategies (ART, PrEP).

Defining Prevention for Serodiscordant Couples

HIV prevention for serodiscordant couples includes a combination of both behavioral and biomedical strategies. An HIV-serodiscordant couple is defined as a couple having an ongoing sexual and/or intimate relationship where one partner is HIV-positive and the other is HIV-negative. HIV prevention studies define who constitutes a “couple” in different ways. The majority of couple-based studies outside the United States (USA) define a couple as two individuals who are married or cohabiting; however, most studies in the United States define a couple by the length of time that the individuals have been together (usually at least 3–6 months). Couple-based behavioral HIV prevention strategies for serodiscordant couples include three major types: (1) couple HIV counseling and testing (CHCT), (2) couple-based HIV sexual and drug risk reduction beyond CHCT, and (3) couple-based adherence to antiretroviral therapy (ART).

Couple-based behavioral HIV prevention strategies may be delivered simultaneously to each member of the couple individually, to the couple together, or to a group of couples together (El-Bassel et al. 2010a, b). Couple-based behavioral HIV interventions are often guided by social cognitive theories and focus on providing information, motivation to reduce or eliminate risk behaviors, and technical, personal, or interpersonal skill building to reduce both sexual and drug-related risk

behaviors between partners (El-Bassel et al. 2010a). These interventions go beyond individual determinants of sexual and drug risk behaviors to include interpersonal dynamics and interactional forces (El-Bassel et al. 2010a). Biomedical couple-based HIV prevention approaches include two major types: (1) use of ART for HIV-positive partners and (2) pre-exposure prophylaxis (PrEP) for HIV-negative partners. These biomedical advances have been classified as “treatment as prevention” because early access to ART and PrEP has been found to significantly reduce the likelihood of HIV transmission among serodiscordant couples.

Couple-Based Behavioral HIV Prevention

Couple HIV Counseling and Testing (CHCT)

CHCT has been widely utilized in Africa and, more recently, in the United States. HIV testing is an important first step in identifying serodiscordant couples. CHCT involves providing informed consent for voluntary HIV testing to individual partners, testing a couple for HIV together, informing them of their test results, and providing a forum to ensure that both partners are aware of their HIV status and able to discuss ways to protect the HIV-negative partner, as well as to access HIV treatment and care for the HIV-positive partner and to identify and provide appropriate referrals to address psychosocial needs of HIV-affected couples. Using CHCT’s pre- and posttest counseling techniques, a trained counselor facilitates the disclosure of HIV (Curran et al. 2012). Serodiscordant couples are also given information about how to correctly and consistently use condoms to reduce chances of HIV transmission to the HIV-negative partner (Padian et al. 1993). Some CHCT approaches also address abstinence, avoiding anal sex, and negotiating risk reduction plans (Padian et al. 1993). The World Health Organization (WHO) recommends that HIV-negative individuals with a known HIV-positive partner retest in 4 weeks to assess for recent HIV transmission and semiannually afterward, if they continue to be

sexually active (WHO 2012). Recent WHO recommendations suggest HIV testing should be conducted every 6 months and informed by the couples “risk assessment,” fertility intentions, as well as by symptoms for acute HIV (see entry “► [HIV Testing and Counseling](#)”). In addition, WHO also recommends educating both partners in strategies to reduce the risk of HIV transmission, such as male circumcision and PrEP, if available. WHO recommendations underscore the importance of clinical follow-up for the HIV-positive partner that should include (1) a CD4 cell count to assess his or her eligibility for ART, (2) technical skills to ensure correct condom use and recommendations to use condoms consistently (see entries “► [Male Condoms](#)” and “► [Female Condoms](#)”), (3) discussions on family planning, safe conception, and pregnancy, and (4) prevention of mother to child transmission (PMTCT) for HIV-positive pregnant women (WHO 2012) (see entry “► [Behavioral Aspects of HIV Mother-to-Child Transmission](#)”).

Benefits: CHCT promotes mutual disclosure of HIV status and can assist partners in providing support for each other, if one or both are HIV positive (Curran et al. 2012). CHCT also increases uptake and adherence to ART, promotes condom use, and, by encouraging behavior change, leads to a reduction in the risks of HIV transmission (Curran et al. 2012). Promotion of disclosure of HIV status in a serodiscordant relationship within a stable partnership has been found to foster family support, improve engagement in HIV care for the HIV-positive partner, and result in high adherence to ART (Curran et al. 2012). Recently, the US Food and Drug Administration (FDA) approved an HIV home test (HT) manufactured by Orasure for over-the-counter sale. The Orasure HT has the potential to promote testing among couples that do not know their HIV status. Home testing can serve as a tool to help couples learn their HIV status discreetly. Moreover, HT may avoid the stigma and barriers associated with going to a clinic for HIV testing and may promote privacy. Despite these advantages, couples in serodiscordant relationships that learn their HIV status through HT would still benefit from CHCT to ensure that they receive concrete referrals and

gain access to ART, are encouraged to use condoms regularly, and address negative reactions that may be associated with disclosure of HIV. This is particularly relevant because some studies have found that disclosure of HIV among serodiscordant couples has been associated with increased risk of divorce, separation, and intimate partner violence. For example, the Partners in Prevention HSV/HIV Transmission Study and Partners PrEP Study found that counselors observed disbelief and misconceptions about HIV results among couples, including skepticism about the accuracy of the test, desire for retesting and hopes for a different outcome, sadness, blame toward the partner for infidelity, and concern about children's HIV status, where couples needed support and counseling (Curran et al. 2012). These findings underscore the need for couple-based HIV prevention that moves beyond CHCT in order to promote risk reduction within stable relationships as well as with other partners and creates prevention approaches that help the couple feel safe to talk about sensitive issues that increase risks (e.g., extra-dyadic affairs and HIV risk behaviors such as bisexuality, sharing drugs and equipment, and sexual violence).

HIV/STI Behavioral Prevention Beyond CHCT

Couple-based HIV interventions that moved beyond CHCT were introduced in the early 1990s, and the first such intervention study was published in the United States in 2001 (El-Bassel et al. 2010a). Couple-based HIV prevention was initiated when the HIV field recognized that serodiscordant couples who learned about their HIV status and participated in CHCT needed additional opportunities to learn how to use strategies together to maintain safer sex practices, reduce drug risk behaviors, and address HIV, STI, and reproductive health issues. The core elements of couple-based HIV prevention include skill building to increase male and female condom use and promote behaviors that reduce sexual and drug risk behaviors (e.g., reduction of sexual concurrency, sex trading [see entry “► [Female, Male and](#)

[Transgender Sex Workers, Epidemiology of HIV/AIDS](#)”], bisexuality, sharing needles); identify and learn ways to confront interpersonal and structural barriers to fight HIV (see entry “► [Multilevel Interventions/Structural Approaches to HIV Prevention](#)”), gender roles and expectations as contributors to unprotected sex, injection, and drug risk behaviors (see entry “► [Harm Reduction for Injection Drug Users](#)”); integrate STI, HIV, and reproductive health services; build healthy relationships; and address goal setting to allow couples to practice risk reduction strategies in real-world settings (El-Bassel et al. 2010a, b). Risk reduction group approaches with serodiscordant couples have been found to be highly acceptable, feasible, and efficacious in reducing sexual and drug risk behaviors in multiple countries (El-Bassel et al. 2010a). Multiple studies have been conducted with serodiscordant couples. For example, a cognitive behavioral group-based intervention for HIV-positive women and their partners in Zambia found higher rates of increased condom use when male partners had a higher level of intervention participation (Jones et al. 2005). A multisite study on HIV prevention in the United States among serodiscordant African American couples found higher rates of consistent condom use as a result of a couple-focused risk reduction approach when compared to an individually focused health promotion intervention (El-Bassel et al. 2010b). A couple-based study with 282 HIV-negative drug-using couples from New York City found, at 12 months follow-up, that there was a 41% reduction in the incidence rate of the number of unprotected sex acts with intimate partners and 39% reduction in unprotected sex acts with other partners when the couple received the seven-session intervention together when compared to one person receiving the intervention alone (El-Bassel et al. 2011).

Benefits: Bringing couples together sends a message that the responsibility for HIV risk reduction falls on both members of the dyad and underscores that both men and women can put each other at risk for HIV. Second, this type of intervention provides a supportive environment that may enable a couple to more safely disclose to each other extra-dyadic sex partners, a history of

STIs, a history of injection drug use, or past experiences in abusive relationships (El-Bassel et al. 2012). Third, it provides an environment in which a couple can learn communication skills and practice them and discuss gender differences (e.g., how men and women discuss sex, the meaning of requesting and/or refusing the use of condoms), gender power imbalances associated with sexual coercion and the inability to negotiate condom use, gender inequalities in risk practices, and sexual expectations (El-Bassel et al. 2010a). Couple-based behavioral prevention is particularly useful for women who are fearful of negotiating condom use, uncomfortable in suggesting safer sex practices, or afraid to refuse unsafe sex because of a partner's reaction and abuse (El-Bassel et al. 2010a, b). Research has shown that when women attempt to negotiate safer sex practices with their partner, they may experience an increased risk of intimate partner violence (El-Bassel et al. 2010a).

Biomedical HIV-Serodiscordant Couple-Based Prevention

A number of recent biomedical advances for serodiscordant couples have made significant contributions to HIV prevention science and provide a dramatic difference in reducing the incidence of HIV. For example, in an HIV Prevention Trials Network study (HPTN 052) of 1,763 serodiscordant relationships where the HIV-positive partner (CD4 count of 350–550 cells per cubic millimeter) received early initiation of ART, there was a 96% reduction in the number of linked HIV-1 transmissions, when compared to those receiving delayed therapy (CD4 count of 200–250 cells). The mechanism by which prevention occurs is likely the suppression of HIV-1 in genital secretions, resulting from ART (Cohen et al. 2011). Another study, which considered the use of suppressive therapy (acyclovir) for serodiscordant couples, found that daily acyclovir therapy did not lead to a reduced risk of HIV transmission, although treatment did reduce plasma HIV-1 RNA levels and the occurrence of genital ulcers (Celum et al. 2010).

Additionally, the Partners PrEP Study with serodiscordant couples (Baeten and Celum 2011) showed that daily oral tenofovir (TDF) and emtricitabine/tenofovir (FTC/TDF) PrEP for the HIV-negative partner reduced risk of HIV acquisition, by 62% and 73%, respectively, in African men and women. Similar efficacy between TDF and FTC/TDF HIV protection effect was robust in both women and men (Baeten and Celum 2011). PrEP has been recommended for the HIV-negative partner in a serodiscordant relationship, or for a partner whose HIV status is unknown or who engages in HIV risk behavior (see entry “► [Pre-exposure Prophylaxis \(PrEP\)](#)”). Moreover, PrEP has been recommended for the HIV-negative partner when the couple decides to conceive.

Benefits: Access to early ART reduces HIV incidence among serodiscordant couples, improves the health and survival of HIV-positive individuals, and significantly reduces infectiousness and the likelihood of transmission to HIV-negative partners. Mathematical modeling predicted that treating HIV-serodiscordant couples, in countries such as Malawi and Lesotho with a high HIV prevalence rate (7.1–19.5%), where a large percentage of couples are serodiscordant (9.8 and 13%), could result in significant reductions in population-level HIV incidence (Dunkle et al. 2008).

These biomedical advances highlight that treatment is prevention and promoting HIV testing and ART (test and treat), as well as linkages to care, is critical in reducing new HIV infections. Treatment as prevention among serodiscordant couples would increase the number of people who are aware of their HIV status, with the subsequent benefit of causing a decline in new infections among serodiscordant couples and reducing community viral load. However, treatment as prevention needs to be complemented with education and behavioral changes to reduce HIV risks and couple-based behavioral prevention approaches.

Adherence to Treatment

As access to antiretroviral therapy (ART) may be insufficient to ensure full adherence to treatment

regimens, behavioral interventions that address barriers, educate, and promote adherence help to ensure that the benefits of ART are fully realized within serodiscordant couple relationships (see entry “► [Behavioral Aspects of HIV Treatment as Prevention](#)”).

Couple-based ART adherence interventions are designed to bring the couple together to learn strategies to ensure that the medication is taken regularly. The responsibility for adherence is placed on both members of the couple. The HIV-negative partner becomes involved in a number of activities with a third party (e.g., counselor, nurse, doctor) to promote adherence to prescribed medication regimens. Currently, there is only one evidence-based adherence study for serodiscordant couples. The Sharing Medical Adherence Responsibilities Together (SMART) Couples Study is the first and only couple-based ART adherence intervention (Remien et al. 2005). The intervention was designed to improve medication adherence among HIV-positive patients by fostering active support from their HIV-negative partner and addressing sexual transmission concerns within the dyad.

Benefits: Adherence to antiretroviral therapy among HIV-positive individuals reduces sexual HIV transmission (Reynolds et al. 2011). There are also a number of advantages to bringing couples together in terms of improved adherence to HIV care and treatment. Intervening with both members of the dyad, rather than with the individual patient alone, can produce increased (1) motivation to maintain good health, (2) understanding of how medications work to control HIV viral load and of the necessity for high levels of adherence to the treatment regimen, (3) commitment to obtain and maintain a steady supply of medications and to provide daily reminders for medication taking, (4) ability to identify and resolve ongoing barriers to optimal adherence, (5) encouragement to confront HIV-related stigma and feelings of isolation which can contribute to treatment avoidance, and (6) ability to communicate and provide mutual care-taking behaviors within the dyad. Furthermore, in the context of HIV infection, it

is important to integrate HIV prevention into HIV care whether couples are HIV serodiscordant or concordant because, as noted above, there is a clear association between viral load control and transmission risk.

Conclusion

Couple-based behavioral and biomedical HIV prevention strategies for serodiscordant couples can play a pivotal role in the fight against HIV/AIDS and have the potential to significantly reduce new incidence of HIV. Given that sexual and drug use behaviors occur in a dyadic context, involving both members of a couple jointly in an intervention to reduce transmission risk and support each other in adhering to ART and other biomedical treatment (PrEP) is paramount to fighting the HIV epidemic where no vaccine is available. A couple-based approach underscores the joint responsibility of both members of the dyad and, in particular, increases men’s awareness of their responsibilities. CHCT combined with behavioral risk reduction strategies and the promotion of condom use remains a strategic prevention priority in the United States and other countries. Even with the FDA approval of over-the-counter sales of home HIV testing, CHCT remains important for couples to deal with negative reactions associated with discovering one’s HIV status. Behavioral prevention that moves beyond CHCT and includes skill building (technical skills, communication skills, and healthy relationship skills) and integration of prevention of STIs, HIV, and reproductive health is needed to address couple’s needs and promote behavioral change. Recent biomedical advances for HIV-serodiscordant couples have made a significant contribution to HIV prevention science and provide new avenues for reducing the incidence of HIV, demonstrating that ART treatment is prevention. A combination of behavioral and biomedical strategies to address risks between serodiscordant couples holds more promise for reducing HIV transmission than either strategy alone.

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HIV Prevention for Stimulant Using Men Who Have Sex with Men

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Definition

HIV and stimulant use are interconnected epidemics among men who have sex with men (MSM) in the USA. Among MSM, stimulant use and its behavioral sequelae are critical targets for interventions across the spectrum of HIV prevention, treatment, and care.

Epidemiologic Profile

HIV Risk Among MSM in the USA

More than 54,000 Americans acquire HIV each year, and men who have sex with men (MSM) represent over half of these new diagnoses.

Representing approximately 2.0–5.0% of the male population in the USA, MSM comprised 68.0% of adult and adolescent men living with HIV in 2013. In the same year, male-to-male sexual contact was attributable to 65.8% of new HIV diagnoses in males (CDC 2015). Furthermore, new infections among MSM have remained steady over the past 5 years (2009 = 21,811; 2013 = 21,498; CDC 2015).

Stimulant Use and Sexual Risk-Taking Among MSM in the USA

In the past decade, stimulants, including cocaine/crack, crystal methamphetamine (meth), amphetamines, and methylenedioxy-methamphetamine (MDMA/ecstasy), and alkyl nitrites (poppers), have become increasingly popular among MSM worldwide (Bourne 2012). In high income nations, such as USA, the prevalence of recent (past 12 months) amphetamine use among MSM is reported to be between 7.2% and 18.8% (Koblin et al. 2007), crystal meth use between 2.8% and 18.0% (Forrest et al. 2010; Spindler et al. 2007), and ecstasy use from 18.5% to 36.7% (Greenwood et al. 2001; Mansergh et al. 2001).

Existing literature highlights the intertwined relationship between stimulant use and sex for MSM. Stimulants are thought to amplify sexual desire and pleasure, decrease sexual inhibition, and enhance sexual endurance. Research demonstrates that for many MSM, sex either “always” or “often” accompanies stimulants. Stimulant use may also involve protracted periods of sexual activity and group sex. MSM who use stimulants are known to engage in high-risk sexual practices, including unprotected anal sex. The physical and psychological effects of the drugs can result in an impaired ability or desire to use condoms, high numbers of sex partners, as well as instances of condom breakage and slippage due to incorrect use and prolonged sexual encounters. Consuming certain stimulants has been shown to double or triple the probability of engaging in high-risk sexual behaviors among MSM (Colfax and Shoptaw 2005; Vu et al. 2015; Mayer et al. 2006).

HIV Infection Among MSM Who Use Stimulants in the USA

Stimulants are highly associated with the acquisition and transmission of HIV and other sexually transmitted infections (STI) among MSM. For instance, a 2015 meta-analysis found that MSM who use amphetamine and/or crystal meth were 1.70 times more likely to be infected with HIV than non-using MSM (Vu et al. 2015). The prevalence of HIV among MSM who use stimulants in the USA has been reported to be as high as 60.0% across epidemiologic studies (Strathdee and Stockman 2010). While overall HIV survival rates have significantly improved over the last two decades, studies have shown that MSM living with HIV who use stimulants experience difficulties with antiretroviral therapy (ART) access and adherence. Stimulants have also been shown to accelerate HIV disease progression independently of adherence to ART. Additionally, immunological failure, increased plasma viral load, and a heightened risk of mortality are associated with stimulant use among MSM living with HIV. The additive effect of increased plasma viral load, the direct physiological effect of stimulants, and increased sexual and drug use risk behaviors significantly potentiates the risk for HIV transmission to sex partners.

Stimulant Treatment Interventions

Treating MSM who use stimulants, especially in the context of sexual risk behaviors, is recognized as a powerful means of preventing both HIV spread and promoting favorable HIV treatment outcomes. The field of stimulant use treatment has made strides in the past decade due to the development and testing of behavioral and pharmacological interventions. However, existing treatment options have proven to be, at best, only moderately effective and there is an insufficient number of randomized controlled trials (RCTs) to support one intervention over another. Below is a summary of the current literature on the development and testing of some of the most promising treatment approaches.

Behavioral Approaches to Stimulant Treatment

At present, behavioral and cognitive interventions are the mainstay of stimulant treatment. Prominent among these approaches is contingency management (CM), a therapeutic technique that is based on the principles of operant condition to encourage specific behavioral goals. In the case of stimulant use, CM involves the delivery of vouchers that are exchangeable for money or commodities. Vouchers are contingent on negative toxicology screens that indicate abstinence from stimulant use. A 2014 meta-analysis of several CM trials to reduce stimulant use found an overall effect size of 0.46. This suggests that CM has a moderately meaningful impact on stimulant use (Benishek et al. 2014). Unfortunately, the treatment gains garnered by CM have not proved sustainable in the long term. The same meta-analysis documented a decrease in effect size from the completion of treatment to the 6-month follow-up. Furthermore, meta-analyses such as these provide limited insight into the efficaciousness of CM with MSM and other key populations for HIV infection due to the small number trials conducted among these groups.

Cognitive behavioral therapy (CBT) is a therapeutic technique that addresses cognitive distortions through a problem-solving based process involving cycles of clinician and patient interaction. CBT provides skills that are valuable in assisting people to stop using drugs and prevent relapse. CBT has been moderately successful at reducing the use of crack-cocaine, crystal meth, and amphetamines (Vocci and Montoya 2009). Among MSM who use crystal meth, CBT interventions have been shown to reduce incidence of receptive unprotected anal intercourse (Shoptaw et al. 2005). While there is a growing body of evidence supporting CM as the more effective treatment option, CBT has been shown to be equally as effective at sustaining treatment effects in the post-discharge period. Studies combining CBT and CM in the treatment of crack-cocaine and crystal meth among MSM and other populations have yielded strong effect sizes as a whole but there is a lack of evidence to support abstinence and maintained sexual risk reduction

gains in the post-treatment period. On the positive side, adding CM to CBT protocols has been shown to improve rates of attrition, suggesting that the integration of these approaches may enhance over all treatment efficiency (Vocci and Montoya 2009).

Behavioral activation (BA) is a front-line, evidence-based treatment for depression and anxiety. BA is intended to provide people who use stimulants with the necessary tools to reengage in positive reinforcement behaviors allowing for gradual increases in potentially rewarding and pleasurable activities that do not involve drug use. While BA interventions to reduce stimulant use among MSM have yet to be tested in a RCT, results from small open pilot trials are encouraging. For instance, Mimiaga and colleagues piloted an intervention for crystal meth use among high-risk sex in MSM in the USA. The intervention included 10 weekly sessions of BA with integrated HIV risk reduction counseling. The mean unprotected anal intercourse episodes decreased significantly from baseline to end of treatment and from baseline to 6 months post-baseline. On average, there was a significant decrease over time in the number of self-reported crystal meth episodes in the prior 3 months and the number of days that crystal meth was used in the prior month. Statistically significant reductions in depressive symptoms and poly-substance use were also maintained (Mimiaga et al. 2012a).

The rationale for using BA in conjunction with CM is supported by formative research which found that MSM continue to engage in crystal meth use as a means to seek out pleasurable experiences because without the drug activities that used to be pleasurable were no longer fulfilling (Mimiaga et al. 2008). In 2012, an open pilot of a BA/CM intervention was conducted among MSM and heterosexual men and women living with HIV who used crystal meth and/or crack-cocaine in the USA. The intervention combined 12 weeks of CM and 10–16 sessions of BA. Reductions in stimulant use (as indicated by negative saliva toxicology screens) and improvements in self-reported ART adherence and engagement with HIV care were observed from baseline to 6 months post-baseline (Mimiaga et al. 2012b). Due to the

small sample sizes and open study designs, further efficacy testing of both the BA and BA/CM interventions is required.

Pharmacologic Options for Stimulant Treatment

An increased focus on the neurobiology of substance use over the past 20 years has contributed to a growth in research on pharmacotherapies to increase stimulant abstinence. There is a solid evidence base suggesting that pharmacotherapies can be developed by altering the pharmacokinetics and pharmacodynamics of stimulants or its effects on the brain. Yet, determining optimal pharmacological targets has been difficult. As with behavioral interventions, no single pharmacotherapy has been found to be broadly effective in clinical trials. Novel methodological approaches will make the process of drug discovery more predictable and efficient (Brensilver et al. 2013; Kampman 2005).

Numerous classes of medication have been studied, mainly in small clinical trials. Medications presumed to have potential for stimulant treatment target dopaminergic, serotonergic, glutamatergic, and opioidergic brain pathways. In addition, medications that enhance cognitive process such as modafinil have gained attention in light of the known cognitive deficits associated with long-term stimulant use (Brensilver et al. 2013; Kampman 2005). In the treatment of crystal meth and amphetamines, promising research has been reported for methylphenidate, naltrexone, bupropion, and mirtazapine. RCTs of these pharmacotherapies have documented higher percentages of stimulant-free urine samples and rates of continuous abstinence as compared to the placebo in subgroups of patients (Brensilver et al. 2013). For crack-cocaine use, disulfiram (also known as antabuse), an established medication for treating alcohol dependence, is one of the most promising treatment candidates to emerge (Kampman 2005). There are now more than 10 published trials showing that disulfiram reduces cocaine use in cocaine-dependent patients.

In recognizing the need for novel treatment and delivery approaches, recent research has focused on the development of “anti-addiction” vaccines

that block the pharmacological effects of cocaine (Kosten et al. 2014a). A trial of a vaccine being developed for the treatment of cocaine use in the USA showed that cocaine-dependent adults who received a high dose of the vaccine produced the most cocaine-free urine samples and had better retention rates than those in the control and low dose study arms. However, differences between the three study arms were found to be insignificant (Kosten et al. 2014b). Further investigations of innovative pharmacological options such as vaccines are necessary, as these approaches may be particularly useful for individuals who engage in activities (e.g., stimulant use) that are barriers to medication adherence and engagement in care.

Settings for Stimulant Use Treatment and HIV Prevention Counseling

Given the interconnections between stimulant use and HIV risk among MSM in the USA, there is a need for further integration of substance use treatment with HIV prevention in places where MSM seek care, including substance use treatment facilities, clinics, and tertiary care centers. The reduction or cessation of substance use is the principal concern of most inpatient programs; however, individuals receiving inpatient treatment may experience co-occurring problems (e.g., depression) that remain unaddressed and may increase HIV risk. Such risk behaviors not only complicate their recovery process but also have broad public health significance with respect to rates of infectious disease transmission. Moreover, at specialized settings serving MSM, a lack of effective treatments for stimulant use and difficulties implementing traditional HIV prevention modalities within the context of stimulant use are a significant barrier to addressing these concomitant health concerns.

Conclusion

By reducing stimulant use, impacts can be made on individual risk behaviors and HIV-related outcomes, subsequently reducing HIV transmission rates vulnerable subpopulations such as MSM. For

MSM who use stimulants in the context of sex, treatment for stimulant abuse and dependence is an essential part of primary and secondary HIV prevention. For MSM accessing HIV care and prevention services, behavioral interventions that address stimulant use in the context of sex will support service engagement, treatment adherence, and safe sex. If recent successes in the treatment of opiate addiction into HIV care clinics are any indication, the integration of pharmacologic options for stimulant use into these settings will likely be beneficial – in terms of promoting retention, reducing sexual risk, and improving health outcomes among MSM.

During the last decade researchers have made strides in identifying and testing pharmacotherapies to treat stimulant use. However, as behavioral treatments are currently the corner stone of stimulant use treatment, pharmacological options should be considered an adjunct rather than a replacement for behavioral approaches. If medications can decrease the cravings and neuropsychiatric effects of stimulant withdrawal, individuals will be better able to engage in behavioral interventions. Over time, these combined modalities may help to sustain treatment gains.

The future of HIV-related behavioral interventions for MSM who use stimulants should include comprehensive programs that provide treatment for mental health problems – with and without pharmacotherapy – along with the use of other evidence-based biomedical and behavioral techniques to decrease HIV risk behaviors. To this end, further research is needed to pilot, test, and refine novel behavioral approaches and pharmacological treatments to establish the best methods for addressing stimulant use in the context of HIV prevention among MSM.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Behavioral Science Highlights of Evidence and Research](#)

- ▶ [Combination Approaches to HIV Prevention](#)
- ▶ [Gay men and other Men who have sex with men \(MSM\), Epidemiology of HIV/AIDS Introduction](#)
- ▶ [HIV Prevention Efforts Within Substance Use Disorder Treatment Settings](#)
- ▶ [HIV Prevention for MSM](#)
- ▶ [Immunological Responses to Antiretroviral Therapy](#)
- ▶ [Positive Health, Dignity, and Prevention \(PHDP\)](#)

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HIV Prevention in Persons 50 and Older

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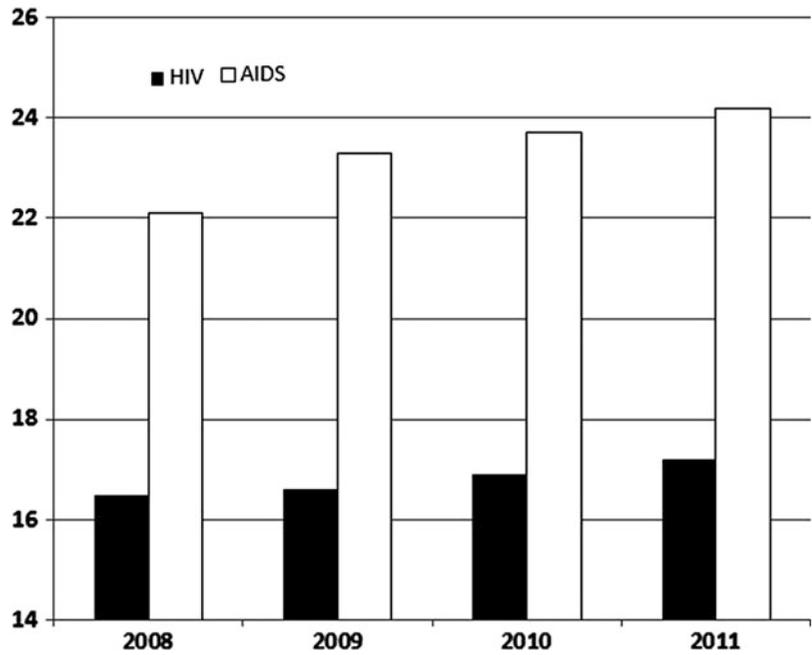
Definition

Primary and secondary HIV prevention efforts targeted to HIV-positive and HIV-negative persons 50 years and older.

The CDC estimates that in the United States, 50% of all people living with HIV (PLWH) will be 50 years and older by 2015 (CDC 2008). Part of this phenomenon, often called the “graying of the epidemic” is primarily driven by the effectiveness of HIV antiretroviral therapy (ART), as well as new HIV infections among older adults (defined as persons 50 and older) (see Fig. 1). For 2010, the CDC reports that 17% of all new HIV diagnoses occurred in people aged 50 and older and 24% of all AIDS diagnoses occurred in this older adult population (CDC 2013; see Fig. 2).

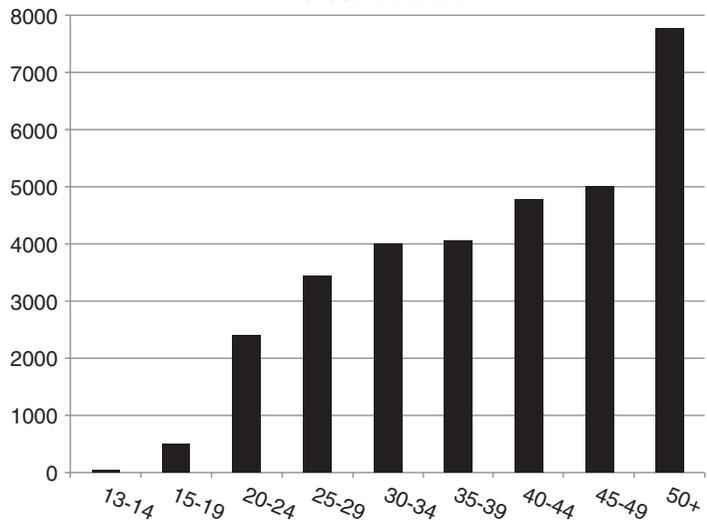
In the beginning of the US epidemic, persons 50 years and older acquired HIV primarily through blood transfusion and most died before the advent of ART. Today, the modes of HIV transmission among older adults, similar to their younger counterparts, occur mostly through sexual contact and injecting drug use (IDU). The groups of older adults most at risk and affected by HIV are men who have sex with men (MSM), women, and injecting drug users. In 2011, 60% of new HIV diagnoses were among MSM, 23% were due to heterosexual contact, 27% were women, and 14% were due to IDU (CDC 2013).

HIV Prevention in Persons 50 and Older, Fig. 1 % of total US new HIV infections for adults aged 50 and older (CDC Surveillance Report 2013)



HIV Prevention in Persons 50 and Older, Fig. 2 US AIDS diagnoses by age for 2010 (CDC 2012)

Number of Newly Diagnosed AIDS Cases by Age in the US 2011
CDC Surveillance Data



Older MSM, more than any other group, who have aged with constant exposure to myriads of HIV prevention messages, represent the majority of new HIV infections over the age of 50 (CDC 2013). In the second decade of the US epidemic, the rate of HIV infection among women ages 50–59 rose by 56%, doubled among women aged 60–65, and tripled among

women over 65 (Collier 2012). These groups are comprised of racial and ethnic minorities. Between 2007 and 2010, older Blacks and Latinos, respectively, had 13 times and 5 times higher rates of HIV infection than older Whites (Linley et al. 2012). In 2010, 46% of the men and women aged 50 years and older were Black, 36% were White, and 16% were Latino (CDC

2013). Racial disparities are particularly pronounced among women: 63% of all women aged 50 years and older living with HIV were Black compared to 18% Latina and 16% White (CDC 2013).

The sexual behaviors of older adults most at risk for HIV infection as well as those living with HIV have not been adequately researched. Few valid HIV prevention interventions have been developed (Negin et al. 2014). This neglect is premised in part on assumptions that older people do not engage in sexual activity and/or HIV risk behaviors. Yet some studies consistently show that older adults with HIV, like their HIV-negative peers, are sexually active and engage in unprotected sex and substance use (illicit drugs, alcohol, and tobacco) (Golub et al. 2010; Lovejoy et al. 2008; Cooperman et al. 2007). Although older adults share similar risk factors with younger groups, there are differences that emerge. As part of the baby boomer generation, older adults at risk for HIV and older PLWH share different norms and values regarding sexual behavior and substance use. Compared to their younger counterparts, older adults get tested for HIV less frequently, have lower knowledge about HIV transmission, and are often less aware of how to protect themselves against HIV. They are also experiencing age-related physiological changes, declining sexual function, as well as long-term relationship changes that may increase vulnerability for HIV infection. There is a clear need for data-driven sexual risk reduction interventions or evidence-based social media prevention campaigns that target older adults. The following summary identifies the different prevention needs of older adults at risk of HIV and older PLWH.

Older Adults at Risk of HIV Infection

In the last 10 years, the rates of sexually transmitted infections (STIs) among 45- to 65-year-olds have nearly doubled and tripled (CDC 2004, 2010). Many attribute the rise of STIs among older adults to generational norms regarding condom use and age-related physiological and sexual

function changes. In one national study of sexuality and health among heterosexual older adults, 93% of those who were sexually active reported inconsistent condom use (Leigh et al. 1993). Another national survey of men and women over 50 years of age found that 14–17% reported penile-vaginal intercourse (PVI) and only 20–25% used condoms during their most recent PVI episode (Schick et al. 2010). Many older women and their partners may be less consistent with condom use because pregnancy is no longer a concern. Additionally, menopausal and postmenopausal women may be at increased risk for STIs and HIV because of physiological changes in the vaginal lining as it becomes thinner and dryer, making them more vulnerable to infection (CDC 2008). The use of erectile dysfunction medications by men has increased sexual activity and sexual risk taking, resulting in increased rates of STIs among the over-50 age group (Jean et al. 2010).

Certain psychological and interpersonal changes exacerbate many of these risk behaviors and reinforce vulnerability to HIV infection. These include changes in relationship status from being married to being separated, divorced, or widowed. Changing relationship factors among older adults, including deaths, children or family members moving away, and friends relocating as they retire, can result in diminished social support and increased mental health issues due to grief, isolation, and concomitant loneliness. To compensate for these “losses,” older adults may use alcohol, tobacco, and/or drugs, further compromising their health and ability to sustain safer sex practices. Interpersonal changes may lead to new intimate relationships, which can lead to increased sexual risk, especially when coupled with psychosocial challenges. One study found that for older women, the acquisition of new partners is accompanied by decreases in safer sex practices due to many older male partners’ difficulty using condoms (Rich 2001).

A barrier to HIV prevention for older adults at high risk for HIV infection is the well-documented observation that many health-care providers underestimate their risk for HIV and STIs (Lindau et al. 2007). HIV symptoms, or

AIDS-related opportunistic infections, are often overlooked among older adults because such symptoms may overlap with other age-related conditions. For example, HIV-related cognitive dysfunction is often misdiagnosed as Alzheimer's. Extreme weight loss and fatigue may be viewed as part of the typical aging process. Few providers encourage older adults to test for HIV and STIs. Consequently older adults are more likely to be diagnosed late in the course of HIV infection with many having progressed to AIDS. When HIV infections are detected late, many older adults develop AIDS within 12 months of their diagnosis, placing them at higher risk for morbidity and mortality (Linley et al. 2012).

Older Adults Living with HIV

Among sexually active older adults with HIV, studies find that between 33% and 42% report unprotected insertive intercourse (Golub et al. 2010; Illa et al. 2008; Aidala et al. 2006). Risk factors for unprotected sex among older adults with HIV include misconceptions about viral load and risk of HIV transmission, recent substance use, and poor psychological well-being (Golub et al. 2010; Karpiak et al. 2006). Evidence has shown that over time PLWH might abandon safer sex practices due to the belief that adherence to ART together with low or undetectable viral loads reduces the risk of transmitting HIV to zero (Sullivan et al. 2007). In the ROAH (Research on Older Adults with HIV) study, a cross-sectional survey of 1,000 older adults with HIV in New York City, 50% of older adults reported sexual activity and a third of sexually active individuals reported unprotected sex in the last 3 months, which was significantly associated with loneliness and recent substance use (Karpiak et al. 2006; Golub et al. 2010).

Depressive feelings and neediness are factors underlying risky sexual behavior. Studies consistently report associations between unprotected sex and negative affect such as depression and anxiety. These elevated levels of depression that are typically accompanied by loneliness, anxiety, and

chronic stress occur across gender, race/ethnicity, and sexual orientation in studies of older adults with HIV. Such data supports the contention that stress and mental health problems are often identified as significant determinants of risk behavior among HIV-positive older adults (Groves et al. 2010; Heckman et al. 2000; Kalichman et al. 2000a, b). In the older adult with HIV, high rates of depressive symptoms persist (Heckman et al. 2000; Asch et al. 2003; Berger-Greenstein et al. 2007; Junqueira et al. 2008; Hoerger et al. 2012; Saito et al. 2012; Bhat et al. 2013; Traeger et al. 2013; Vance 2013) with clinical depression being the most commonly observed mental health disorder in that population (Goodkin et al. 1996, 2003; Heckman et al. 2011; Relf et al. 2013). These rates are almost five times higher than the general New York City population (Havlik et al. 2011; Golub et al. 2010; Groves et al. 2010). Depression has been highly correlated with lack of engagement in care and poor adherence to ART medications, which causes HIV viral levels to rise, thereby increasing infectivity (Gonzalez et al. 2011).

Loneliness and recent substance use among ROAH participants were significantly associated with unprotected sex among sexually active older adults with HIV (Golub et al. 2010; Brennan et al. 2011). Substances most often used with sex were alcohol (32%), marijuana (20%), crack (17%), cocaine (15%), and poppers (11%). Those who report current use of alcohol or drugs were more sexually active (58%) compared to those who did not use substances (42%). Almost half (47%) of sexually active HIV older adults reported using alcohol or drugs during sex. Unprotected insertive sex occurred more frequently (40%) when alcohol or illicit drugs were used with sex (Karpiak et al. 2006; Brennan et al. 2009; Golub et al. 2010).

Prevention Challenges

Very few HIV prevention efforts are aimed at people over 50, and most social media and health promotion campaigns primarily use images of young adults, creating the false perception that



HIV Prevention in Persons 50 and Older, Fig. 3 Examples of HIV and older adults social media campaign in New York City developed by the AIDS Community Research Initiative of America (ACRIA)

older adults are not at risk (Negin et al. 2014). Age-appropriate social messaging campaigns are uncommon. One campaign: “Age is Not a Condom” has been developed and deployed in New York City bus shelters by the AIDS Community Research Initiative of America (ACRIA) in New York City for that past 4 years (2011–2014) (see Fig. 3). The *Older Adults and HIV Toolkit* developed by the government’s AoA (Administration on Aging) and the website of the National HIV/AIDS and Aging Awareness (NHAAA) day are other sustained prevention campaigns for older adults.

Programs are needed to increase HIV testing among older adults at risk for HIV infection. To increase older adults being tested for HIV, the CDC recently recommended that routine HIV testing in adults be extended up to 64 years of age (Branson et al. 2006). Making testing routine for older persons may initiate discussions about risk behavior between health-care providers and older patients. Few older adults report that they discuss their sexual health with their health-care provider (Abrass et al. 2012; Hughes 2011; Working Group for HIV and Aging Consensus Project 2012). Providers must be better trained to recognize and act (e.g., recommend testing) when an

older patient evidences clinical symptoms that are indicative of HIV infection and encourage those who are at elevated risk to have regular HIV tests.

There is growing evidence that among PLWHA, there exists prevention fatigue or complacency (Valdiserri 2004; Spire et al. 2008). This is manifested by a return to unsafe sexual behaviors based on the rationale that HIV is easily treated and akin to managing a chronic illness (Sullivan et al. 2007). The need to implement the recent CDC initiative (HIP – High Impact Prevention) must be extended to the older adults with HIV. The recent approval of the daily use of an antiretroviral (Truvada) for HIV prevention strategy is historic (CDC 2014), with some suggesting that there are parallels to the introduction of “the pill.” Providers and their older adult patients must familiarize themselves with this newest approved prevention option. The impact on the sexual health of older adults with and without HIV infection may be momentous. When combined with sustained connection to care and adherence to ARVs, we may begin to witness the beginning of the end of AIDS (CDC 2014). Older adults must be included in the HIP effort.

Primary and secondary HIV prevention for older PLWH and older adults at risk of HIV

should have as a goal reduced transmission risk. HIV prevention efforts have largely adopted a pathogenic perspective, identifying psychological factors that increase HIV risk behavior. There is a need to implement salutogenic models that engage health-promoting factors that are positive. Placing emphasis on positive psychological health factors may result in more sustained safer sex practices. This approach necessitates that health-care providers acknowledge the psychological resources of their clients, many of whom are long-term HIV survivors exhibiting a high level of resiliency (Halkitis et al. 2012). Prevention efforts should include addressing psychosocial and interpersonal factors that may undermine health and well-being as individuals get old. Psychological distress and poor mental health, weak social networks, and loneliness are especially frequent among older PLWH and are common determinants of sexual risk taking (Groves et al. 2010). For example, in secondary HIV prevention for PLWH, also known as *Prevention for Positives*, support should be provided to assist older PLWH in how best to disclose their HIV status to new sexual partners. Disclosure issues are one of the challenges created by pervasive and detrimental effects of AIDS-related stigma (Emlet 2006; Hult et al. 2012).

Ageism, HIV-related discrimination, and stigma create barriers to HIV prevention efforts. Older adults at risk of HIV infection may not be comfortable disclosing their sexual behaviors or substance use to others, thereby making it difficult for them to access prevention information and support. These barriers result in delayed testing, diagnosis, and treatment. HIV/AIDS-related stigma may be more severe among older persons, leading them to hide their diagnoses from family and friends and new sexual partners (CDC 2008). Failure to disclose HIV infection limits access to potential emotional and practical support and can result in self-imposed defensive social isolation that may also manifest itself in depression.

To address the aging needs of older PLWH and older adults at risk for HIV, some have suggested integrating the *Healthy Aging* model into HIV prevention. *Healthy Aging* programs focus on helping older adults develop and maintain optimal

physical, mental, spiritual, and social well-being so that they can remain active, connected, and engaged (CDC 2014; Hansen-Kyle 2005; Vance and Robinson 2004). Such integration may strip away the stigma of ageism and help older PLWH and older adults at risk for HIV to reduce risk, effectively by using available health services and by encouraging and supporting relationships.

Conclusion

The aging of the HIV epidemic has generated awareness that HIV risk behaviors are not exclusive to the young. Already over 20% of all new AIDS diagnoses occur in the 45 and older group, and 1 in every 6 new HIV diagnoses is in the over-50 population (see Fig. 2; CDC 2013).

Educating older adults, especially those in high incidence groups (MSM, women, IDU), about the risk of HIV and other STIs is necessary for HIV prevention. Additionally, it is critical to educate physicians and health-care providers to interact with their older adult populations, engage them in conversation about their sexual health, and encourage regular HIV and STI testing. Educating older adults and their care providers regarding HIV risk is a needed structural prevention intervention that will assuredly reduce new HIV infections as well as late detection of HIV and AIDS.

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HIV Prevention in the Correctional System

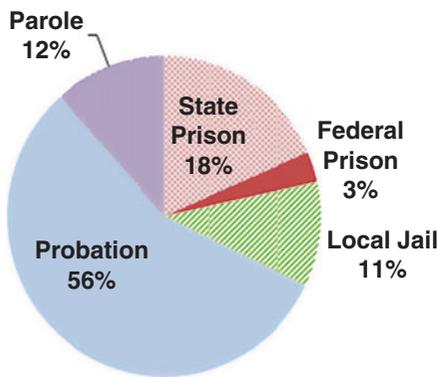
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Definition

The Correctional System: Prisons Versus Jails

The criminal justice system has many components, and HIV prevention takes on different forms according to venue. All countries under the rule of law have a system for confining those who break laws. In the USA, the nation with the highest incarceration rate in the world, there are

divisions by status (convicted or awaiting trial, confined in a locked facility, or followed in the community) and age (adult versus juvenile). Prisons incarcerate adults convicted of felony offenses, which result in sentences for at least a year up to life. Those serving time for breaking federal laws go to a prison run by the Federal Bureau of Prisons; most felony convictions are for state offenses, punished by serving in a state-run prison. Jail systems, operated by counties or cities, hold individuals who are awaiting trial or have been convicted of a misdemeanor. The median length of stay in a jail is 2–5 days; the mean is a couple of weeks. It is rare that a person stays in jail for more than a year, but legal preparation for a complicated trial may prolong pretrial detention. The number of persons confined inside a locked facility is dwarfed by those under community supervision, by either the probation or parole systems. For the relative proportions of the various components of the adult US criminal justice system, see Fig. 1; the number of juveniles held in confinement on a given day is considerably smaller than the number of adults. In other countries, the relative size of the various components can differ. For example, if a court system processes people slowly, a higher proportion of persons will be detained awaiting trial. Also, not all countries separate the awaiting trial from the sentenced.



HIV Prevention in the Correctional System, Fig. 1 US Correctional populations, 2011 (Source: Bureau of Justice Statistics, Publication NCJ239972)

Fundamentals of HIV in the Correctional System

The USA has both the highest incarceration rate in the world and the greatest number of persons confined (International Centre for Prisons Studies 2012), accounting for approximately 2.2 million Americans serving time behind bars in jails and prisons on any given day (Glaze and Parks 2012). In 2006, one in six persons (16.9%) in the USA infected with HIV was incarcerated at some point over the course of the year (Spaulding et al. 2009). Incarcerated individuals are three times more likely to be living with HIV disease at all stages, including AIDS, compared to the general adult population (Maruschak 2012). This concentration of the HIV epidemic in jails and prisons has at least two explanations. By disproportionately incarcerating men over women and Blacks over non-Blacks, the criminal justice system draws from portions of the population with the highest rates of HIV (see Table 1). Social factors epidemiologically associated with high HIV risk include poverty and homelessness (Braithwaite and Arriola 2003); these demographic factors are also overrepresented in the correctional system. Additionally, some of the risk behavior that puts an individual at higher risk of HIV (e.g., intravenous drug use, commercial sex work) is illegal, thus putting persons engaging in these activities at higher risk of confinement.

Most incarcerated men and women who are infected with HIV acquire their infection while in the community, before entering a correctional facility (Centers for Disease Control and Prevention 2009, Braithwaite and Arriola 2003). High-

HIV Prevention in the Correctional System, Table 1 Disproportionate share of men and minorities confined in the USA, 2009

Characteristic	US general population (%)	Prison/jail population (%)	Americans living with HIV (%)
Male sex	50%	91	75
Black race	14%	39	46
Hispanic ethnicity	16%	21	17

Sources: West (2010), Centers for Disease Control and Prevention (2012)

risk behaviors such as unprotected sexual encounters are not infrequent among persons who subsequently enter a correctional facility. Among newly diagnosed HIV-positive jail detainees, 29% admitted to participating in unprotected anal sex in the 30 days prior to incarceration (de Voux et al. 2012). Furthermore, a high prevalence of sexually transmitted diseases (STDs) has been found among inmates, placing them at an increased risk for HIV transmission and acquisition (US Department of Health and Human Services 2011).

Drug and alcohol use is common among persons who enter correctional facilities (Braithwaite and Arriola 2003). Injection drug use can directly lead to both HIV infection and incarceration; injection drug users represented 11% of new HIV-positive cases among a group of jail detainees (de Voux et al. 2012). Substance abuse besides injection drug use can lead to behavior disinhibition which promotes high-risk activity, making users of cocaine and methamphetamine through other routes than intravenous, as well as abusers of alcohol also vulnerable to HIV infection.

Special Considerations for Jails

While not commonly conducted, HIV testing and linkage to medical care and treatment is feasible in the fast-paced environment of jails. In 2003, the states of Florida, Louisiana, New York, and Wisconsin received funding from the CDC to conduct HIV testing in jails. These four programs successfully performed 33,211 tests and identified 269 new HIV cases. With the use of rapid HIV testing, 99.9% inmates received their test results before discharge from jail. Among those with reactive rapid HIV tests, many reported no risk behavior that was considered high risk, such as including injection drug use, male-to-male sex, transactional sex, sexual assault, STDs, or sex with an at-risk partner. Therefore, new cases may be missed if inmates were only offered testing based on risk behaviors; these testing programs demonstrated that jail-based testing should be offered as universal opt-out testing.

A total of 440 known and newly diagnosed HIV-positive inmates with a reactive rapid HIV test were referred to medical care, treatment, and prevention services (Macgowan et al. 2009), demonstrating that jails provide a setting to offer HIV testing and treatment, if necessary, to thousands of persons daily.

The median length of stay in many jails is 48 h or less (Spaulding et al. 2012). In a jail setting, inmates may be released soon after being diagnosed as HIV positive; therefore, inmates may not have the opportunity to receive a pretreatment evaluation and begin antiretroviral treatment in jail. Considering the short length of stay for most jail inmates, few persons newly diagnosed in this setting would begin costly treatment before release (de Voux et al. 2012).

For newly diagnosed cases with a longer length of stay, pretreatment evaluation can be completed. Community-based medical care can be offered to those with a short length of stay to ensure treatment begins. Occasionally, the stay is long enough to commence highly active antiretroviral treatment (HAART). Linkage to HIV medical care and treatment in the community is critical to ensure that these HIV-positive individuals begin or continue to adhere to treatment after release (see “[Linkage to Medical Care after Release](#)”).

For those individuals who have been involved in medical care and treatment prior to entering jail, the detainment may be long enough to break appointments and interrupt therapy. Jails need a protocol to continue treating patients who report taking a reasonable HAART regimen prior to confirmation with outside providers.

Special Considerations for Prisons

HIV-positive persons incarcerated in prisons have sentences longer than a year, providing adequate time to conduct testing, deliver results, and start HAART. Incarceration can be an opportunity for prisoners to receive quality healthcare from medical staff and to adhere to medical treatment in a controlled environment. Sustaining HIV treatment allows HIV-infected individuals to achieve undetectable viral loads, which can greatly

decrease the risk of HIV transmission to sexual or drug injection partners. An increased CD4+ count in a HIV-infected person allows preservation of the immune system to help the infected person avoid life-threatening medical conditions. When inmates are provided with adequate care, health outcomes can be comparable to those in the community setting. The Bureau of Justice Statistics found that between AIDS-related deaths in state prisons decreased from 24 deaths per 100,000 inmates in 2001 to 5 per 100,000 in 2010 (Maruschak 2012). This massive decrease in correctional AIDS-related deaths parallels the decrease in HIV-/AIDS-related deaths nationwide.

Due to the variation of healthcare services currently offered in correctional facilities, health outcomes vary from facility to facility. A study completed in Texas found that approximately half of HIV-infected inmates receive HAART during imprisonment (Baillargeon et al. 2009). Inmates have the right to refuse treatment and may do so for reasons such as stigma associated with HIV, adverse side effects, or poor prognosis.

Prevention of HIV by Testing

Why is Screening Warranted?

Since nearly 16.9% of Americans infected with HIV pass through correctional facilities annually (Spaulding et al. 2009), jails and prisons are logical venues for testing. In the general US population nationwide, approximately 18% of HIV-positive persons are unaware of their infection (Centers for Disease Control and Prevention 2012). There is no evidence that the ratio of diagnosed to undiagnosed infection is any better in the correctional setting. Thus, HIV testing of asymptomatic persons in a correctional setting has the ability to identify cases that may otherwise go undiagnosed and untreated.

Just as HIV testing is an HIV prevention strategy in the outside community, HIV diagnosis may help prevent further HIV transmission in jails and prisons. After learning their status, HIV-positive persons tend to decrease risk behavior such as unsafe sex; the prevalence of unprotected anal

and vaginal intercourse can drop by more than fifty percent after HIV diagnosis (Marks et al. 2005). The same phenomenon has been observed among inmates who tested HIV positive during incarceration; receipt of an HIV diagnosis is associated with lower rates of anal intercourse among male inmates (Jafa et al. 2009). Furthermore, HIV diagnosis improves the health of the inmate, by allowing the inmate to access necessary medications. Early access to HAART prolongs the lives of those diagnosed with HIV infection and reduces the likelihood of transmission. Additionally, HIV testing in correctional facilities, either during or after entry, not only benefits those incarcerated but also those in the community as well. Partner services conducted by the local health department (Altice et al. 2010) may be able to locate former sexual and injecting drug partners of incarcerated HIV-positive individuals, allowing those in the community to seek testing; if positive, these individuals can obtain clinical care and HIV/AIDS education. For serodiscordant couples, vigilance with HAART on the part of the infected person can lower the risk of transmission to the uninfected one. Thus, testing is an opportunity to decrease the transmission of HIV/AIDS and to increase HIV/AIDS knowledge in the community, extending the potential benefits of HIV testing in the correctional system.

Without routine testing, undiagnosed HIV-positive individuals will continue to remain untreated and engage in riskier behaviors, increasing the risk of transmitting the virus. Routine opt-out testing has the ability to normalize HIV management in correctional facilities and communities, reducing the stigma and fear associated with HIV testing and treatment if testing reveals a positive result.

Testing Procedures in the Correctional System

The Centers for Disease Control and Prevention (CDC) published the *HIV Testing Implementation Guidance for Correctional Settings* in 2009. The CDC recommends the adoption of a universal, routine, opt-out testing approach to improve HIV detection among the incarcerated population. This approach includes incorporating consent for HIV screening into the general consent form for

medical services within the institution and HIV testing all inmates unless an inmate refuses. Inmates should be given information about HIV/AIDS before testing occurs, and the decision to test must be noncoercive (Centers for Disease Control and Prevention 2009). Although HIV/AIDS diagnosis in the correctional system has its benefits, some inmates may delay or refuse HIV/AIDS related services in correctional facilities for reasons such as mistrust of staff and/or fear of discrimination from peers.

Despite CDC recommendations, in many prisons and most jails, HIV testing is provided only upon request. In a harsh environment such as a correctional facility, inmates may not feel comfortable stepping forward and requesting an HIV test during an opt-in entrance screening program. Inmates may feel less threatened about submitting to testing when collecting specimens is a routine practice (Centers for Disease Control and Prevention 2009).

Universal opt-out testing at entry may not be perceived as an option in some facilities when budgets are tight or where custody staff are unable to provide security services for a prolonged medical encounter in the intake area. Alternatives to the optimal approach of universal testing by nursing at entry include screening during subsequent medical encounters or risk-based screening. Risk-based screening constitutes targeting HIV testing to inmates who have the highest-risk characteristics (injection drug use, men who have sex with men, sex with an at-risk partner, transactional sex, multiple sex/drug partners, and STDs) within the last 12 months. These approaches allow correctional facilities to test those individuals that may be at the highest risk of HIV infections; however, these methods may preclude the testing of most infected individuals. Studies of jail detainees newly diagnosed with HIV reveal that the plurality had no risk for HIV acquisition other than heterosexual sexual relations (Macgowan et al. 2009). Thus, limiting HIV screening to those who report injection drug use or men who have sex with men is less effective in identifying HIV cases compared to universal testing. Partnerships with local health departments and/or community-based organizations can aid in carrying out HIV

testing and counseling in correctional facilities where further resources are needed.

As of late 2012, most long-term correctional facilities use HIV enzyme immunoassay (EIA) in HIV screening; any positive HIV screening test is followed by a second, confirmatory test using a Western blot assay (Centers for Disease Control and Prevention 2009), though the algorithm for confirming positive tests may be changing in the near future. This traditional HIV testing requires venipuncture, so a fear of needles may be a barrier to testing. Additionally, this testing also requires that the specimen is processed in an external laboratory; results can take several days to be returned to the staff of the correctional facility. Inmates who are detained for only a short stay might not receive their test results. However, this screening strategy may be a practical, low-cost option for inmates with a longer length of stay such as those found in prisons (see *Prisons*).

In correctional facilities with rapid population turnover such as jails, newer rapid HIV tests, although more expensive than traditional tests, can offer the advantages of quicker turnaround compared to screening with EIA and Western blot (see *Jails*). Rapid testing is performed either by swabbing the oral mucosa or sticking the finger; results of these tests are available in as little as 10–30 minutes, a period of time that fits with the hectic pace of a jail (Altice et al. 2010). The medical staff can quickly inform inmates of a preliminary result shortly after the testing, rather than bringing the person back to the healthcare setting at a later time, in a situation where every movement of persons uses the resources of correctional officers. Preliminary positive results from rapid testing require confirmatory testing with conventional assays.

Treatment in Correctional Facilities

Principles of treatment for a person newly diagnosed with HIV in correctional setting should mirror those in the general community. For example, the first step to treatment is HIV education to ensure that the inmate is aware of necessary lifestyle changes and the importance of adherence to

treatment (US Department of Health and Human Services 2011). Before initiating antiviral medications, untreated infected persons need genotype testing to assess for viral resistance; some jail physicians have mistakenly started persons on medications prior to genotype testing in a rush to initiate medications before discharge. HIV-infected persons may need to be referred to an HIV specialist depending on the complexity of the inmate's medical issues and the primary care physician's familiarity with HIV management. Many inmates suffer from comorbidities such as mental illness, viral hepatitis, tuberculosis, and STDs. Medical staff in some correctional facilities may lack HIV specialists or persons with adequate HIV experience and education; these facilities can develop links to HIV specialists in the community and contract with providers to provide the necessary consultative care (Centers for Disease Control and Prevention 2009). Medical staff in the correctional facility who learn that an inmate has HIV and has been prescribed antiretroviral medication prior to incarceration should continue or resume treatments with assistance from the previous provider and medical records.

The two methods of administering HIV medications in facilities include directly observed therapy (DOT) and keep-on-person (KOP) therapy. The DOT system involves nurses administering medications in pill lines or by visiting cells; patients are known to have better adherence using this system, but an inmate may fear the loss of confidentiality. Conversely, KOP therapy requires an inmate to be responsible for taking his/her own medication on schedule. This method can aid in the adherence of HIV treatment after release, though as the infected person is not monitored by the medical staff, adherence to medical treatment while incarcerated will be unknown.

There are many aspects of HIV treatment in correctional facilities that can lead to breaches in confidentiality. For example, other inmates may learn of a positive HIV status if the number of pills fellow inmates receive is observed or if only HIV-infected inmates are receiving a medication with special handling, such as refrigeration. Most correctional facilities have abandoned the prior and unnecessary practice of segregating those

with HIV, but when selected inmates are called to the infirmary on the days the HIV specialist visit, an HIV diagnosis may be disclosed. It is important for facilities to create methods to guarantee inmates' medical privacy during HIV treatment delivery. In a setting where HIV may be stigmatized and the risks of violent acts are high, inmates may not feel safe while disclosing and receiving medical care from the medical staff. Trust between the staff and inmates is vital to encourage the treatment of HIV and to persuade HIV-positive inmates to initiate and adhere to the recommended treatments during their sentence and when released back into the community (Centers for Disease Control and Prevention 2009). Ultimately, the medical staff and the custody staff must work as a team to keep safe inmates being tested and treated for HIV.

Primary HIV Prevention in the Correctional System

More risk behaviors for HIV transmission such as substance abuse and unprotected sex takes place in the community than during incarceration (Braithwaite and Arriola 2003); however, the potential to transmit HIV and other infectious agents to others during incarceration exists. High-risk behaviors associated with HIV are present in correctional facilities, even though sexual activity and drug use are banned. A study completed in Georgia found 67 new cases of HIV during incarceration among inmates who tested negative at prison-entry HIV testing (Jafa et al. 2009). Precise data on HIV incidence and methods of transmission are not known. The incidence of HIV likely varies by facility.

Prevention programs for HIV in correctional facilities have met with controversy in some jurisdictions. Even providing education about HIV can face resistance among jail and prison staff. To decrease the concern that some curricula may contradict the rules and policies of the correctional system, such as using condoms for sex, educational messages to reduce HIV transmission may be presented in a way as it applies to hypothetical situations "once released from a correctional

facility.” In this way, correctional staff would not have to deviate from the rules in their facilities, and inmates would still have the opportunity to learn how to protect themselves and sexual and/or drug injection partners from HIV transmission (Braithwaite and Arriola 2003). Demonstration programs have shown that inmates and staff of jails and prisons can be reached with HIV education programs successfully. Education received in correctional facilities can benefit inmates during incarceration and in the community, upon release.

In 2006, the World Health Organization and the Joint United Nations Programme on HIV/AIDS published *HIV/AIDS Prevention, Care Treatment and Support in Prison Settings*, which strongly recommends the use of harm reduction strategies such as the distribution of clean needles and syringes or diluted bleach, along with instructions for cleaning injection equipment to reduce HIV transmission during drug injection, tattooing, or skin piercing, and condoms in correctional facilities (The World Health Organization and The Joint United Nations Programme on HIV/AIDS 2006). These prevention programs are not been politically feasible in the USA, despite the potential benefits to the population. Permitting condoms and syringes acknowledges that banned risk behaviors continue to take place despite official proscription. Condoms are available in state prisons in only Vermont and Mississippi and in jails in five cities (New York City, Philadelphia, San Francisco, Los Angeles, and Washington DC). Implementing strategies to reduce harm associated with injection drug use behind bars has largely occurred only outside of the USA.

Linkage to Medical Care After Release

Over nine million individuals are released from confinement to the community each year. Over 150,000 of these releasees have HIV. Medical services offered during incarceration may be the individual’s first link to primary and mental healthcare, as well as HIV/AIDS care. However, former inmates may encounter a host of social and medical challenges upon release. In a population

with an exceedingly high number of substance abusers, relapse to drug use is common upon release; homelessness and poverty often burden releasees as well. It is common for incarcerated persons to return to poor communities with inadequate access to HIV medical care. Upon release, inmates must find a doctor, make and keep an appointment, and fill prescriptions. These tasks require money, transportation, and motivation. As there are a considerable number of obstacles immediately after release, adherence to HIV treatments may not be a high priority for many releasees while trying to gain stability in the community. Any improvements in an inmate’s health observed during incarceration may quickly diminish if adequate care and treatment is not continued. Poor adherence to HAART can lead to disease progression and emergence of drug-resistant viral HIV strains (US Department of Health and Human Services 2011).

Prerelease discharge planning allows inmates to develop a plan to access medical care and treatment upon reentry into the community. A study in the Texas prison system showed that assistance with completing an AIDS Drug Assistance Program application increased the percentage of releasees who filled a HAART prescription before running out of 10 days of medications given at release from 2.5% to 7.9% (Baillargeon et al. 2009). HIV case managers assist with discharge planning to link recent releasees to healthcare and resources to alleviate social burdens in the community. Comprehensive discharge planning involves making an appointment with a community care provider, education about the importance of adherence to medications, transfer of medical records, assistance with insurance applications, and linkage to community HIV case management services. Upon release, former inmates should be provided with an adequate supply of medication to avoid interruption in treatment until an initial appointment in the community (Centers for Disease Control and Prevention 2009).

Starting in 2006, the Rollins School of Public Health at Emory University and Abt Associates, Inc., coordinated the evaluation of the Enhancing Linkages to HIV Primary Care and Services in Jail

Settings Initiative (EnhanceLink). This initiative successfully provided HIV testing in 20 US jails, resulting in 822 new diagnoses among 212,464 inmates (0.4%) who agreed to HIV testing. Services were provided to not only address linkage to medical care but also to address social needs upon release such as mental health and substance abuse treatment and linkage to housing. Transitional services were accepted by 82% of the HIV-positive persons in the program offered services (Spaulding et al. 2012). A substantial portion of these releasees linked to care were retained in care and were virally suppressed 6 months later; the study design permitted a cost analysis to demonstrate cost-effectiveness.

Conclusion

With a large proportion of HIV-positive individuals passing through jails and prisons in the USA, correctional health services have the opportunity to play an instrumental role in the diagnosis, clinical care, and linkage to services of HIV/AIDS patients. Improved HIV educational programs, HIV testing, and linkage to community care are needed to ensure that better health outcomes are observed in this population while incarcerated and after release to the community. The length of stay in a correctional facility determines what services can feasibly be delivered in correctional settings. Although several challenges are associated with HIV treatment and prevention in correctional facilities, demonstration projects have shown that public health involvement in jail and prison healthcare provides an opportunity to improve the lives of those who pass through the criminal justice system.

Cross-References

- ▶ [Behavioral Science Highlights of Evidence and Research](#)
- ▶ [HIV Counseling and Testing, Prevention of HIV](#)

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HIV Prevention in Transgender Persons

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Definition

The term “transgender” applies to a range of diverse individuals whose sex, gender identity, and/or gender expression are not concordant with the sex they were assigned at birth. The term “transgender women” will be used here to refer to individuals who were assigned male at birth and identify as female (sometimes described as “male-to-female” or “MTF”), and the term “transgender men” will be used to refer to individuals who were assigned female at birth and identify as male (sometimes described as “female-to-male” or “FTM”). Some transgender people do not identify as male or female but have identities

that fall outside of the binary; they may identify as “genderqueer” or as a nontraditional gender.

Although HIV is a concern for transgender communities in general, extremely high rates of HIV have been documented among transgender women of color in particular. However, research demonstrating efficacious HIV prevention strategies for transgender populations has lagged, due in part to the complexities of research with this population. Transgender identity can be difficult to assess due to variations in terminology, defining and reaching this hidden population, and varying preferences among transgender people regarding the disclosure of their transgender status.

Data about transgender people are currently not collected on a national level in the United States; therefore, there is currently no accurate way to estimate the number of transgender people living in the United States. Due to limitations on how data are collected in public health settings, transgender people are usually either deleted from datasets or misclassified. For example, transgender women are routinely included in the risk group “men who have sex with men” (MSM). While the MSM data then include HIV rates among transgender women, the programs that are then funded based on these data are not inclusive of transgender women’s needs and concerns. HIV prevention research with this population has also faced barriers related to “syndemic dynamics” or numerous co-occurring and interrelated epidemics that influence HIV risk among transgender people, such as mental health issues, substance abuse, and victimization (Brennan et al. 2012; Operario and Nemoto 2010).

HIV Prevalence Among Transgender People

Although national-level data are not available, many regional studies have been conducted around the United States to estimate rates of HIV among transgender people, to document risk factors, and to determine HIV prevention needs and strategies. Most research has been conducted with transgender women as opposed to transgender

men, due to the disproportionately high risk for HIV among transgender women. A meta-analysis of 29 studies found that in the four studies that included HIV testing, 28% of transgender women tested positive for HIV (Herbst et al. 2008), with higher rates (56%) found among African-American transgender women. In the 18 studies that relied on self-report only, 12% of transgender women reported living with HIV. Less is known about rates of HIV among transgender men due to the paucity of studies focused on this population; rates that have been reported thus far range from 0% to 3% (Herbst et al. 2008).

Despite high rates of HIV among transgender women, multiple barriers to HIV testing exist for transgender populations, including low levels of knowledge, comfort, and skills among health and social service providers, lack of transgender-friendly testing sites, and a lack of transgender-specific HIV prevention programs providing education about risk and the importance of HIV testing (Sevelius et al. 2011).

Factors that Contribute to High Rates of HIV Among Transgender Women

Social and Structural Issues

Transphobia

The negativity that transgender people face in society is referred to as “transphobia” and is defined as the discrimination against or devaluation of someone due to their gender presentation and/or transgender identity. Many transgender people endure transphobia from a very young age, often emerging as conflict with their families and at school (Brennan et al. 2012). Young transgender women are especially vulnerable to harsh treatment in our society where effeminate men are often targets of ridicule and abuse. Subsequently, many young transgender people, especially young transgender women, drop out of school and live on the streets, leaving them with few job skills and the prioritization of basic survival (Sugano et al. 2006). Data suggest that young transgender women who experience more transphobia are also more likely to engage in behavior that put

them at high risk for HIV infection (Sugano et al. 2006).

Internalized Transphobia and Mental Health

Experiences of discrimination and victimization negatively impact mental health by increasing anxiety, depression, and suicidality (Clements-Nolle et al. 2001; De Santis 2009). Internalized transphobia occurs when transgender people incorporate society’s negative beliefs about being transgender into their own self-concept. Internalized transphobia may lead to low self-esteem and high levels of depression and anxiety (Melendez and Pinto 2007), which in turn can lead to (higher rates of) unprotected sex (De Santis 2009). Internalized transphobia may also contribute to transgender people seeking gender affirmation from sexual partners in ways that jeopardize their health (Melendez and Pinto 2007; Sevelius 2012). Transgender women have reported having unsafe sex to affirm their female gender identities, thereby decreasing sexual negotiation and communication (Nuttbrock et al. 2009). A new model that conceptualizes how the need for gender affirmation contributes to risk behavior may inform future research and interventions to address sexual risk due to the need for gender affirmation among transgender women and girls (Sevelius 2012).

Incarceration

Rejection from families of origin, being homeless or marginally housed, and experiencing unemployment are some primary reasons why transgender women may engage in survival activities, such as sex work. Sex work may lead to incarceration (Garofalo et al. 2006), which partially accounts for the overrepresentation of transgender women in prisons and jails. When released from custody, transgender women face continued employment and housing discrimination and often become caught in cycles of sex work, drug use, and incarceration (Operario and Nemoto 2010).

Like shelters, most prisons and jails in the United States sex-segregate prisoners according to genitalia, and many transgender women do not have access to genital surgery (even if they would choose it) due to the high costs of these

procedures. For this reason transgender women are usually housed with male inmates, which can lead to violence, sexual assault, and harassment while incarcerated, directly increasing their HIV risk (Grant et al. 2011). Transgender women often do not receive trans competent health care while incarcerated, and transgender inmates are often denied medically necessary hormone replacement therapy (Grant et al. 2011).

Behavioral Risk Factors

The severe stigma, discrimination, and violence that transgender women face underlie many of the HIV-related risk behaviors frequently reported in this population. Risk behaviors for transgender women include multiple sex partners, unprotected receptive anal intercourse, sex work, sex under the influence of alcohol and drugs, and unsafe needle use for injecting drugs and gender-related hormones or silicone (Brennan et al. 2012; Operario and Nemoto 2010). HIV risk behaviors among transgender women appear to be disparately distributed along racial and ethnic lines. In several studies, African-American and Hispanic transgender women reported greater risk behaviors compared with white and Asian and Pacific Islander transgender women (Herbst et al. 2008; Nuttbrock et al. 2009).

Despite high HIV prevalence rates, transgender women frequently underestimate their risk of acquiring or transmitting HIV (Herbst et al. 2008). The previously mentioned meta-analysis found that almost half (44%) of transgender women reported unprotected receptive anal intercourse, with the highest rates being reported with sex work clients (39%) and primary partners (37%) (Herbst et al. 2008). Sex under the influence of drugs and/or alcohol is one of the most commonly cited sexual risk factors among transgender women as it is often unprotected (Wilson et al. 2009).

While current rates of HIV among transgender men are lower compared with rates among transgender women, initial evidence suggests that some transgender men who have sex with non-transgender men may be at higher risk (Sevelius 2009). In addition, some transgender men engage in sex work and substance use,

although these rates are also significantly lower than those among transgender women (Clements-Nolle et al. 2001).

Sex Work

Experiences of employment and housing discrimination are common among transgender women and lead directly to the need to engage in survival sex work for many who are denied opportunities for education, job training, and basic social services due to their transgender identity and/or gender presentation (Brennan et al. 2012; Grant et al. 2011; Nuttbrock et al. 2009).

Sex work is common among transgender women, with reported rates of involvement ranging from 26 to 80% (Operario et al. 2008). Sex work may be perceived as the only means of survival, due to lack of other forms of social support, pervasive employment discrimination, and issues with legal documentation of gender identity. There may also be a generational effect in which older transgender women sometimes introduce younger women into sex work practices (Wilson et al. 2009). Young transgender women are often quickly introduced to the financial rewards of sex work as a means of survival, receiving much needed support from older transgender people and being validated in their chosen gender identity by both their colleagues and their clients (Brennan et al. 2012; Wilson et al. 2009). Young transgender women of color are particularly vulnerable.

The financial incentive to engage in sex work is especially high for transgender women who pursue gender confirmation procedures, such as taking hormones and undergoing surgeries (i.e., facial feminization, breast augmentation, and genital reconstruction), which are expensive and usually not covered by health insurance. The financial need created by expensive gender confirmation procedures may lead some transgender women to engage in particularly risky behaviors because sex work clients will sometimes pay more for barrier-free sex or to have women inject drugs with them.

Transgender women often report using substances to cope with sex work and other stressors associated with the stigma of being transgender,

thereby decreasing their desire for and self-efficacy to negotiate safer behaviors. In particular, sex work has been consistently linked with use of methamphetamines as a means of coping with the stress of sex work and maintaining stamina. Drug use can in turn increase sex workers' risk of acquiring HIV through needle sharing and sex under the influence of drugs. Indeed, it has been demonstrated that transgender female sex workers have higher rates of HIV than non-transgender male or female sex workers; a recent meta-analysis of studies examining HIV status of transgender female sex workers found that 28% were HIV-infected (Operario et al. 2008).

Substance Use

Substance abuse has been consistently linked to HIV risk among transgender people (Herbst et al. 2008). Among transgender women, unprotected sex under the influence of substances seems to be especially prevalent with primary partners, but is also reported with paying and casual partners (Melendez and Pinto 2007). In San Francisco, transgender people who reported injection drug use were almost three times more likely to be HIV positive than those who did not report injection drug use (Clements-Nolle et al. 2001).

Despite high rates of reported substance use and associated negative health outcomes, there are few recovery or detoxification programs that are perceived as safe for transgender women to access and designed to address trans-specific treatment needs. Few training programs prepare substance abuse treatment professionals by providing the necessary knowledge and skills to support transgender people in treatment. Often, when transgender people seek substance use treatment, they are forced to choose between expression of their gender identity or staying in treatment, disclosing or forgoing the use of hormones, and tolerating or combating gender-based harassment from staff and/or other residents. If transgender people find a treatment program willing to serve them, they are often housed according to birth sex and not according to their gender identity and expression. As a result of these negative experiences, many transgender people avoid treatment settings altogether. Anti-discrimination policies in

drug treatment settings, arranging appropriate accommodations, increasing the sensitivity of providers, and honoring the experiences, preferences, and self-expression of transgender people in treatment are all important ways to begin creating trans-inclusive substance abuse treatment programs.

Best Practices for HIV Prevention with Transgender Populations

It is vital that any HIV prevention efforts with transgender populations are grounded in the transgender community through partnerships, advisory boards, and opportunities for transgender people to contribute. By involving transgender people in shaping and promoting HIV prevention efforts, the acceptability and relevance of the programs is enhanced dramatically. Programs should acknowledge that transgender people of color are affected differentially by HIV, and prevention programs should address issues that are relevant to their local communities of color, when appropriate. Multicomponent interventions that address multiple co-occurring issues that influence HIV risk among transgender people are likely to be most effective (Operario and Nemoto 2010). Another important aspect of HIV prevention with transgender people is advocacy to identify and mitigate structural barriers, such as lack of access to safe housing, employment, addiction recovery services, and health care.

Based on the high proportion of undiagnosed HIV infection among those tested, transgender people represent an important community for enhanced HIV testing and prevention efforts. Yet, HIV prevention programs often do not adequately represent and reflect the lives and experience of transgender people. In addition, most HIV prevention programs that do prioritize transgender people tend to exclude transgender men. Many HIV prevention providers assume that transgender men only have sex with non-transgender women and are therefore at low risk for contracting HIV. Efforts should continue to develop novel strategies to overcome barriers and provide HIV testing and prevention services

to transgender people. Based upon current clinical findings and evidence pertaining to the frequency of HIV risk-related behaviors, transgender people should be encouraged to have an HIV test at least annually, or more often if indicated.

Conclusion

Public health intervention research has produced few culturally specific, evidence-based HIV prevention interventions for transgender people. Of the published interventions that have been implemented with transgender women, none have yet been controlled trials or were rigorously evaluated for efficacy or effectiveness. The Centers for Disease Control and Prevention has funded programs to adapt evidence-based interventions for transgender women, but these interventions were developed for other target populations and do not account for the unique cultural context of risk behaviors among transgender women. Given the complex sexual risk factors present among transgender populations, HIV prevention programs developed for other populations that are simply adapted to include new language will not ultimately address the cultural context in which risk behaviors occur and in which protective factors develop. HIV prevention interventions need to be based on the culture and context that most influence the lives of transgender people (Sevelius et al. 2011).

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HIV Prevention in Youth

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Definition

HIV prevention in youth refers to programmatic efforts that aim to prevent new HIV cases among individuals younger than 25 years of age. Youth between the ages of 15 and 24 account for a disproportionate amount of worldwide HIV incidence (United Nations Children's Fund (UNICEF) 2011). In order to halt the HIV epidemic, numerous interventions have been designed to prevent the transmission of HIV among youth. Evaluations of these interventions indicate that they are especially effective in changing young people's HIV-related knowledge, attitudes, and skills but less effective in generating behavioral change. A continuum of prevention that targets not only youth but also their sociocultural context (► [Multilevel Interventions/Structural Approaches to HIV Prevention](#)) throughout adolescence and early adulthood is necessary for the reduction of HIV incidence among this population.

Epidemiology of HIV Among Young People

In 2009, youth aged 15–24 accounted for 16% of the people living with HIV and 42% of new infections among all people aged 15 and older (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2010). The characteristics of the HIV epidemic among youth vary greatly by country and area of the world. In developing and least-developed countries, HIV prevalence is over twice as high among young women as it is among young men. By contrast, in industrialized countries, prevalence is generally higher among young men (UNICEF 2011).

Modes of transmission also differ by region. In sub-Saharan Africa, heterosexual activity is the primary driver of the epidemic among young people. In Central and Eastern Europe, injecting drug users (IDUs) account for about 80% of all new infections (Interagency Youth Working Group 2008). In the Americas and western-European Countries, young men who have sex with men (YMSM) (► [HIV Prevention for MSM](#)) are particularly affected by the epidemic (UNICEF 2011). In the United States, for instance, nine of every ten HIV-positive young men become infected through male-to-male sexual contact (CDC 2012).

Developmental Considerations and HIV Risk

Adolescence refers to the transitional period between childhood and adulthood. Although the specific age ranges are not well established, it is generally agreed that adolescence starts with puberty and ends when the person becomes an independent adult. UNICEF divides the developmental period corresponding to adolescence into young adolescence (between ages 10 and 14), older adolescence (ages 15–19), and young adulthood (ages 20–24).

Because morbidity and mortality rates are generally lower during this period than during other life stages, adolescence has traditionally been seen as a healthy period of life. However, the physical, psychological, and social changes that occur at this age can place young people at increased risk for HIV. Prevention of HIV infection among youth requires an understanding of how the characteristics unique to this developmental stage may increase young people's risk for HIV infection. These include individual characteristics, such as physical and psychological changes; characteristics of the relationships with close others, like peers, parents, and partners; and characteristics of the broader social context, such as the legal system.

Individual characteristics. Early in adolescence, individuals undergo pubertal changes that result in the maturation of the sex organs. The

ability to produce sperm (spermarche) and ova (menarche) signals the young individual's potential for sexual reproduction. As these biological changes take place, the adolescent begins establishing his or her own identity or sense of self (Arnett 2010). Adolescence is a time of exploration that allows shifting identities across contexts and experimentation with new behaviors that may increase one's HIV risk.

Sexual activity and drug use usually occur for the first time during adolescence. However, both physiological as well as cognitive characteristics may increase the likelihood of experiencing negative outcomes from these experiences. For example, neuroscience research has provided evidence that the prefrontal cortex, which is associated with higher-level processing, self-regulation, and decision-making, is not fully developed until young adulthood (Arnett 2010). As a result, many youth start using drugs or become sexually active before their ability to evaluate the long-term consequences of their behavior is fully developed.

In terms of cognitive development, adolescence is characterized by egocentric thinking or the belief that one is unique and thus not susceptible to the negative outcomes that befall others (Arnett 2010; Pedlow and Carey 2004). This form of optimistic bias results in lowered risk perceptions regarding one's chances of acquiring HIV. Furthermore, the availability of highly active antiretroviral therapy (HAART) (► [cART and Supportive Care for HIV-Associated Malignancies](#)) may minimize the perceived severity of HIV (The Foundation for AIDS Research (amfAR) 2010).

Relationships with close others. Although parental figures are generally very important for adolescents, peers and sexual partners become increasingly relevant in this stage. Much research has shown that perceiving that one's peers are engaged in sexual activity and drug use can increase one's own likelihood of engaging in similar risky behaviors. It is important to recognize that, although peers may influence the adoption of unhealthy behaviors (such as drug use), they may also encourage healthy behaviors (like condom use).

The characteristics of the relationships with sexual partners are also relevant for HIV risk among youth. Adolescents tend to engage in serial monogamy or several short-term monogamous relationships. This can lead to several sexual partners in a short period of time, which increases the risk of HIV acquisition. Furthermore, adolescents may have older sexual partners, making it harder for them to refuse sex and negotiate condom use. This is particularly a concern for young women, especially when males are accorded more social and political power and gender-based violence is prevalent (UNAIDS 2010; UNICEF 2011).

Broader social context. Poverty, limited access to education, and lack of employment opportunities are structural characteristics that can also increase the likelihood of HIV infection among adolescents. Young people in impoverished conditions are less likely to have accurate knowledge about HIV and how to prevent it. Additionally, homeless youth and out-of-school youth have increased likelihood of engaging in high-risk behaviors, like drug use and sex work, often as a means of survival.

Laws and policies are important contributors to adolescents' engagement in safe or risky practices. In several countries, parental consent is legally required for youth seeking access to health services such as HIV testing, contraceptive counseling, and harm reduction programs. However, young people are often unwilling to discuss their risk behaviors with their parents and thus are forced to forgo these services. The stigma associated with HIV-risk behaviors, such as male-to-male contact (► [HIV prevention for MSM](#)), sex work, and drug use, may further hamper youth's access to services (Access to Care) and programs, even in countries that have provisions allowing minors to access services without parental consent.

HIV Prevention Interventions for Youth

Awareness of the threat to adolescent health posed by HIV has resulted in the development and implementation of several risk reduction interventions targeting preadolescents, adolescents, and young adults. Because the majority of HIV

infections among youth are acquired through unprotected sexual intercourse, most of these interventions provide information and skills to enable youth to delay sexual onset and/or decrease sexual risk behaviors. Schools across the world provide a convenient location for such interventions because they have the infrastructure to serve large proportions of youth before and, in many cases, after sexual debut (Gallant and Maticka-Tyndale 2004). Nonschool-based programs are also fundamental in meeting the needs of at-risk subpopulations like IDUs or YMSM (► [HIV Prevention for MSM](#)).

School-based interventions. To achieve the greatest reduction in HIV incidence, school-based sex education programs that promote knowledge about HIV and positive health beliefs, skills, and behaviors should be implemented early, as they are particularly effective among youth who have not yet become sexually active (UNICEF 2011). Much research has shown that youth are not likely to return to abstinence after sexual debut. Thus, school-based interventions must target both sexually active and nonsexually active adolescents with prevention messages. Comprehensive risk reduction programs that encourage abstinence but provide information about medically accurate risk reduction strategies are likely to reach more students than abstinence-only programs. Research has shown comprehensive sex education programs to result in increased condom use (► [Male Condoms](#); ► [Female Condoms](#)) as well as reduced current sexual activity, frequency of sexual activity, number of sex partners, frequency of unprotected sex, and reduced pregnancy and STI rates. Abstinence education programs may reduce current sexual activity but fail to show significant effects on any other behavioral outcomes (Chin et al. 2012).

HIV education in primary and secondary schools is quite common: 64% of primary schools and 88% of secondary schools in 137 reporting countries included HIV in their curricula in 2007 (UNICEF 2011; United Nations). It is important to note that content and provision of sex education is often dictated by policies at multiple levels of government. Furthermore, school-based interventions are often delivered by teachers or health

educators who may alter important content or refuse to deliver components to students (Gallant and Maticka-Tyndale 2004; Poobalan et al. 2009).

Although schools provide an ideal setting to reach large numbers of youth throughout the process of sexual and social maturation, it can be difficult to engage youth from diverse backgrounds with various levels of experience in programming activities. School-based programs may not be well suited to reaching youth that are most at risk of HIV, such as YMSM (► [HIV Prevention for MSM](#)) and IDUs. By nature, tailored interventions targeting such high-risk populations are not relevant for all youth and thus are more likely to be implemented in community settings familiar to the targeted population than to be implemented in school settings. The impact of school-based interventions is further limited when schools are unable or unwilling to provide condoms and HIV tests.

Nonschool-based interventions. School-based interventions are an important component of the prevention continuum. However, given the aforementioned limitations of school-based programs, it is fundamental to implement preventive activities in other settings such as health care systems, youth-serving organizations, and the broader community.

Provision of health care services, including condom distribution and HIV testing (► [HIV Testing and Counseling](#)), is central for HIV prevention among youth. Making these services “youth friendly” and providing outreach or out-of-facility services for those youth who are unwilling or unable to attend health care settings have been found to be effective (Denno et al. 2012). Youth-serving organizations that understand the needs of high-risk youth are well suited to offer tailored harm reduction interventions. For example, it may be difficult to identify lesbian, gay, bisexual, and transgender (LGBT) youth in school settings, but organizations serving LGBT youth provide a safe place to talk about sensitive issues such as sexual behavior.

Community-wide interventions can be useful in providing education, changing social norms regarding sexual activity, and decreasing the stigma of HIV among the public. There is some

evidence to suggest that theory-based mass communication (► [Mass Media and HIV Prevention](#)) campaigns that target specific audiences with health-promoting messages can increase condom use and HIV testing intentions and behavior (Noar et al. 2009). Multicomponent (► [Multilevel Interventions/Structural Approaches to HIV Prevention](#)) campaigns that include efforts to change individual behavior as well as promote awareness are likely to be most effective.

Characteristics of Successful Interventions

HIV prevention programs aimed at youth measure success in terms of behavioral outcomes such as delayed sexual onset (► [Delayed Sexual Debut](#)), decreases in frequency of sexual activity, increases in condom use (► [Male Condoms](#); ► [Female Condoms](#)), and decreases in number of sexual partners. Cognitive antecedents of behavior change, such as attitudes and knowledge, are also frequently used as indicators of program success. Several reviews of HIV risk reduction interventions have found that they are more likely to have a positive impact on sexual knowledge and attitudes than on sexual behavior in general (Poobalan et al. 2009). When interventions do influence behavior, they are more likely to change sexual risk behaviors than level of sexual activity (Pedlow and Carey 2003). A systematic review of HIV prevention interventions found large effect sizes for sexual risk communication skills (.50), condom use skills (.30), and quantity of sexual risk communications (.27), but only small effect sizes for condom use (► [Male Condoms](#); ► [Female Condoms](#)) (.07) and reduced sexual frequency (.05) (Johnson et al. 2003).

The approaches taken to achieve these outcomes vary considerably. However, there is some consensus regarding the characteristics necessary to effect positive change. Reviews of successful HIV prevention programs targeting youth in developed and developing countries suggest at least four key components: (1) a focus on HIV, (2) structured learning activities based on a

theoretical framework, (3) developmentally appropriate information, and (4) consideration of the needs of the target audience (Gallant and Maticka-Tyndale 2004; Kirby et al. 2006; Poobalan et al. 2009).

HIV focus. The prevailing recommendation for HIV risk reduction interventions is to narrowly focus on behaviors that can reduce the likelihood of HIV acquisition, such as condom use (► [Male Condoms](#); ► [Female Condoms](#)), and communicate clear messages in progressive stages to encourage increases in the desired health behaviors and reductions in the undesired risk behaviors (Kirby et al. 2006; Poobalan et al. 2009). An alternative approach is offered by positive youth development programs that do not focus on a single health risk behavior but rather target the well-being of adolescents by promoting the development of confidence (including optimism about the future), caring, connection, character, and competence (Lerner et al. 2003).

Theoretical framework and structured learning activities. Successful interventions include learning activities that are guided by theory. Because health behavior theories specify determinants of behavior and behavior change, they are useful in identifying targets for intervention. The most common theoretical basis for HIV risk reductions targeting adolescents is social cognitive theory. This theory posits that behavior is the result of learning that is acquired through interactions between the individual and his or her environment. Additionally, the theory notes that belief in one's ability to execute the recommended behavior (e.g., condom use) is critical to enactment of the behavior. Programs based on social cognitive theory frequently employ skill-building activities that are appropriate for the developmental stage of the participants (DiClemente et al. 2008). For example, intervention programs often include role-playing activities that allow youth to develop their communication, negotiation, decision making, and condom application skills (Pedlow and Carey 2004). These types of active learning activities are more effective than passive learning activities such as watching films (Poobalan et al. 2009).

Developmentally appropriate. Programs should focus on delaying sexual initiation (► [Delayed Sexual Debut](#)) of nonsexually active youth and aim to increase safe sex practices among sexually active youth (Malow et al. 2007), especially as research has shown abstinence among sexually active youth to be unlikely (Pedlow and Carey 2004). Interventions targeting preadolescents and those who are not yet sexually active have been found to be most effective in decreasing sexual risk (Poobalan et al. 2009), perhaps because they can aid in the development of safe sexual behaviors before patterns of sexual risk behaviors are established (Pedlow and Carey 2004). However, programs targeting sexually active adolescents are also important, as adolescents tend to have several short-term monogamous relationships in close succession (resulting in multiple partners) and are not likely to consistently use condoms (Pedlow and Carey 2004; amfAR 2010). Furthermore, given the importance of peers during adolescence, interventions that target youth within their social networks may be particularly effective. Group interventions, such as those that occur in school settings, are poised to leverage social influences such as reinforcement, norm change, and interpersonal learning (Pedlow and Carey 2003). Additionally, booster sessions after the formal conclusion of interventions have been identified as an effective means of providing youth with meaningful information at various stages of sexual experience (Pedlow and Carey 2004).

Tailoring. Successful interventions are tailored to meet the needs of the adolescent population of interest (Malow et al. 2007). In addition to age, programs should be sensitive to the differential influence of gender roles and sexual orientation (Sexual Orientation). It has been found, for example, that interventions targeting condom use (► [Male Condoms](#); ► [Female Condoms](#)) are more effective among young men than among young women, arguably because it is harder for young women to negotiate condom use and to refuse sexual intercourse (Michielsen et al. 2010).

Interventions targeting most-at-risk subgroups should clearly be tailored to address the specific

needs and risk patterns of such groups. In the United States, for example, despite the fact that YMSM (► [HIV Prevention for MSM](#)) are disproportionately affected by HIV, only about 5% of HIV interventions among youth addressed same-sex sexual activity or gay/bisexual sexual orientation (Harper and Riplinger 2013). IDUs are another group at high risk for HIV infection. Prevention among this group should not only include harm reduction (► [Harm Reduction for Injection Drug Users \(IDUs\)](#)) strategies (like access to needle-exchange programs) but should also aim to reduce high-risk sexual practices (such as inconsistent condom use) frequently linked to substance use (UNICEF 2011).

Approximately 7.5 million children and youth under the age of 25 were living with HIV in 2009 (UNICEF 2011; United Nations). Interventions for HIV-positive youth should focus on testing (► [HIV Testing and Counseling](#)) and treatment as most infected youth are not aware of their status. For example, it is estimated that 20% of infected youth in the United States do not know their status. Of those that do know their status, only 30% have access to adolescent health services (Access to Care) (amfAR 2010). Promoting adherence to treatment as well as safe sexual practices has the potential to decrease transmission of HIV among youth. “Treatment as prevention” (► [Prevention for People Living with HIV](#)) efforts should be accompanied by community-wide programs that aim to decrease stigma and discrimination (Stigma and Stigmatization) towards HIV-positive youth (UNICEF 2011).

Future Directions

Researchers have called for better measures of behavioral outcomes such as condom use (► [Male Condoms](#); ► [Female Condoms](#)) (which has been measured in terms of frequency at first sexual encounter, at last sexual encounter, and numerous other ways) as well as biological markers to confirm the presence or absence of STIs and HIV (DiClemente et al. 2008; Michielsen et al. 2010; Pedlow and Carey 2003). More research on, and programs for, vulnerable

populations such as YMSM (► [HIV Prevention for MSM](#)) are needed (amfAR 2010). There is also a need for better understanding regarding the impact of contextual factors on youth's beliefs and behaviors. Viewing HIV as a health outcome that is influenced by relational, familial (► [Family Interventions](#)), community, and societal contexts (DiClemente et al. 2008) may encourage the development of multilevel interventions (► [Multilevel Interventions/Structural Approaches to HIV Prevention](#)) that allow for a more comprehensive explanation of behavior and lead to lasting behavioral change.

Conclusion

A world without AIDS requires prevention of HIV among today's youth. Although adolescence is widely considered a period of good health characterized by relatively low disease burden, the health risk behaviors of adolescents merit special attention given their contribution to current and future health. Comprehensive sex education programs that provide medically accurate information regarding condoms and contraception hold particular promise for reaching youth with HIV educational messages and skill-building activities before and after sexual debut. However, these types of programs may not be welcomed by politicians, school administrators, and parents. Efforts to reach particularly vulnerable populations such as YMSM (► [HIV Prevention for MSM](#)) and IDUs will require tailored interventions that address their social and cultural context. Progress has been made in the global fight against AIDS and continued HIV prevention efforts targeting youth can contribute to a world with zero HIV infections, zero discrimination, and zero AIDS-related deaths (UNAIDS 2010).

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HIV Reservoirs in Lymph Nodes and Spleen

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Definition: The Lymphatic Reservoir

The lymphatic reservoir is constituted by subsets of the CD4+ T lymphocytes, dendritic cells, and cells of monocyte–macrophage lineage which become infected by HIV. After infection, these cells harbor transcriptionally silent but replication-competent HIV DNA, beyond the reach of host immune responses and antiretroviral therapy (ART) (Shan and Siliciano 2013). These cells are generally long-lived and maintain the capacity for viral replication throughout their life spans.

Introduction

After entry into the body, HIV rapidly spreads to regional lymph nodes and other lymphoid tissues. This early exploitation of the lymphatic system is mediated by specific host cells and defines the long-term course of HIV infection (Brenchley et al. 2004; Kramer-Hammerle et al. 2005; Moir et al. 2015). CD4+ T lymphocytes, dendritic cells, and cells from the monocyte–macrophage lineage are principal players in these events, and subsets of these cells become latent reservoirs of the virus within the lymphatic system (Blankson et al. 2002; Vicenzi et al. 2007).

The Lymphatic System

The lymphatic system consists of a complex network of vessels connecting primary lymphoid organs (bone marrow and thymus) to secondary lymphoid organs such as spleen, tonsils, lymph nodes, and gut-associated lymphoid tissue (GALT). Among other roles, the lymphatic system serves essential functions in immune system regulation and as the channel of transport for leukocytes, T lymphocytes, macrophages, and dendritic cells throughout the body (Angeli and Randolph 2006; Platt and Randolph 2013a, b). Lymphatic capillaries (also known as initial lymphatics) are lined with a permeable endothelium of a single cell layer and permit the migration of leukocytes between the lymphatic system, interstitial tissues, and bloodstream. Lymphatic capillaries unite at larger-caliber lymphatic vessels, which may contain muscle cell layers and unidirectional valves to facilitate lymph transport (Baluk et al. 2007). Lymph arrives via afferent lymphatic vessels to lymph nodes and other lymphoid organs and tissues.

Lymphatic vessels are closely coupled to blood vessels throughout the body, with the exception of the brain. Due to strict volume and pressure environment of the brain's enclosed space as well as the selective permeability of the blood–brain barrier, the brain lacks its own lymphatic network. Nevertheless, lymphatic vessels at the cribriform plate play an integral role in cerebrospinal fluid

drainage, with cervical lymph nodes serving as important sites for immunologic responses of CSF-derived leukocytes (Koh et al. 2005; Weller et al. 2006; Wagshul and Johnston 2013).

Lymph enters the subcapsular sinus of lymph nodes and flows through the sinusoidal spaces, where antigens from the periphery, carried mainly by the antigen-presenting dendritic cells, elicit a cascade of immune responses. Follicles within the lymph node cortex are sites of antigenic stimulation and are comprised mainly of B lymphocytes. T lymphocytes mostly comprise the deeper paracortex, while the even deeper medulla is the site of plasma cell maturation (Miranda et al. 2013).

The migration of leukocytes through the lymphatic system is a highly selective process requiring regulated interactions between leukocyte surface proteins and endothelial cell receptors (Cyster and Schwab 2012). The majority of leukocytes transported by afferent lymphatic vessels are lymphocytes, while up to 20% are dendritic cells, mostly derived from mononuclear phagocytes but distinct from macrophages (Smith et al. 1970). Other types of leukocytes, including plasma cells, neutrophils, eosinophils, and basophils, make up a minority of the afferent lymph leukocytes (Smith et al. 1970). Of the T lymphocytes in the afferent lymph, CD4⁺ T lymphocytes are overwhelmingly more abundant than CD8⁺ T lymphocytes, with a ratio several times higher than their ratios in the bloodstream (Mackay et al. 1990). Efferent lymph cellularity is mostly comprised of lymphocytes (as dendritic cells generally remain in lymph nodes until cell death) which are dispersed back into the bloodstream (Kamath et al. 2002; Braun et al. 2011).

Lymph Nodes as an Epicenter of HIV Pathogenesis

Viral dissemination occurs via infected lymphocytes and dendritic cells circulating throughout lymph nodes and lymphoid tissue (Moll 2003). Subsequent production of various pro-inflammatory cytokines (IL-1 α / β , IL-2, IL-6, IL-7, IL-12, IL-15, TNF- α) stimulates lymph

node cell proliferation; this is the fundamental etiology of the diffuse lymphadenopathy of acute and asymptomatic HIV infection (Levy 2007; Vicenzi et al. 2007). Lymph nodes at this stage are characterized by robust follicular hyperplasia with prominent germinal centers. The expansion of B lymphocytes within the lymph node sinuses also contributes to lymphadenopathy (Miranda et al. 2013). Accumulation of lymphocytes within lymph nodes disrupts germinal centers, resulting in follicular lysis and hemorrhage. The intense inflammatory responses secondary to cellular migration and pro-inflammatory cytokine production lead to an alteration of the lymph node structure, with additional cellular recruitment, angiogenesis within the lymph node, and irregular collagen deposition (Miranda et al. 2013). In advanced HIV infection, follicles undergo atrophy and are dominated by follicular dendritic cells, with scant presence of lymphocytes as they are gradually depleted (Miranda et al. 2013). As such, lymph nodes in advanced HIV are fibrotic and exhibit a distorted architecture.

Throughout the course of HIV infection, CD4⁺ cell counts decline as infected lymphocytes are destroyed by cytopathic effects of HIV, cytotoxic lymphocytes, and apoptosis. Furthermore, infection and destruction of thymocytes impairs repletion of the lymphocyte pool (Vicenzi et al. 2007). Eventually, the dendritic cell network deteriorates as well (Burton et al. 2002).

Resting Memory CD4⁺ T Lymphocytes as Reservoirs

CD4⁺ T lymphocytes, containing integrated HIV DNA and circulating between peripheral lymph nodes, are a stable reservoir, which has been extensively studied and represents a major barrier to HIV cure (Chun et al. 1997; Moir et al. 2015). In addition to lymph nodes and the spleen, GALT tissue harbors this reservoir. The reservoir burden varies between different tissues with GALT CD4⁺ T lymphocytes harboring HIV DNA at levels several times higher compared to peripheral blood, even in the setting of effective ART (Moir et al. 2015). Of the different CD4⁺ T cell subsets,

resting or unactivated, memory CD4⁺ T lymphocytes are the main reservoir due in part to their long life spans. The role of infected naïve CD4⁺ T lymphocytes is less clear though there is evidence to suggest that they may also serve as a reservoir (Wightman et al. 2010). Integrated viral genome (provirus) in CD4⁺ T lymphocytes remains in an archived state and does not contribute to viral replication unless the cell is activated.

It is not well understood how resting T lymphocytes become reservoirs of HIV. One possibility is that HIV simply infects resting T lymphocytes; however, this mechanism would be inefficient because cellular activation is generally required for HIV genome integration (Moir et al. 2015). Unactivated, resting CD4⁺ T lymphocytes have intrinsic barriers to HIV infection, including low CCR5 expression and inefficient capabilities for reverse transcription and integration (Shan and Siliciano 2013). Alternatively, HIV may infect activated T lymphocytes to initiate the reservoir. Activated T lymphocytes possess a cytoplasmic and nuclear environment conducive to efficient reverse transcription and integration and are primary drivers of viral replication. Unlike resting T lymphocytes, however, activated T lymphocytes are generally short-lived; if not destroyed by cytopathic effects of HIV or by cytotoxic T lymphocytes, they are destined for apoptosis. Activated T lymphocytes infected by HIV occasionally escape the pathways that lead to cell death and instead revert back to a resting memory state through poorly understood mechanisms (Shan and Siliciano 2013). Regardless of the mechanism, establishment of T-lymphocyte reservoirs likely occurs most abundantly during the viremia peaks of acute infection, but viremia in chronic infection may replenish or expand the reservoir.

Only a small fraction of resting T lymphocytes become reservoirs of HIV. Indeed, infected resting T lymphocytes are relatively scarce during effective ART, measuring only about one per million resting T lymphocytes. Nonetheless, this means there may be 1×10^7 resting T-lymphocyte reservoirs in an individual (Chun et al. 1997). Recently, it was suggested that the size of the latent reservoir may be up to 60-fold higher than

previous estimates (Ho et al. 2013). CD4⁺ T lymphocytes thus provide an expansive, stable reservoir, which can persist for a significant period of time. The half-life of resting memory T lymphocytes has been estimated to be 44 months, theoretically requiring approximately 70 years for this reservoir to fully decay naturally, provided 100% effective ART and limited reservoir clonal expansion (Siliciano et al. 2003; Moir et al. 2015).

Follicular Dendritic Cells as Reservoirs

Dendritic cells are crucial to adaptive immunity. They trap and process foreign antigens on their dendritic processes and migrate to secondary lymphoid sites, where they induce strong T-lymphocyte activation and proliferation in an antigen-specific manner (Soumelis et al. 2007). Multiple lineages of dendritic cells exist, with plasmacytoid dendritic cells (derived from lymphoid precursors) and myeloid dendritic cells (derived from myeloid precursors) being the major peripheral dendritic cells (Soumelis et al. 2007). Both express CD4, CCR5, and CXCR4 to varying degrees and can become infected by HIV, but display limited capacity for viral replication (Schmidt et al. 2007). Immature myeloid dendritic cells are likely the first lymphoid cells to come into contact with HIV during sexual transmission, given their presence at sites necessary to bind antigens in the anal, vaginal, and foreskin epithelium. They bind HIV viral protein gp120 to a unique surface protein, dendritic cell-specific ICAM-3-grabbing nonintegrin 1 (DC-SIGN), and then disseminate the virus via migration to lymph nodes (Cunningham et al. 2007; Del Cornò et al. 2007). Follicular dendritic cells (FDCs) reside within lymph node follicles and are particularly important participants in the establishment of the HIV reservoir. As with any other antigen, they trap intact, circulating HIV, usually in the form of antigen-antibody complexes. The trapped viruses may remain infectious for several months despite the production of neutralizing antibodies, suggesting that FDCs conformationally change

the antigen–antibody interaction (Smith et al. 2001; Burton et al. 2002).

Early in infection, lymph node FDCs become extensively coated with HIV virions trapped on their surfaces, which accounts for the majority of the virus particles in an acutely infected person (Tenner-Racz and Racz 1995). Unlike myeloid and plasmacytoid dendritic cells, FDCs are not known to become infected with HIV; viral DNA has not been isolated from FDCs (Keele et al. 2008), perhaps because the virus cannot enter FDCs or undergo reverse transcription within this cell type. Rather, FDCs preserve infectious HIV particles on their surfaces during and following periods of viremia in the vicinity of trafficking T lymphocytes. FDCs intimately interact with T lymphocytes, an important immunologic relationship which HIV exploits. FDCs present the virus to T lymphocytes, haplessly infecting them (Keele et al. 2008). Though infectious virus can remain tied to FDC surfaces for several months, it is likely that any period of viremia (even very low-level viremia) in chronic infection can replenish this infectious potential of FDCs.

Despite effective ART, HIV RNA can be found in the subcortical regions of lymph nodes, which has been attributed in part to the activity of FDCs (Moir et al. 2015). Unlike other HIV reservoirs, where each infected cell harbors on average one virus, a single FDC may trap and retain multiple, genetically diverse, replication-competent virus particles (Keele et al. 2008).

Cells of the Monocyte–Macrophage Lineage as Reservoirs

Tissue macrophages are derived from circulating monocytes and constitute another important latent reservoir for HIV within the lymphatic system. Although they mainly reside in specific tissues and organs, macrophages do constitute a small minority of lymph cellularity (Platt and Randolph 2013b). Like T lymphocytes, they express CD4 and chemokine receptors necessary for HIV entry (Szabo et al. 1990). While most

T lymphocytes become activated upon infection and are then destined for apoptosis, macrophages are more resistant to the cytopathic effect of HIV and can persist for longer periods (Le Douce et al. 2010). Generally, very low levels of lymph node macrophages (0.05%) are latently infected with HIV in chronic infection (Redel et al. 2010). Nonetheless, macrophages possess the characteristics of a latent reservoir; they are quite long-lived and achieve viral replication upon activation. Activation of these macrophages may be driven by pro-inflammatory cytokines or other infections, such as herpesviruses (Caselli et al. 2005; Kumar et al. 2014). Macrophage HIV reservoirs are not limited to the lymphatic system; central nervous system and gastrointestinal tract macrophages, for example, also serve as important HIV reservoirs (Kumar et al. 2014).

Lymphatic Reservoirs as a Barrier to Cure

The rebound of viremia after ART interruption confirms inability of ART to eradicate the latent viral reservoir (Levy 2007). Even during ART, very low viremia persists chronically, often far below the detection limits of commercial assays. Intermittent viral release (from reservoirs) without complete rounds of replication is a source of such low-level viremia (Sahu 2015), although cryptic viral replication in long-lived reservoirs cannot be excluded (Shan and Siliciano 2013). “Sterilizing” HIV cure thus requires eradication of all latent reservoirs, but no scalable strategies have been identified to date.

Conclusion

The lymphatic system is one of the main theaters of HIV pathogenesis. Circulating and tissue-based reservoirs are established very early in infection within this system, predominantly in resting memory CD4⁺ T lymphocytes, FDCs, and macrophages. The lymphatic system

reservoir is a part of the formidable barrier against HIV cure.

Cross-References

- ▶ Cellular Cofactors for HIV-1 Transcription
- ▶ Central Memory CD4 T Cells
- ▶ Immunology of Latent HIV Infection
- ▶ Lymphocyte Apoptosis
- ▶ Role of Dendritic Cells in HIV-2 Pathogenesis
- ▶ T-Cell Homeostasis

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HIV Reservoirs in the Central Nervous System

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Definition

The central nervous system (CNS) is a unique anatomical compartment that is shielded from the rest of the body by the blood-brain barrier (BBB). Exchange of substances between the CNS and the blood is controlled and limited by the BBB. Human immunodeficiency virus (HIV) enters the CNS early after viral transmission and, in some cases, replicates independently of that in the blood. Viral replication in the CNS could be supported in multiple cell types including CD4⁺ T cells, macrophages, microglia, and potentially astrocytes or other cells lacking CD4 receptor expression. Following productive infection of permissive cells, it is plausible that viral species persist in the CNS in the form of a reservoir, or dormant state of infection that is capable of reactivation and production of new viral particles. Analyzing such a reservoir in the context of the CNS is challenging, given the fact that brain tissue can only be sampled at death. Yet, studies using cerebrospinal fluid (CSF) as a surrogate for analyzing HIV infection of the CNS have described four states of infection, which will be discussed in this section. Importantly, physiological and viral factors influence the outcome of HIV replication in the CNS, which affects the composition of a persistent viral reservoir within an individual. Future work aimed at a total cure to HIV infection will need to address all potential viral reservoirs, including those that reside within the CNS.

Introduction

Human immunodeficiency virus type 1 (HIV-1) is the cause of acquired immunodeficiency

syndrome (AIDS), where uncontrolled infection in the blood and lymphoid organs depletes CD4⁺ T cells and diminishes immune competence over time. Although antiretroviral therapy (ART) controls viral replication and disease progression, these drugs must be taken throughout life to suppress infection. A major focus of current research is to eradicate HIV-1 and cure infected patients. HIV-1 persistence in cellular and anatomical reservoirs precludes a cure; thus, efforts to characterize these reservoirs are an important part of developing a strategy for eradicating all forms of HIV-1.

In order to discuss reservoirs of HIV-1, it is important to understand how the virus utilizes different cell surface receptors for productive infection. HIV-1 infects cells that express CD4 (the viral receptor) and CCR5 or CXCR4 (viral coreceptors). Preferential infection of a specific cell type by HIV-1 (cell tropism) is defined by receptor usage. Most transmitted/founder virus strains are CCR5-utilizing and infect CD4⁺ T cells (R5 T cell-tropic), but over time, HIV-1 can evolve to utilize CXCR4 expressed on CD4⁺ T cells (X4 T cell-tropic) due to the depletion of CCR5⁺ CD4⁺ T cells from untreated infection. Additionally, HIV-1 can evolve to infect macrophages, which express CD4 and CCR5 (R5 macrophage-tropic). R5 macrophage-tropic viruses are predominantly found in the central nervous system (CNS) as an evolutionary adaptation to the paucity of T cells in the CNS. The composition of the latent reservoir could differ between individuals depending on nadir CD4, although probably all permissive cell types are infected at all times, at least at low level (reviewed in Joseph 2014b).

The central nervous system (CNS) is an anatomic compartment that harbors potentially multiple cellular reservoirs of HIV-1. The CNS is a unique anatomical site as it is relatively “immune privileged” due to the physiology of the blood–brain barrier (BBB), a selectively permeable barrier that controls communication between the CNS and the periphery. It is difficult to investigate CNS infection, as brain tissue is only available at autopsy. The cerebrospinal fluid (CSF), which bathes the CNS, is a surrogate for studying

CNS infection, but these studies are limited in their ability to characterize CNS tissue reservoirs of HIV-1. To understand potential CNS reservoirs of HIV-1, it is important to discuss the following: the dynamics of viral and permissive cell entry into the CNS; the conditions that favor viral replication and contribute to reservoir stability; and the potential for viral egress from cells constituting the CNS reservoir.

Defining a Viral Reservoir

Two essential criteria exist to define a viral reservoir of HIV-1 (reviewed in Blankson et al. 2002). First, a reservoir must preserve replication-competent virus in some form (i.e., viral particles or viral genomes) so that the virus can reestablish productive infection in the future. Second, a reservoir must have mechanisms of longevity. For example, reservoirs composed of virions would require escape from biochemical decay, as seen with virion particles that become trapped extracellularly in dendritic cell processes. Cell-associated viral reservoirs, however, require cell survival and escape from immune control including cytotoxic T cell (CTL) activity. Latent infection, a state with no active replication, is the best characterized cellular reservoir of HIV-1, but another type of reservoir could be composed of productively infected cells with slow turnover. The concept of a reservoir is further complicated by the detection of cells that clonally expand in vivo through transactivation of cellular growth-promoting genes by integrated viral DNA (Maldarelli et al. 2014).

A reservoir will most likely occur in cells that are normally infected with HIV-1. This virus infects cells that express the viral receptor CD4 and coreceptor (CCR5 or CXCR4). Activated CD4⁺ T cells are the most permissive cell type for HIV-1. Latently infected resting memory CD4⁺ T cells are the hallmark reservoir of HIV-1 infection and are thought to predominantly arise from productive infection of activated CD4⁺ T cells as they are transitioning back into a resting state (reviewed in Siliciano and Greene 2011). Latently infected T cells contain replication-

competent HIV-1 genomic DNA (provirus) integrated within the human genome in the absence of ongoing viral replication. Although dormant, latently infected resting T cells can be induced to become activated and thereby transcribe integrated DNA to generate new progeny virions capable of productive infection. It is important to note that integrated DNA can be either intact or defective; thus, many infected T cells contain HIV-1 DNA that is incapable of producing functional virions (Ho et al. 2013). Latently infected cells are established early after a person becomes infected, and these cells persist even in the presence of ART-mediated viral suppression. Persistence of the latent reservoir is due in part to the virus escaping immune surveillance, as integrated viral DNA in latently infected cells is likely transcriptionally silent and does not produce antigens that signal immune attack (reviewed in Archin et al. 2014).

A second type of HIV-1 reservoir could be a productively infected cell with slow turnover. After the onset of suppressive ART, which prevents new infections without affecting previously infected cells, the blood viral load decreases rapidly (1–2 weeks) due to rapid turnover of short-lived infected T cells. For most people, the same pattern of rapid viral decay is observed in the CSF. However, occasionally the viral load decays much more slowly in the CSF than in the blood, which suggests that cells with a longer half-life than T cells can support HIV-1 replication in the CNS (reviewed in Joseph 2014b). Potential CNS cell types infected with HIV-1, including T cells as well as longer-lived cells, will be discussed later in this chapter.

The BBB and BCSFB: Barriers to HIV Infection of the CNS

The CNS, consisting of the brain and spinal cord, is an anatomical compartment isolated from the rest of the body by the blood–brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB). The BBB and BCSFB are semipermeable barriers that limit exchange of substances between the CNS and peripheral blood. These physiologically

distinct barriers influence the composition of CNS fluid, including the CSF and CNS interstitial fluid, which differs greatly from the blood. For example, concentrations of white blood cells, albumin, and immunoglobulin G (IgG) in the CSF are all <1% of that in the blood in spite of the fact that the water portion of the CSF is derived from the blood plasma. Key to barrier function of the BBB and BCSFB are intercellular tight junctions, which connect cerebrovascular endothelial cells (BBB) or choroid plexus epithelial cells (BCSFB). Otherwise, the BBB and BCSFB differ in physical composition and function (reviewed in Ransohoff and Engelhardt 2012).

The BBB is present along CNS blood vessels throughout the brain. Tight junction-connected BBB endothelial cells are separated from the brain parenchyma (the brain tissue proper) by two basement membranes: the endothelial and parenchymal. At the capillary level, these membranes are fused, but at all other vascular levels, these membranes separate to delineate the CSF-filled perivascular space (reviewed in Engelhardt and Ransohoff 2012). The BBB exhibits specialized function based on its position within the overall CNS vasculature: Nutrient transport occurs primarily at capillaries that lie in close proximity to neurons, whereas immune modulation occurs at the postcapillary venule (a small vessel that blood flows through after leaving the capillaries) where the perivascular space can accommodate the presence and movement of cells (reviewed in Obermeier et al. 2013).

The BCSFB resides in the choroid plexus located in each of the four brain ventricles. The choroid plexus is composed of fenestrated, permeable blood vessels (vessels containing endothelial pores) surrounded by tight junction-connected epithelial cells that are directly exposed to the CSF. Ependymal cells of the choroid plexus produce CSF from arterial blood. Newly produced CSF fills the brain ventricles, circulates around the exterior surfaces and perivascular spaces of the brain, and ultimately is reabsorbed into venous blood at the meninges. CSF flow is mediated by pulsation of the choroid plexus and action of ependymal cell cilia. Interstitial fluid of the brain parenchyma is also drained into the CSF,

a fluid that acts as a surrogate for lymph by mediating immune surveillance throughout the CNS (reviewed in Spector et al. 2015).

The CSF is considered to be an immunologically active fluid as it houses T cells, B cells, and monocytes, cells which have limited access to the CNS. During physiologic conditions, T cells are primarily restricted to the CSF. However, monocytes can exit blood vessels, enter the brain, and differentiate into macrophages that primarily concentrate around CNS vasculature (perivascular macrophages) but can also be found in the meninges (meningeal macrophages) and choroid plexus (choroid plexus macrophages). CNS myeloid cells, as well as microglia (resident macrophages of the brain), have antigen-presenting capability and are considered important for immune surveillance and interaction with circulating central memory T cells (reviewed in Ransohoff and Engelhardt 2012).

Viral Entry and Cell Migration into the CNS

HIV-1 is found in the CNS very early after transmission, although the associated mechanisms of entry are not well understood (Sturdevant et al. 2015). One theory, which is the most favored, proposes trafficking of HIV-infected CD4⁺ T cells into the CNS as part of routine immune surveillance or in the context of neuroinflammation. Alternatively, HIV-1 virions could cross the BBB/BCSFB, likely in the setting of high blood viral load. Consistent with this hypothesis, some studies suggest that HIV-1 can specifically interfere with the production of proteins involved in the maintenance of tight junctions thereby disrupting the integrity of the BBB (reviewed in Zayyad and Spudich 2015). In contrast, the work of Price and colleagues has shown that, in the absence of neurocognitive symptoms, the CSF viral load decreases late in infection, while the viral load in the blood is on average increasing. This disparity in viral load occurs as CD4⁺ T cells are being depleted in the blood and white blood cell (WBC) count drops in the CSF, the latter of which suggests that T cells in the CSF

are also reducing in number. These data suggest that the reduction in CSF viral load is due to a loss of CD4⁺ T cells that come from the blood, and thus virus enters the CNS in the form of trafficking T cells (reviewed in Price et al. 2014). Regardless of how HIV-1 enters the CNS, persistent infection in the brain requires a population of permissive cells that reside in or are trafficked to the CNS.

Peripheral immune cells, including T cells and monocytes, leave the blood and enter the CNS through a sequential mechanism of cell binding to the vascular endothelium, rolling along the endothelial lining, and extravasation into the CSF and/or CNS. First, circulating immune cells transiently bind blood vessel endothelial cells through receptor-ligand interaction involving either the selectin family of adhesion molecules or α 4-integrins (reviewed in Ransohoff and Engelhardt 2012). Through these interactions, immune cells roll along the endothelium until they come into contact with chemokines that bind to their G-protein-coupled receptors on the immune cell surface. Chemokine binding induces signals that lead to increased affinity and avidity of cell surface integrins for their ligands and arrest of rolling cells. Immune cells then crawl along the endothelium by exposing protrusions that search for sites to cross, a process mediated by additional adhesion molecule interactions. Finally, the cell traverses the endothelium, either between endothelial cells or through endothelial pore-like structures, into the CSF-filled spaces of the brain. The cell then crosses the parenchymal basement membrane and underlying glia limitans, a membrane composed of astrocyte cell foot projections, to enter the parenchyma (reviewed in Engelhardt and Ransohoff 2012).

Brain pathology affects barrier function of the BBB/BCSFB, and as an associated immune response is mounted, this increases the number of CNS-infiltrating immune cells. During inflammation, CNS inflammatory cells secrete leukocyte-attracting chemokines and endothelial cells upregulate expression of adhesion molecule receptors, thereby aiding immune cell recruitment and extravasation into the CNS. Neuroinflammation can be a protective immune response to CNS tissue damage or infection, but immune pathology

can also compromise the BBB/BCSFB, which alters CNS homeostasis and the proportions of immune cells in the brain (reviewed in Obermeier et al. 2013).

HIV-1 infection is associated with increased inflammation and immune activation both systemically and in the CNS. Neuroinflammation and BBB/BCSFB integrity both appear to affect the population of HIV-1 present in an infected CNS in a multifaceted, dynamic manner. Theories behind some of this complexity have been proposed based on studies comparing viral populations in the CSF, a surrogate for examining the CNS in living people, with those in the peripheral blood of the same individuals. First, in people who have very low or undetectable CSF viral load, it is likely that virus is not replicating, at least not appreciably, in the CSF or CNS but perhaps gains transient entry to the CNS. CSF virus likely comes from infected CD4⁺ T cells in the blood that cross the BBB/BCSFB and release viral progeny in the CNS. Second, in people who have elevated levels of HIV-1 in the CSF that is genetically similar to virus in the peripheral blood, it is likely that CSF virus also comes from migrating infected T cells but now in higher levels as part of an immune response associated with increased white blood cell count in the CSF (pleocytosis). Low-level, focal replication in the CSF or CNS could also account for increased viral load with or without pleocytosis, although without establishment of a persistent CNS infection. Third, in people who have detectable CSF virus that is genetically distinct from that in the peripheral blood, CSF virus could come from transient, clonal amplification of certain viral species in the CSF or CNS that may or may not establish persistent infection over time. The term “compartmentalized” viral replication is used to describe independent replication of HIV-1 within a given bodily compartment and is illustrated by genetic differences in the viral population between compartments, such as the CSF/CNS and blood. “Equilibrated” viral replication defines a state where HIV-1 populations are genetically similar between two compartments due to ongoing or recent intercompartmental movement of viruses (Sturdevant et al. 2015).

Roughly 30% of acutely HIV-1 infected people have pleocytosis, which generally correlates with higher viral load in the CSF. Thus, increased viral burden in the CSF could result from an influx of infected cells in response to neuroinflammation. Consistent with this hypothesis, BBB dysfunction, indicated by an increased CSF/blood albumin ratio, often accompanies pleocytosis. A loss of barrier integrity in the context of enhanced immune cell trafficking to the CNS could allow more infected cells and/or cell-free virus to enter the CNS. Viral replication in the CSF/CNS also increases viral load in the CSF, irrespective of pleocytosis. Yet, pleocytosis occurs in a fraction of people with compartmentalized CNS replication and in an even greater proportion of people with equilibrated replication. How pleocytosis affects viral replication in the CNS is unclear, and it is plausible that pleocytosis occurs as a consequence of CNS HIV-1 infection. On the other hand, an influx of permissive cell types for infection could also promote viral replication in the CNS (Sturdevant et al. 2015; Spudich et al. 2005).

CNS Cell Types as Potential Reservoirs

CD4⁺ T cells

The primary target of HIV-1 infection is the CD4⁺ T cell; however, there are relatively few T cells in the healthy CNS. The concentration of T cells found in the CSF is less than 1% of that found in blood and even fewer, if any, are seen in the brain parenchyma. Despite the low absolute number of T cells present, the CSF has a relatively large proportion of permissive T cells; the CSF cellular composition includes primarily T cells (90% of total CSF cells), which are mostly of memory phenotype (central and effector) and recently activated (CD69⁺) (reviewed in Ransohoff and Engelhardt 2012). As noted above, pro-inflammatory conditions promote immune cell influx into the CSF/CNS, thus increasing the number of potential target cells for HIV-1 replication.

The traditionally described cellular reservoir of HIV-1 is the latently infected T cell. For such a cell to contribute to a CNS reservoir of HIV-1, the cell must reside over time in the CNS. CD8⁺ T cells

have been shown to persist in the CNS of mice infected with vesicular stomatitis virus (VSV). These cells are CD103+, which is an integrin found on tissue-resident CD8+ T cells, and expression of CD103 follows antigen recognition in the brain. Furthermore, CD103 appears to be important for retention of CD8+ T cells in the CNS, as knockdown of this molecule resulted in reduced accumulation of CNS T cells. Interestingly, CNS-resident CD8+ T cells in the brain parenchyma were shown to form clusters, some of which contained CD4+ T cells (Wakim et al. 2010). Although CNS CD4+ T cells were not analyzed thoroughly in this study, another study showed that tissue-resident CD4+ T cells in the skin are antigen-experienced and express CD103 (Watanabe et al. 2015). Taken together, these data suggest that a population of CNS-resident CD4 + CD103+ T cells could exist in the CNS and harbor HIV-1.

Some evidence exists in support of HIV-1 replication in CNS T cells. In some cases there is elevated viral load in the CSF sufficient to indicate HIV-1 replication in the CSF/CNS and rapid viral decay in the CSF upon ART initiation, suggesting that this virus was replicating in a short-lived cell, such as a T cell. Some people with rapid CSF viral decay have compartmentalized replication of HIV-1 in the CSF, and CSF virus is T cell-tropic, meaning that the virus replicates best in T cells compared to other cell types. Such individuals likely have a CNS-derived population of HIV-1 arising from infected T cells, a population that differs from T cell-tropic virus in the blood (Joseph 2014a). Alternatively, people with relatively high CSF viral load, equilibrated viral population, and evidence of pleocytosis may also have HIV-1 replicating in CNS T cells due to an increase in CSF/CNS T cell concentration but in a manner that does not result in a distinct population of virus in the CSF compared to the blood (Sturdevant et al. 2015).

Macrophages

Slow decay of CSF virus with ART suggests that, in this case, HIV-1 is being produced from a longer-lived cell type than a T cell. HIV-1 cell tropism depends at least in part on CD4 receptor

expression density on the surface of a cell. R5 T cell-tropic virus replicates robustly in cells that express high levels of CD4 (T cells) but poorly in cells that express low levels of CD4, including macrophages, which have a similar number of cell surface CD4 molecules to T cells, but the molecules are less densely packed due to the larger surface area of macrophages (Joseph 2014a).

The ability to use low levels of CD4 for cell entry is an evolved feature of the viral envelope gene that cannot be attributed to a single mutation (Arrildt 2015). Rather, macrophage tropism likely evolves as an adaptation to the lack of CD4-rich target cells in the CNS, and the evolution of macrophage tropism appears to involve multiple genetic changes that differ between people. Pleocytosis further complicates macrophage-tropic evolution as it may alter the relative proportion of permissive cell types in the CNS thereby supporting viral replication from either T cell- or macrophage-tropic lineages. Indeed, a rhesus macaque animal model of HIV-1 infection showed that infection causes activation of bone marrow-derived monocytes and increased traffic of activated monocytes to the CNS with subsequent differentiation into CNS macrophages (Burdo et al. 2010).

The CNS is rich in macrophages that could serve as a reservoir for HIV-1. Perivascular macrophages, choroid plexus macrophages, and meningeal macrophages are all bone marrow-derived and are named for anatomical regions in which they reside. These cells could be infected by macrophage-tropic virus or with much lower efficiency by an R5 T cell-tropic virus. Perivascular macrophages are likely exposed to cell-free or cell-associated virus that crosses the BBB. Such virus could come from either the blood or CSF, depending on barrier physiology at the point of entry. Indeed, immunohistochemical staining of autopsied brain shows the presence of HIV-1 nucleic acid and protein in perivascular macrophages. Similarly, meningeal macrophages, located at the superficial brain meninges, are likely also exposed to blood or CSF virus that crosses the leptomeningeal BBB. Choroid plexus macrophages, on the other hand, are likely exposed to predominantly blood virus, as these

macrophages are located in the choroid plexus stroma, which harbors fenestrated capillaries that provide blood for the production of CSF (reviewed in Joseph 2014b).

A rhesus macaque animal model of HIV-1 infection suggested that the virus could migrate between the CNS meningeal and parenchymal regions or replicate autonomously in each of them. The rapid migration of genetically homogeneous virus throughout the brain was associated with faster disease progression and widespread encephalitis. Furthermore, compartmentalized replication in the meninges versus parenchyma was associated with localized detrimental inflammation within these respective brain regions. One macaque with compartmentalized replication in the meninges versus parenchyma was suggested to have macrophage-tropic virus present in both regions, an observation that exemplifies how regional macrophages may contribute to CNS infection and disease. Collectively, these data suggest that the uncontrolled replication of HIV-1 in different brain regions, and potentially in regional macrophages, may be detrimental for local physiology through induction of pathological inflammation (Matsuda et al. 2013).

Microglia

Microglia are resident macrophages of the CNS and the predominant immune cell type in the brain parenchyma. Unlike macrophages, microglia are not bone marrow-derived, rather they arise during embryonic development and are maintained throughout adulthood via local proliferation. Microglia have immune functions including phagocytic ability, inflammatory cytokine secretion, and weak antigen presentation. Studies using HIV-infected human brain tissue at autopsy show that microglia can contain HIV-1 nucleic acid and protein. Furthermore, as with monocyte-derived macrophages, HIV-1 can infect microglia in vitro, suggesting that this cell type is permissive to HIV-1 infection. Like macrophages, microglia have low surface densities of CD4, so virus capable of successfully infecting these cells is likely macrophage-tropic (reviewed in Joseph 2014b). Microglia are thought to have very long life spans, even longer than CNS bone marrow-derived

macrophages, thus persistent infection of these cells could constitute a CNS reservoir of HIV-1. Alternatively, persistent replication in this cell compartment deep in the brain parenchyma could maintain an active reservoir even in the face of poorly penetrating anti-HIV-1 therapy.

Astrocytes

Astrocytes provide mechanical and metabolic support for neurons and are the most abundant cell type in the brain. Viral DNA has been detected in astrocytes of HIV-infected people, and astrocytes can be infected at low levels in vitro (reviewed in Joseph 2014b). However, it is unclear whether astrocytes are productively infected in vivo, as they express no CD4, the viral receptor. Indeed, an analysis of macrophage-tropic HIV-1 *env* genes from individuals with HIV-associated dementia failed to detect CD4-independent infection (Joseph 2014a). Yet, CD4-independent infection was characterized in another study where rhesus macaques were infected with chimeric human/simian immunodeficiency virus (SHIV) that contains an R5 T cell-tropic HIV-1 *env* in the context of an SIV backbone. Still, only one HIV-1 *env* clone isolated from the CNS of a single infected macaque was able to infect CD4⁺ cells in vitro, so it is difficult to draw definitive conclusions from a single observation (Zhuang et al. 2014). An alternative explanation for the presence of HIV-1 nucleic acid in astrocytes is that these cells have phagocytic ability and could ingest infected T cells (reviewed in Joseph 2014b). The extent to which HIV-1 can enter cells in the absence of receptor and/or coreceptor is a poorly studied issue that deserves more attention given the number of cells without viral receptors present in a person and the concern that these alternative cell types could contribute to the reservoir.

Assessing the Contribution of CNS Reservoirs to Viremia

The defining criterion of a reservoir is that the virus is preserved in some form that allows for reestablishment of productive infection. In the

case of the blood reservoir, latently infected CD4 + T cells can be isolated from the blood and used in a viral outgrowth assay to directly assess the prototypical latent reservoir (reviewed in Archin et al. 2014). Such methods are essentially moot for analyzing the CNS reservoir due to the logistics of collecting viable CNS cells postmortem in a timely manner. An alternative method of determining whether HIV-1 in the CNS can reestablish infection is to characterize virus in the CSF that emerges following ART treatment interruption (rebound virus).

HIV-1 rebound virus appears in the CSF roughly 2 weeks after the detection of virus in the blood (de Almeida 2005). Phylogenetic analysis of rebound viral populations in the CSF versus blood could be used to determine if populations in these two compartments differ, indicating that “compartmentalized” CSF rebound virus comes from the CNS and thus illustrates the presence of a CNS reservoir of HIV-1. Furthermore, if viral species previously confined to the CSF arise in the blood during rebound, then reestablishment of systemic infection would be influenced by the CNS reservoir. Although it would be difficult to prove what cell type recrudesced CNS virus originates from, the use of *in vitro* infectivity assays would illustrate cell tropism of rebound virus. The phenotype of CSF rebound virus likely depends on the state of viral replication in the CNS prior to initiation of ART; therefore, studies are required to characterize viral populations throughout the brain of ART-naïve as well as experienced individuals.

Although evidence greatly supports the concept of HIV-1 persistence being attributable to latency, the immune privileged CNS may represent a unique anatomical reservoir, as crosstalk with the periphery is limited due to the BBB, and many ART drugs are relatively poor at penetrating the CSF/CNS. Treatment intensification using ART drugs with optimal CNS penetrance (relative to others) does not reduce levels of HIV-1 RNA in the CSF (Yilmaz et al. 2010). These data suggest that low-level viral replication does not account for the presence of residual CSF virus. However, treatment intensification studies are limited in informing our understanding of latency in the

CNS. Examination of CSF rebound virus could help fill this gap in knowledge. CNS viral persistence is further complicated by the fact that the cellular composition of the CNS is macrophage-rich with limited exposure to T cells, which reside primarily within the CSF. Thus it is possible that mechanisms of HIV-1 persistence differ between the CSF/meninges and the CNS/parenchyma and these mechanisms are affected by the presence of neuroinflammation, which alters the interaction between these bodily compartments. Finally, we do not understand the extent to which viral replication in the parenchyma is “reported” as virus in the CSF.

Recently the concept of “immune privilege” of the CNS has been confounded with the discovery of meningeal lymphatic vessels in the brain. These vessels appear to drain fluid and immune cells from the CSF and connect with deep cervical lymph nodes, which are part of the peripheral lymphatic system (Louveau 2015). The discovery of meningeal lymphatic vessels complicates our understanding of how the cerebrospinal, blood, and lymph fluids are linked. The classical mechanism of fluidic drainage from the CSF to the blood occurs across meningeal arachnoid villi, which are microscopic projections of the arachnoid mater, the middle of the three CNS membranes that enclose the parenchymal tissues and CSF (meninges). Furthermore, CSF fluid is thought to cross the cribriform plate, a paper-thin, perforated bone in the nasal cavity, to drain to the deep cervical lymph nodes (reviewed in Ransohoff and Engelhardt 2012). Meningeal lymphatic vessels comprise a potential bypass of the known CSF draining mechanisms and could contribute to HIV-1 dissemination from the CNS to the rest of the body.

Conclusion

Many aspects of HIV-1 infection within the CNS are poorly understood, but several key features of CNS infection can be gleaned from studies to date. It is evident that virus enters the CNS early by one or more mechanisms and that permissive or semipermissive cell types are present to support

productive infection. Furthermore, the CNS is rich in long-lived macrophages and microglia, which could house virus in a similar manner to resting memory CD4⁺ T cells, although studies have not shown that infection of macrophages can have a persistent, dormant state. Studies using CSF rebound virus could inform our understanding of CNS viral persistence. Likewise, animal models are a valuable platform for addressing mechanistic questions of CNS infection. Defining the CNS reservoir is an important aspect of HIV-1 cure strategies and current research is aimed at clarifying our understanding of HIV-1 persistence in the CNS.

Cross-References

- ▶ [Attachment/Binding](#)
- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [Integration](#)
- ▶ [Macrophage-Specific Aspects of HIV-1 Infection](#)
- ▶ [Neuroinflammation and HAND: Therapeutic Targeting](#)
- ▶ [Neuro-AIDS, Immunopathogenesis of](#)
- ▶ [Overview of HIV CNS Infection](#)

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HIV Reservoirs Within the Lungs

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Definition

Over recent years, efforts aimed at understanding HIV persistence have intensified, with the hopes that this understanding will provide the platform for developing strategies to eliminate HIV reservoirs. Traditionally, efforts have focused on the gut-associated lymphoid tissue, which is the largest HIV reservoir within the body, and the genitourinary tract due to implications for HIV transmission. The central nervous system has also been a focus of study due to interest in the ability of the blood-brain barrier to protect the brain from various pathogens, clinical observations surrounding HIV-associated neurocognitive disorders, and questions regarding antiretroviral penetration into the cerebrospinal fluid and brain parenchyma. The lungs as HIV reservoirs received greater attention in the pre-highly active antiretroviral therapy (HAART), whereby patients had indications for bronchoscopy due to active lung symptoms, compared to the HAART era whereby opportunistic infections are less frequent. However, with increasing rates of chronic lung disease and a significant burden of bacterial pneumonia in HIV-infected individuals on suppressive HAART, further consideration for the lungs as sanctuaries for HIV is merited.

HIV as an Independent Risk Factor for Chronic Lung Disease

Despite the efficacy of HAART, HIV-infected individuals continue to suffer from a disproportionate

burden of chronic lung disease and non-opportunistic infection (Feikin et al. 2004; Gingo et al. 2014). Bacterial pneumonias were found to be up to 25 times greater in HIV-infected individuals with suppressed peripheral viral loads on HAART compared to HIV-uninfected counterparts (Feikin et al. 2004). Furthermore, although smoking has traditionally been blamed as the primary cause for chronic diseases such as chronic obstructive pulmonary disease (COPD), the important contribution of other factors is becoming increasingly recognized. HIV appears to be an independent risk factor for COPD, pulmonary hypertension, and lung cancer (Gingo and Morris 2013). Uncontrolled HIV has been associated with worse results on spirometry and diffusing capacity measurements and has been shown to hasten decline in lung function by 55–75 ml/year (Drummond et al. 2013). Interestingly, a study conducted in perinatally acquired HIV-infected children found that perinatally acquired HIV was associated with increased airways resistance and frequent abnormal results on spirometry testing and that the severity of airway resistance related more to duration of HIV infection rather than history of respiratory complications (de Martino et al. 1997). Although HIV may not infect pulmonary vascular endothelial cells, lung endothelial cells appear to be a target for HIV's gp120, a protein which has been implicated in apoptosis and inducing release of endothelin-1, a vasoconstrictor (Barnett and Hsue 2013). HIV nef and tat are also thought to play deleterious roles in pulmonary artery endothelial cells, supporting a direct role for HIV damage to the blood vessels of the lungs (Barnett and Hsue 2013).

Perturbations in Pulmonary Immunology

During acute infection, HIV typically gains entry into cells via interactions with CD4 receptors and CCR5 chemokine receptors, and in later stages of disease, HIV tends to gain entry via interactions with the CD4 receptor and the CXCR4 chemokine receptor. Following primary infection, HIV traffics from peripherally infected lymphocytes and

monocytes to the lungs through its vascular blood supply (Clarke et al. 1998). Within the lungs, infected monocytes differentiate into alveolar macrophages (AM) which proliferate (Clarke et al. 1998). Intrapulmonary HIV also infects CD4 and CD8 T cells in addition to fibroblasts as well as cell-free bronchoalveolar lavage (BAL) fluid (Beck 2013; Costiniuk and Jenabian 2014).

The presence of HIV within the lungs results in various immune aberrancies. In addition to distorting the numbers of various cells in BAL (with increased AMs, neutrophils, and eosinophils and reduced CD4/CD8 ratios observed in the BAL fluid of HIV-infected individuals), there are also functional abnormalities observed (Beck 2013; Costiniuk and Jenabian 2014). These have been most extensively characterized in AMs, which have impaired accessory cell function required to initiate cellular responses toward T cells (Koziel et al. 1993). Increased AM production of several pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF)- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), in addition to others, has been described (Beck 2013; Costiniuk and Jenabian 2014). AMs also display impaired oxidative burden and reduced ability to bind and internalize organisms due to mannose receptor impairment (Koziel et al. 1993). Polyclonal B-cell activation results in nonspecific polyclonal gammopathy and reduced pathogen-specific antibodies and may contribute to disrupted Th1/Th2 balance (Beck 2013; Costiniuk and Jenabian 2014). Ratios of Tregs (CD4 + CD25^{high}FoxP3^{high} cells) from BAL fluid of HIV-infected individuals are increased compared to HIV-uninfected individuals (Jambo et al. 2011). Given that these cells play a key role in limiting immune activation and T-cell function in cancer and chronic viral infections, disruption of Treg homeostasis may render the lungs vulnerable to disease processes. In HIV-uninfected individuals, abnormal Treg function has been observed in COPD, and murine studies suggest that Tregs protected against hypoxia induced pulmonary hypertension (Tan et al. 2014; Chu et al. 2015).

Peripheral immune cells which have become increasingly recognized as important players in

bridging innate and adaptive immune responses are mucosal-associated invariant T (MAIT) cells. These are mostly CD8 cells which express CD161 + and the invariant V α 7.2 T-cell receptor segment, and their responses are activated through antigen presented by MHC-related protein 1 (MR1) (Eberhard et al. 2014). In peripheral blood of HIV-infected individuals, MAIT cells are reduced in number, including in elite controllers and long-term non-progressors (Eberhard et al. 2014). In the context of HIV, peripheral MAIT cells have been observed to be activated and functionally exhausted, with reduced levels independently predictive of disease progression (Leeansyah et al. 2013; Eberhard et al. 2014). Furthermore, it has been shown that intrapulmonary MAIT cells are increased in the lungs during pulmonary tuberculosis in both HIV-infected and HIV-uninfected individuals and thus may redistribute to the lungs, suggesting an important role for these cells in pulmonary immunity (Wong et al. 2014).

Recent advancements in molecular biology have provided the platform for exploring the effect of the microbiota on health and disease. In HIV-uninfected individuals, changes in pulmonary microbiota have been described in the context of cystic fibrosis and COPD (Lynch 2014). Pulmonary pathogens induce inflammatory responses within the lungs, and commensal organisms play a role in regulating these responses and protecting the lungs against inflammatory and oxidative stress (Lynch 2014). Although studies are currently ongoing, relatively little is known about the composition of the microbiome in HIV-infected individuals. Thus far, it appears that colonization with *Pneumocystis jirovecii* is associated with more severe airflow limitation and obstructive lung disease in HIV-infected individuals (Morris et al. 2009).

Pulmonary Reservoirs of HIV

HIV's propensity to infect AMs, with their resistance to apoptosis, enables HIV to persist for prolonged periods within the lungs (Perno et al. 1998). The virus' propensity to infect CD4 cells, some of which later transition to a resting

memory state, also facilitates its ability to persist for prolonged time periods. A portion of this integrated provirus is replication competent, and interactions between appropriate HIV regulatory proteins and cellular transcription factors result in reversal of latency following exposure to antigens and stimulatory cytokines. Like the gut, the lungs are exposed to pathogens and a wide array of exogenous materials which may induce inflammatory responses and provide antigenic stimulation, reactivating HIV from latently infected cells. Nicotine has been shown to induce HIV replication within AMs and in peripheral blood, while coinfection can also contribute to the maintenance of the HIV reservoir (Clarke and Israel-Biet 1996; Costiniuk and Jenabian 2014). Lipoarabinomannan from *Mycobacterium tuberculosis* activates the long-terminal repeat promoter and induces TNF- α and IL-6 production, contributing to HIV replication (Collins et al. 2002). Coinfection may also upregulate CD4 expression on cells, rendering them more susceptible to HIV infection and weaken host immune responses (Ablashi et al. 1995; Beck 2013). Although never formally examined, rise in disinhibited cell growth and pro-inflammatory cytokines observed in neoplasia plausibly contribute to HIV pulmonary replication in the setting of lung cancer. Similarly, it is plausible that inflammation associated with other chronic lung diseases influences HIV persistence.

Although the lungs are highly vascular and do not have a specific barrier preventing entry of certain agents, they nonetheless are a unique anatomical compartment. Notably, the millions of closely packed cells promote cell-to-cell spread of virus which enables for escape from host immune defenses and exposure to antiretrovirals (Sigal et al. 2011). Evidence from both simian and human studies shows that HIV burdens in lungs often, but not always, exceed than in peripheral blood. In a simian study whereby primates had suppressed peripheral viral loads, the lungs and gut had approximately equal amounts of HIV (Horiike et al. 2012), and various human studies, involving heterogeneous groups of HIV patients, showed marked differences in quantities of HIV in lungs versus peripheral blood (Costiniuk and Jenabian 2014). In one of the largest early studies

examining 78 HIV-infected patients undergoing bronchoscopy for respiratory symptoms or abnormal chest X-rays, Clarke et al. documented a mean DNA copy number in BAL of 2791/10⁶ cells, mean HIV proviral DNA copy number in non-adherent BAL of 1746/10⁶ cells, and mean HIV proviral DNA copy number in adherent BAL of 1478/10⁶ cells (Clarke et al. 1994). In contrast, mean DNA copy number in adherent peripheral blood leukocytes was 391/10⁶ leukocytes, HIV proviral DNA copy number in peripheral blood lymphocytes was 303 copies/10⁶ lymphocytes, and mean HIV proviral DNA number in monocytes/peripheral blood-derived macrophages was 606 copies/10⁶ cells (Clarke et al. 1994).

Some studies have also found genotypic variability between HIV in the lungs and peripheral blood. Using DNA sequence analyses of HIV gp120 V3–V5, Nakata et al. demonstrated full separation of HIV lineages from lungs and blood in four out of five patients (Nakata et al. 1995). HIV within AMs has also been shown to evolve to a greater degree than HIV in peripheral monocytes (Itescu et al. 1994). Coinfection may also influence HIV evolution in anatomical compartments. Using phylogenetic and phonetic analysis of the C2–C3 coding regions of HIVenv, Collins et al. found compartmentalization of HIV quasi-species between blood and the pleural space in four out of eight patients, with migration between the blood and pleura (Collins et al. 2002). They found a trend toward more genetic heterogeneity within the pleural space, which they suggest could be due to increased HIV replication due to coinfection or local selection pressure (Collins et al. 2002). Finally, HIV co-receptor usage within the lungs is CCR5 tropic, while HIV in peripheral blood may be both CCR5 and CXCR4 tropic, although secondary co-receptor usage does not differ between HIV from blood versus lungs (Singh et al. 1999).

Effect of HAART on HIV Reservoirs and Pulmonary Immunity

The majority of studies which have examined HIV burden between lungs and peripheral blood

were performed in the pre-HAART era, whereby many individuals either were not on therapy or were on zidovudine or didanosine monotherapy and many individuals had indication for bronchoscopy such as pulmonary symptoms or abnormal chest imaging (Costiniuk and Jenabian 2014). Many of the studies were confounded by smoking (Costiniuk and Jenabian 2014). In the HAART era, Twigg III et al. examined the effect of HAART after 1 month and 6 months of treatment. They observed a decline in BAL fluid HIV in addition to a decline in CD8 T cells, although intrapulmonary HIV still remained detectable, especially in DNA in BAL CD4 T cells (Twigg III et al. 2008). Interestingly, the rate of HIV decay within the lungs was more rapid than that observed in peripheral blood (Twigg III et al. 2008). In another study by Knox et al., they found that BAL CD4 lymphocytes were not depleted in gross quantities in chronic HIV infection that CD4 BAL lymphocytes begin to reconstitute 1 month after HAART initiation and reconstitute to an even greater extent after 1 year of HAART (Knox et al. 2010). They suggest that the mechanism of reconstitution is due to proliferation of local CD4 T cells rather than redistribution (Knox et al. 2010). They also found that pulmonary CD4 T cells are more polyfunctional than peripheral blood CD4 T cells (Knox et al. 2010). With regard to peripheral CD161+ MAIT cells, Eberhard et al. found that peripheral MAIT cells did not recover to levels observed in HIV-uninfected controls despite HAART (Eberhard et al. 2014). In terms of antiretroviral penetration into the lungs, to date, little research has been conducted. In one study involving 24 HIV-infected individuals, assays for a variety of antiretrovirals were performed following 4 and 24 weeks of therapy initiation (Twigg III et al. 2010). Efavirenz, the only non-nucleoside reverse transcriptase inhibitor (NNRTI) examined, was detectable in 63% of BAL fluid measurements, while nucleoside reverse transcriptase inhibitors (NRTIs) were detectable in 44% and protease inhibitors (PI) in 46% of BAL fluid measurements (Twigg III et al. 2010). Furthermore, BAL fluid concentrations of NRTIs, the NNRTI

efavirenz, and PIs were 60–73%, 31%, and 66–200% of those observed in plasma, respectively (Twigg III et al. 2010). Of note, efavirenz had the longest half-life and was the most lipid soluble of all the antiretrovirals examined (Twigg III et al. 2010).

Future Areas of Study for the Lungs as HIV Reservoirs

An important first step for the study of the lungs as HIV reservoirs will be to better quantify the burden of pulmonary disease among HIV-infected individuals via heightened screening for individuals with clinical symptoms or risk factors for lung disease. It will also be important to screen individuals at risk of developing future lung disease, in an attempt to detect subclinical disease and attempt to halt disease progression. This may take the form of screening programs involving spirometric and diffusion capacity assessment. In smoking HIV patients, there may be a need for screening CTs to help identify early signs of cancer and continued emphasis on smoking cessation. Identification of the role of different pulmonary immune cells involved in the control of pulmonary HIV will also be important. Although they have not yet been examined in the lungs of HIV-infected patients to date, tissue resident memory T cells (Trm) will likely gain attention in upcoming years. These cells initiate cytokine release following infection and induce antimicrobial response through pattern recognition receptors (PRR). It will also be important to refine our understanding of the role of AMs as in maintaining the pulmonary HIV reservoir given their relative resistance to apoptosis and expression of drug efflux transporters, limiting entry of HIV into these cells (Perno et al. 1998). Similarly, it will be important to better understand the role of HAART-induced reduction in pulmonary inflammation in BAL fluid and its effects on pulmonary immune cell function and the intrapulmonary HIV reservoir. In order to understand the HIV reservoir, mucosal lung biopsies in order to quantify the level of HIV, compared with other

compartments, may be informative. However, bronchoscopy itself is invasive. Although noninvasive techniques are available to study pulmonary inflammation, such as sputum induction and exhaled breath condensate, they are not always reliable. Furthermore, exhaled breath condensate does not allow for the collection of cells in order to quantify the pulmonary cellular reservoir.

Characterization of the pulmonary microbiome may also shed insight into HIV-associated chronic lung disease and maintenance of the pulmonary HIV reservoir. This will likely change in different geographic situations and will be influenced by coinfection with pathogens such as *Mycobacterium tuberculosis*. Furthermore, understanding the implications of the pulmonary reservoir on defined clinical outcomes will be of paramount importance. Finally, although most work on the lungs as reservoirs of HIV have been conducted in North America, where clade B virus predominates, understanding different viral clades, and especially clade C which is endemic in the sub-Saharan Africa, would broaden our understanding of the HIV reservoir on a global scale.

Conclusion

With increasing longevity, HIV-infected individuals are developing more chronic lung diseases. Epidemiological studies have revealed that HIV itself is a risk factor for chronic lung disease. Pulmonary HIV is associated with various immune perturbations, not all of which normalize with HAART. Evidence for the lungs as HIV reservoirs comes from studies demonstrating different HIV burdens and distinct HIV variants in lungs versus peripheral blood. HIV persistence is also supported by its propensity to infect AMs, long-lived cells relatively resistant to apoptosis, and the high density and close proximity of millions of cells within the lungs which enable cell-to-cell spread of HIV. Understanding the role of the lungs as HIV reservoirs is important as we continue to invest efforts toward HIV eradication strategies.

Cross-References

- ▶ [Bacterial Respiratory and Invasive Pneumococcal Infections and HIV](#)
- ▶ [Central Memory CD4 T Cells](#)
- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [Lung Cancer](#)
- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [Macrophage-Specific Aspects of HIV-1 Infection](#)

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HIV Testing and Counseling

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Definition

HIV testing and counseling (HTC) has been a mainstay of HIV prevention since the late 1980s

in the USA and worldwide. Since its inception, HTC has provided the opportunity for individuals to learn their status, develop a plan to reduce their risk of contracting or transmitting HIV, and, if necessary, link to HIV services. Expanding coverage of HIV testing continues to be an urgent national and global priority (Joint United Nations Programme on HIV/AIDS 2014; White House Office of National AIDS Policy 2015; Office of Disease Prevention and Health Promotion 2016). There has been a growing recognition both in the United States (USA) and internationally that there are a sizeable number of persons living with HIV who are not aware of their HIV status. In the USA, it is estimated that one in eight HIV-positive individuals are unaware of their status (Centers for Disease Control and Prevention 2016a), and globally, it is estimated that the majority of adults are unaware of their HIV status. In 2015, only 44.1% of adults in the USA had tested for HIV at some point in their lifetime (Kaiser Family Foundation 2016). This represents a modest increase since 1997, when 31.8% of adults had ever tested for HIV (Centers for Disease Control and Prevention 2011). Young people (defined as aged 13–24) are at particular risk, accounting for 22% of all new HIV diagnoses in 2014 (Centers for Disease Control and Prevention 2016b). Yet, this age group exhibits low rates of testing, and young people currently have the highest rates of undiagnosed HIV as well as the lowest rates of linkage to care and viral suppression, in the USA. For example, in 2012, it was estimated that 57,200 young people aged 18–24 were HIV positive in the USA, of which 25,300 (44.2%) were not diagnosed (Centers for Disease Control and Prevention 2016b). According to data from the US Centers for Disease Control Youth Risk Behavior Surveillance System, only 22% of sexually active high school students have ever received an HIV test (Coeytaux et al. 2014).

In addition, the 2006–2010 National Survey of Family Growth found that less than 30% of men aged 15–44 who reported high-risk behaviors had not been HIV tested in the prior year (Handel et al. 2015). In sub-Saharan Africa, the geographic region most heavily burdened by HIV/AIDS, some countries have implemented national campaigns that have helped expand testing (AVERT

2016); for example, the number of individuals tested in Kenya increased from less than one million in 2008 to over six million in 2013 (National AIDS Control Council of Kenya 2014). Yet, in other sub-Saharan regions like Ghana, Guinea, Niger, and Liberia, the prevalence of individuals ever tested is less than 10% (Staveteig et al. 2013). Increased HIV testing coverage is therefore needed in order to implement appropriate interventions that may link HIV-positive individuals to antiretroviral treatment, reduce HIV transmission, expand access to HIV care for persons living with HIV/AIDS, and access to preventive interventions such as pre-exposure prophylaxis (PrEP).

History of Testing and Counseling

In 1985, the enzyme-linked immunosorbent assay (ELISA), or enzyme immunoassay (EIA), became the first licensed HIV antibody test and was widely used in clinical-care settings, blood banks, plasma-collection centers, and health departments. The United States Public Health Service (USPHS) also sponsored 874 alternative test sites, where individuals at high-risk could learn their status, by the end of 1985 (Centers for Disease Control and Prevention 2006). In 1986, the Centers for Disease Control (CDC) issued the first official guidelines on testing and counseling, which emphasized the importance of voluntary testing and counseling for individuals at high risk for HIV/AIDS, and stressed the inclusion of risk-reduction strategies and clear explanations of positive and negative results. These guidelines also highlighted the urgent need for confidentiality in order to increase rates of testing and counseling at a time when discrimination against HIV-positive individuals was increasingly widespread. One year later, CDC guidelines were revised in order to expand testing further. The 1987 guidelines aimed to reach individuals engaging in high-risk behaviors by recommending HIV testing for all individuals seeking treatment or testing for sexually transmitted infections (STIs) and/or individuals seeking treatment for injection drug use. These guidelines cited concerns about confidentiality as a major barrier to testing and

emphasized the importance of maintaining confidentiality.

In 1993 and 1994, the CDC issued new guidelines for HTC, outlining the need for a client-centered approach to counseling, which included an interactive risk assessment and a personalized risk-reduction plan during pretest counseling. In client-centered counseling, the provider aims to interact with the individual in an objective, non-judgmental manner, in order to communicate messages of risk reduction that are relevant and meaningful to the individual. The ultimate prevention goal of client-centered counseling is to develop a plan to reduce behaviors that put clients at risk for contracting or transmitting HIV. During this time, HIV antibody testing typically involved two visits: one visit to draw blood and one visit to receive test results. Because of the sensitivity of the ELISA test, tests were confirmed with the Western blot blood test, which lead to a wait time of up to 2 weeks until the second visit to receive test results. Pretest counseling occurred during the first visit and typically included a client-centered risk assessment, the development of a personalized risk-reduction plan, and preparing the client for understanding their test result. The second visit included the delivery of the test result along with posttest counseling, which generally consisted of helping the client to interpret their test result, integrating that knowledge into their personalized risk-reduction plan, and referral of support services (Centers for Disease Control and Prevention 1994). This second visit presented a significant barrier to the receipt of test results – up to 50% of individuals testing at clinics failed to return to receive their results (Branson 2003).

Rapid HIV tests have the advantage of potentially delivering test results at the point of care, which guarantees increased receipt of test results. Therefore, the implementation of rapid HIV point-of-care (POC) testing was a critical development to HIV prevention efforts. In 1992, the first rapid HIV-1 test called single-use diagnostic system for HIV-1, or SUDS, was developed and approved by the Food and Drug Administration (FDA). However, it was found to be unsuitable for point of care because it requires centrifugation

which must be performed in a laboratory. In 2002, a new rapid HIV-1 test, OraQuick Rapid HIV-1 Antibody Test, was approved by the FDA and granted a 2003 waiver under the Clinical Laboratory Improvement Amendments (CLIA), allowing OraQuick to be used as a point-of-care testing strategy using a blood stick. The CLIA waiver allowed the OraQuick test to be used in non-laboratory settings, an important element in the expansion of HIV testing. While CDC protocols mandated confirmatory testing for all rapid HIV testing with a Western blot or immunofluorescence assay (IFA), rapid testing offered a drastic reduction in wait times for results, and OraQuick could deliver results in 20–45 min. In 2004, the first rapid oral fluid test was approved by the FDA and granted a CLIA waiver. This OraQuick Advance HIV-1/2 test was approved for use with oral fluid and plasma specimens (Branson 2003). Again, the OraQuick Advance HIV-1/2 test was to be confirmed by a Western blot or IFA. The development of blood stick and oral fluid rapid HIV testing allowed the implementation of HIV in nontraditional settings, expanding HIV testing even further.

In 2006, the CDC released new guidelines that recommended the expansion of HIV screening as a routine part of healthcare for all adults (Centers for Disease Control and Prevention 2006). By recommending an opt-out routine screening system, the CDC hoped to reduce HIV transmission by identifying and counseling individuals with HIV, linking persons with HIV to care, reduce perinatal transmission, promote early detection of HIV, and normalize HIV testing (Centers for Disease Control and Prevention 2006). The 2006 CDC-recommended model included information provision as an element of screening and encouraged counseling for individuals at high risk, at the discretion of the provider (Holtgrave 2007; Bartlett et al. 2008). This shift was fostered by the lack of scientific consensus at the time regarding the benefits of HIV counseling for HIV-negative individuals and the high levels of general HIV knowledge in the US population (Janssen 2007).

The recommendation to integrate HIV testing into routine healthcare was met with strong

approval. However, critics noted the potential ramifications of testing without respect to risk, issues of consent including patient consent and conflicting state regulations regarding consent, problems with third-party reimbursement of HIV screening, cost-effectiveness of opt-out testing versus targeted HTC, and a possible lack of adequate counseling (Holtgrave 2007; Lyons et al. 2007; Bartlett et al. 2008; Mahajan et al. 2009). Additionally, previous studies have raised concerns over the feasibility of integrating HIV screening into primary care settings, such as lack of time, perceptions of patient nonacceptance and discomfort, lack of training for providers, and low awareness of or agreement with screening guidelines (Koester et al. 2007; White et al. 2014; Zheng et al. 2014). Recent research has demonstrated that in spite of these potential barriers, integrating HIV screening into primary care increases patients' feelings of empowerment and reduces stigma associated with HIV and HIV testing (Simmons et al. 2011). Physicians who have been trained to deliver HIV screening have demonstrated increased levels of self-efficacy, comfort, and willingness to discuss prevention issues with patients (Dreisbach et al. 2014; Thrun et al. 2009). Furthermore, trained physicians reported perceiving HIV screening to be within their mission as primary care providers (Myers et al. 2012).

The 2006 CDC recommendations provided a foundation to alter the traditional model of client-centered HTC and facilitate expanded HIV testing to other nontraditional healthcare facilities, such as the dental setting (Pollack et al. 2014; Durall et al. 2015), substance use disorder treatment programs (Pollack and D'Aunno 2010; Metsch et al. 2012), and emergency departments (Brown et al. 2007). The CDC's Expanded Testing Initiative has supported these recommendations by funding programs to increase testing in new clinical, non-healthcare settings in US jurisdictions that are disproportionately impacted by HIV/AIDS (Centers for Disease Control and Prevention 2015). Finally, the Affordable Care Act (ACA), enacted in March 2010, mandated that non-grandfathered health insurance plans cover,

without additional cost-sharing, preventive services that are given an A rating by the United States Preventive Services Task Force (USPSTF), which includes HIV testing for individuals aged 15–65 years as well as younger and older individuals who are at heightened risk (U.S. Preventive Services Task Force 2016). By minimizing patient financial burden, the ACA helped enhance access to healthcare and recommended preventive and treatment services, including HIV testing (Viall et al. 2016). While the impact of the ACA on HIV testing uptake has not yet been systematically assessed, projections estimate that by 2017, the ACA will account for an additional 466,153 individuals receiving testing and almost 2,600 new HIV diagnoses (Wagner et al. 2014).

Effect of HIV Testing and Counseling

Studies of HTC provide a complicated portrait of its effectiveness. Researchers have found it difficult to measure the effects of HTC together, as opposed to the simple effect of knowing one's status. Reductions in risk behaviors in the 1990s may have been attributable to a general increase in knowledge and awareness of HIV/AIDS prevention among high-risk populations. Several studies have demonstrated substantial risk reduction among HIV discordant couples or couples in which one partner is HIV positive, and one partner is HIV negative. For other populations, studies demonstrating the effectiveness of HTC have been mixed.

In the 1990s, several studies were released that demonstrated the limited effects of counseling on various populations. In 1991, a prominent meta-analysis found that counseling had greater effects on reducing risk behaviors in HIV-positive individuals than HIV-negative individuals (Higgins et al. 1991). In a 1999 meta-analysis, a different group of researchers argued that HIV counseling was not an effective primary prevention strategy or method of reducing HIV transmission among HIV-negative individuals, asserting instead that counseling could be perceived as a *secondary*

prevention strategy or a method to reduce risk behavior among individuals who had tested positive for HIV (Weinhardt et al. 1999). Their main finding supported the conclusion that HIV-negative individuals who participated in counseling did not modify their sexual risk behavior any more than individuals who had not participated in counseling, while HIV-positive participants and HIV discordant couples (e.g., counseled together) reduced unprotected sex and increased condom use relative to HIV-negative and untested participants. However, CDC researchers subsequently argued that the majority of the studies included in the 1999 meta-analysis had taken place prior to the adoption of the 1993 client-centered counseling approach, and therefore did not provide an accurate evaluation of its effects (Holtgrave and McGuire 2007). CDC researchers asserted that Project RESPECT, an ongoing multicenter randomized control trial (RCT), demonstrated the effectiveness of their client-centered counseling approach (Kamb et al. 2000).

Project RESPECT was the first RCT to report findings that demonstrated the reduced incidence of STIs (including HIV) among participants who had engaged in HTC since the 1993 CDC-recommended client-centered approach had been implemented. It is important to note, however, that Project RESPECT enrolled only persons at risk for heterosexual transmission and did not account for men who have sex with men (MSM). Findings from Project RESPECT indicated that interactive, client-centered HIV and STI counseling led to a reduced STI incidence among HIV-negative participants, both men and women (Kamb et al. 1998). Additionally, Project RESPECT was conducted among busy, public, inner-city clinics, demonstrating that client-centered counseling was not only feasible among publicly funded clinics but also successful and effective. Further reports from Project RESPECT found client-centered HTC to be effective among high-risk groups, such as teenagers, individuals with STIs at enrollment, and drug users (Bolu et al. 2004; Semaan et al. 2010). However, a 2008 systematic review of behavioral counseling HIV/STI prevention interventions found that of 15 RCTs, Project RESPECT was the only study

of moderate-intensity counseling to document an impact on subsequent STI acquisition (Lin et al. 2008).

The 2009 HIV Rapid Testing and Counseling Study, which assessed the use of on-site HIV testing at 12 US community-based drug treatment programs, showed that the addition of counseling to on-site HIV testing offered no significant effect on testing or sexual risk behaviors (Metsch et al. 2012). In addition, a cost analysis of the study demonstrated that risk-reduction counseling provided no added benefit to the patient, accounting for projected life expectancy, lifetime costs, and quality-adjusted life years; researchers concluded that on-site rapid testing with information provision only was the most cost-effective approach (Schackman et al. 2013). In 2010, Project AWARE evaluated the effectiveness of HIV testing in STI clinics, comparing brief patient-centered risk-reduction counseling with information provision only; researchers found no significant difference in cumulative STI incidence after 6 months, demonstrating that counseling at the time of testing provides no additional benefit to the patient (Metsch et al. 2013). Given the additional resources and financial costs associated with counseling at the time of testing, these findings indicate a need to reevaluate the role of risk-reduction counseling offered at the time of HIV testing (Haukoos and Thrun 2013).

Other innovative testing and counseling interventions have been developed for focused high-risk populations, such as men who have MSM and drug users. For MSM, studies have demonstrated the effectiveness of personalized cognitive counseling (PCC) in producing significant behavior changes (Dilley et al. 2007). PCC includes targeting “self-justifications” that individuals utilize in their decisions regarding high-risk behavior. These self-justifying thoughts, attitudes, and beliefs are identified and discussed with a counselor, with the primary goal of developing alternative ways of thinking in order to affect behavior change. Couples-based voluntary HIV counseling and testing (CVCT) has also been developed for MSM partnerships and evaluated the association between relationship characteristics, such as

relationship satisfaction and monogamy and positive testing behaviors (Mitchell 2014).

Global Developments in HIV Testing and Counseling

Global HIV testing uptake has increased dramatically in the past decade. In 2007, the WHO and UNAIDS recommended the adoption of provider-initiated testing and counseling (PITC) as a standard of care in an effort to expand global HIV testing coverage and encouraged the routine offering of PITC in clinical settings such as antenatal care services, tuberculosis (TB) clinics, and STI clinics in high-burden areas (World Health Organization 2007). Along with the expansion of PITC, the implementation of other testing strategies, such as community- and home-based testing and the use of rapid testing to deliver same-day test results, contributed to a substantial increase in HIV testing coverage between 2005 and 2015, as the percentage of people living with HIV worldwide who knew their status increased from 12% to 60% (World Health Organization 2015a, 2016). While these numbers represent significant progress in global HIV testing coverage, 40% of people living with HIV globally remain unaware of their status, and the WHO has called for the expansion and improvement of HIV testing services in order to reach people living with HIV who are unaware of their status and link them to HIV care and treatment programs (World Health Organization 2015b).

In 2014, UNAIDS released new global targets for HIV diagnosis and treatment, known as the “90-90-90” plan, proposing that by 2020, 90% of all people living with HIV will know their status, 90% of all people living with HIV will receive sustained antiretroviral therapy (ART), and 90% of all people receiving ART will achieve viral suppression (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2014). In response to these ambitious goals, the WHO updated and expanded their guidelines on HIV testing and counseling in 2015, releasing “Consolidated Guidelines on HIV Testing Services 5Cs: Consent, Confidentiality, Counseling, Correct Results

and Connection.” These new guidelines replace the term “HIV testing and counseling” with “HIV testing services (HTS),” in order to embody the full range of services that accompany HIV testing: pretest information; posttest counseling; linkage to prevention, care, and support; and laboratory quality assurance to ensure correct results (World Health Organization 2015c). Guidelines also affirm the need for expanded HTS, noting that additional approaches to HTS are needed to increase testing uptake among men in high prevalence settings, adolescents (10–19 years of age), infants and children, and couples and partners, including serodiscordant couples. Additionally, WHO guidelines call for increased efforts to expand HTS for key populations such as men who have sex with men, people in prisons, people who inject drugs, sex workers, and transgender individuals and for vulnerable populations in high prevalence settings such as migrant workers, refugees, and other displaced populations (World Health Organization 2015c).

While 2015 WHO guidelines maintain the importance of counseling as an integral part of HTS, counseling recommendations have been updated to adapt to resource limitations and new HIV testing strategies, such as rapid diagnostic tests (RDT), which deliver same-day test results. Guidelines recommend the provision of pretest information and discourage the use of individualized risk assessment and individualized counseling during the pretest period. After testing, guidelines recommend that individuals who test HIV-negative receive brief health information about their HIV status, future HIV prevention, and how to link to HIV prevention services as appropriate; individuals who are diagnosed as HIV-positive or who are part of a couple where one or both individuals are diagnosed HIV-positive should still receive post-test counseling and linkage to care and treatment (World Health Organization 2015c).

Innovative approaches to HTS are needed to increase HIV testing coverage globally. The WHO calls for expansion of PITC, particularly in family and pediatric healthcare settings to increase early infant diagnosis, and recommends the adoption of new testing tools and strategies,

including self-testing (with confirmatory clinic testing), the use of lay health providers to administer rapid diagnostic tests (RDTs), and a new model of community-based testing called “test for triage,” in which individuals are tested via RDT and then linked to clinical care for confirmatory testing and treatment (World Health Organization 2015c). Recent studies of HIV testing uptake interventions have demonstrated promise with various strategies such as community-, home-, mobile-, and work-based testing (Bateganya et al. 2010; Chamie et al. 2016; Corbett et al. 2006; Labhardt et al. 2014; Sabapathy et al. 2012; Suthar et al. 2013), index patient testing approaches (offering testing to household members of high-risk individuals) (Shapiro et al. 2012; Velen et al. 2016; Were et al. 2006), partner testing (offering testing to partners of high-risk or HIV-positive individuals) (Onyango Osofi et al. 2014; Plotkin et al. 2016), and self-testing (Figueroa et al. 2015; Pant Pai et al. 2013).

Moving Forward

Efforts to expand HIV screening have utilized new technologies in order to increase the uptake and effectiveness of screening. Researchers have found rapid testing to be highly acceptable as a screening tool and more effective than standard HIV testing at communicating results, due to the reduced need for a return visit (Pottie et al. 2014); individuals are 1.5–2.2 times more likely to receive their results by using rapid testing than standard HTC (Hutchinson et al. 2006). Other innovative approaches have included integrating HIV screening and services into substance use disorders treatment programs, which was found to be highly feasible and effective, and implementing routine HIV screening in hospital emergency departments, which was found to be feasible and cost-effective and was well-received by patients (Brown et al. 2007; Gunn et al. 2005). Incorporating rapid HIV testing into dental visits has been suggested as an opportunity to reach patients that may not have contact with other clinical providers (Pollack et al. 2010, 2014). In June 2012, the CDC announced their 2-year plan

to train pharmacists and staff at 24 community pharmacies and retail clinics with high HIV prevalence or significant testing needs, in order to deliver confidential rapid HIV testing (Centers for Disease Control and Prevention 2012b). Studies have since demonstrated the feasibility and acceptability of rapid HIV testing in the pharmacy setting in both rural and urban communities in the USA (Darin et al. 2015; Weidle et al. 2014). Strategies such as these represent new resourceful approaches that work to expand HIV screening by reaching untapped populations and environments.

In 2012, the US Food and Drug Administration (FDA) approved the first over-the-counter at-home rapid HIV test kit, the OraQuick In-Home HIV Test, capable of testing for both HIV-1 and HIV-2. The OraQuick In-Home HIV Test is an oral fluid test that captures results in 20–40 min, and does not require a laboratory analysis. The FDA has approved the kit for use by adults over 17 years of age and has asserted the need for confirmatory testing. The creator of the test kit, OraSure, has set up a 24-h toll-free telephone-based consumer support center that will offer information for referrals to care and any questions about the test. The approval of this at-home rapid test kit may normalize HIV testing; however critics have voiced concerns regarding the lack of provider guidance and in-person counseling.

Finally, HIV testing technologies are advancing quickly. Immunoassays that detect antibodies earlier are now approved for HIV diagnosis by the FDA, which will allow for earlier diagnosis and linkage to care (Branson 2012). Given the limited sensitivity of the Western blot, alternative confirmatory strategies have been suggested, such as the combination of rapid tests and/or the use of nucleic acid amplification tests (NAATs), which detect HIV RNA (Daskalakis 2011). In 2013, the FDA approved rapid diagnostic tests that are able to detect the HIV-1 antigen in addition to HIV antibodies and distinguish between acute versus chronic HIV infection, leading to faster results and facilitating early HIV diagnosis (U.S. Food & Drug Administration 2013). Emerging technologies include multiplex tests to identify multiple pathogens (e.g., HIV and hepatitis) simultaneously and POC nucleic acid tests, which utilize

targeted RNA/DNA amplification to facilitate early identification of acute infections (Wesolowski et al. 2017). Alternative confirmatory strategies in rapid HIV testing could facilitate significant increases in HIV testing and early diagnosis, receipt of results, and HIV prevention and linkage to care.

Conclusion

New technologies, approaches, and policies to HIV testing and counseling are rapidly being developed and implemented in order to expand HIV screening, both in the USA and globally. More research is needed on unconventional strategies and locations for HIV testing and counseling and/or screening, in order to develop feasible and effective interventions. It is important to remain vigilant regarding the possible ramifications of expanding HIV screening, by continuing to research the role of counseling in HIV prevention. By expanding coverage and increasing access to HIV screening, more individuals will be able to learn their status, reduce risk of transmission, and seek care.

Cross-Reference

- ▶ [HIV Prevention Efforts within Substance Use Disorder Treatment Settings](#)
- ▶ [HIV Prevention for MSM](#)
- ▶ [Preexposure Prophylaxis \(PrEP\)](#)
- ▶ [Prevention Counseling and Other Strategies in the HIV Care Setting](#)
- ▶ [Prevention for People Living with HIV](#)

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HIV Transmission in Female Commercial Sex Workers and Host Protective Factors

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Introduction

HIV currently infects over 33 million people globally and the major route of transmission remains heterosexual intercourse. Already at the beginning of the epidemic, it was noted that a minority

of highly HIV-exposed seronegative (HESN) individuals seemed to resist the infection and remain uninfected despite continuous contacts with the virus over years. These individuals include sex workers, HIV-serodiscordant couples, children born to HIV-infected mothers, and other individuals that are at risk of acquiring HIV. For more than 25 years, the scientific community has tried to define correlates of protective immunity to HIV studying these cohorts. One of the strategies was to assess blood and mucosal fluids from men and women with high-risk behavior living in endemic communities. These samples have been compared to those of individuals who are at low risk of infection as well as to samples of HIV-infected individuals. Although a number of markers have been found to differ between these three groups, there is only one, a deletion in the CCR5 co-receptor for HIV (CCR5 Δ 32), that has been demonstrated to mechanistically account for resistance against infection (Liu et al. 1996). This mutation is however rare in the global population and almost absent in Africa, where most HIV infections occur; therefore, it cannot account for the approximately 5% of high-risk individuals that seem to be relatively resistant to infection. Despite intensive research efforts that have been spent, very little has been known about the specific immune mechanisms and/or genetic background responsible for decreased susceptibility to HIV infection. It is most likely that a combination of factors are necessary to confer protection and no single explanation can be found, with the exception of the CCR5 Δ 32 mutation, that strongly accounts for HIV resistance by itself. By studying a specific risk group, female commercial sex workers (CSW), several correlates of resistance have been suggested and more unified patterns of protection have finally been starting to emerge.

Defining Study Groups for Assessing Correlates of HIV Resistance

If the search for correlates of resistance to HIV infection is narrowed down to the situation of heterosexual transmission during vaginal intercourse, the most common transmission route

globally, female CSW can be used as a study group. These women may have a different genital microenvironment than non-CSW due to frequent unprotected sexual encounters with multiple clients. In general they also have a higher risk of acquiring other sexually transmitted infections, and their immune responses may be chronically activated by the continuous exposure to components and pathogens present in seminal fluid as well as frequent vaginal hygienic practices. The sex work as such may thus influence the immune parameters in addition to the exposure to HIV and must be taken into account when selecting appropriate control groups. The control groups available are usually non-sex-working low-risk subjects from the same geographical area. To control for the influence of sex work, it is common to include sex-working HIV-seronegative women who do not have sufficient infection pressure to be considered HIV resistant. Although a few of these may actually belong to the resistant phenotype, it is likely that at least 90% of them are susceptible to HIV infection. Nevertheless, in order to find true correlates of HIV resistance, the HESN CSW groups must have a very high rate of exposure to HIV since only an estimated 1:200–1:2,000 sexual encounters between HIV-serodiscordant men and women result in HIV infection (Powers et al. 2008). With an increasing condom use among CSW and a higher number of clients being on antiretroviral treatment, the exposure to HIV will likely be too low to allow the identification of truly HIV-resistant individuals and define appropriate study cohorts in the future.

HIV Entrance into the Female Genital Tract

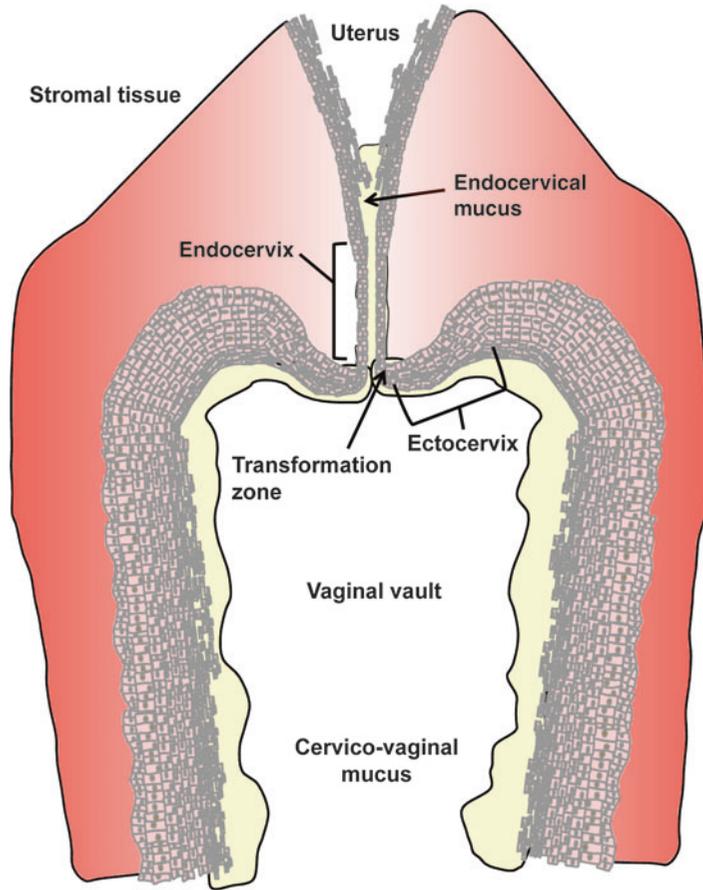
To identify host protective factors against HIV infection in female CSW, it is important to understand the early events in viral transmission to the female genital mucosa. The female genital tract is endowed with anatomical and biological defensive mechanisms, such as a robust epithelium covered by mucus (Fig. 1), several types of immune cells, and soluble immune factors

(Fig. 2). Together this barrier is normally effective in preventing infection from invading microorganisms including HIV. The virus can be present in both blood and seminal fluid of HIV-infected men and can be transmitted to the female genital mucosa during sexual intercourse (Southern 2013). The risk of transmission is increased in the presence of inflammation of the female genital mucosa which may be caused by preexisting infections, altered microflora, and particular hormonal conditions, associated with different stages of the menstrual cycle or the use of hormonal contraceptives. Genital inflammation results in the local recruitment of immune cells and may also impair the epithelial barrier, favoring viral penetration into the subepithelial layer of the mucosa. Although an increased number of immune cells within the mucosa may enhance the defenses against HIV, activated CD4+ T cells are among the preferential target of the virus and can thus promote local amplification and spread of the infection. HIV can also directly bind and pass through epithelial cells and be captured by macrophages, Langerhans cells, or dendritic cells to subsequently be transferred to CD4+ T cells. Once the founder pool of infected CD4+ T cells in the mucosa starts to expand, it is hard to hinder further spread of the virus to regional lymph nodes followed by systemic infection and establishment of reservoirs of latently infected cells (Fig. 2).

The physical barriers and the anatomical structure of the female genital tract, including the thickness of the epithelium and quality of epithelial junction proteins, have not been specifically evaluated in HESN CSW, although they could be an important defense factor against HIV transmission through sexual intercourse. To date most of the studies on this cohort aimed to identify correlates of protection within the immunological responses and genetic background of the host, which will be reviewed in the following sections.

Genital Immune Responses Against Primary HIV Infection

In general, whether it is an allergic reaction or a response to an invading pathogen, the outcome of

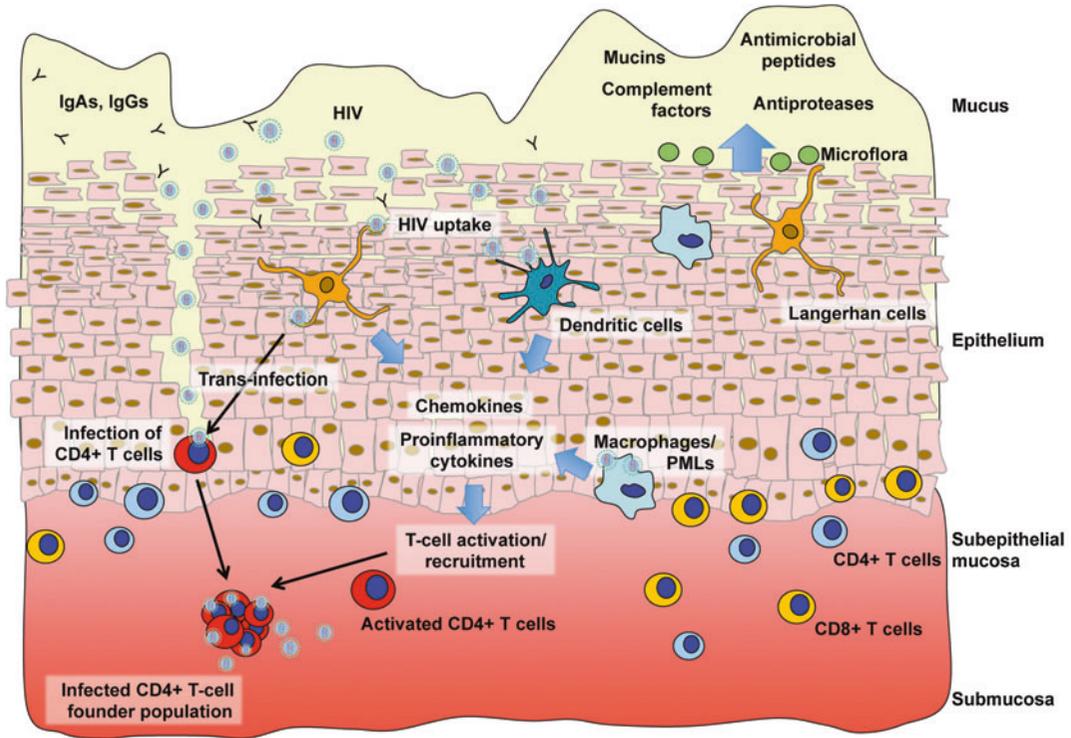


HIV Transmission in Female Commercial Sex Workers and Host Protective Factors, Fig. 1 Anatomy of the lower female genital tract. The cervix represents the lower part of the uterus. The endocervix lines the lumen of the canal that provides communication between the cavity of the body of the uterus and that of the vagina. The protruding portion of the cervix projects into the vagina through the upper anterior vaginal wall and is referred to as ectocervix. The epithelium of the cervix is varied. The ectocervix is covered by stratified squamous nonkeratinized epithelium, which is a continuation of the vaginal epithelium. Usually, this type of epithelium extends from a very short distance into the cervical canal, where it forms a rather abrupt junction with the simple

columnar epithelium lining the rest of the endocervical canal, called transformation zone. Unlike the mucosa of the body of the uterus, the endocervical mucosa does not slough off at menstruation. It does, however, respond to cyclic changes in the levels of the ovarian hormones. It secretes up to 60 mg of mucus a day throughout much of the cycle, but near the time of ovulation, the secretion rate increases tenfold and the abundant clear mucus fills the cervical canal. The mucus that covers the ectocervix and the vagina is a mix of locally produced secretions and endocervical mucus. The stroma beneath the epithelium consists primarily of dense collagenous connective tissue and only about 15% of its substance is smooth muscle

an immune reaction is strongly dependent on the balance between the pro-inflammatory (active) and the anti-inflammatory (dampening) response. A number of different immune cells and soluble molecules can have either one or both of these properties depending on timing, localization, and concentration in tissue, blood, or mucosal

secretions. In the case of HIV, it is even more complex since the same cellular subset (e.g., CD4⁺ T cell) and molecule (e.g., CCR5-binding chemokines) can act as friend and foe of the host, by enhancing the anti-HIV defense and the recruitment and activation of HIV target cells at the same time, as mentioned above. Studies on



HIV Transmission in Female Commercial Sex Workers and Host Protective Factors, Fig. 2 HIV infection and defenses of the female genital mucosa.

The pluristratified epithelium represented in the figure is characteristic of the ectocervical-vaginal mucosa. The mucosa comprises the epithelium and the stroma immediately beneath the epithelium. The deeper layers of stromal tissue represent the submucosa. HIV can infect the mucosa of the vagina as well as that of the uterine cervix, either the ectocervical or endocervical mucosa, although differences, if any, in the contribution of these three sites to the global transmission rate via vaginal intercourse remain unknown. The layer of mucus covering the epithelium mechanically hinders the invasion of HIV virions, which can also be neutralized by the soluble components present therein. The composition in immunological soluble factors of mucus varies. For instance, changes in the local microflora and invading microorganisms can be sensed by epithelial, polymorphonuclear leukocytes (PMLs) and antigen-presenting cells, in particular Langerhans cells, through

an array of receptors able to discriminate between pathogens and commensals, which represent an important link between innate and adaptive immunity. Local inflammation can weaken and damage the epithelium, promoting the penetration of HIV into the subepithelial layer of the mucosa, where most CD4+ T cells reside. Micro-abrasions of the epithelium also normally occur during sexual intercourse. Production of pro-inflammatory cytokines and chemokines, associated with preexisting inflammation or induced by recognition of HIV and co-transmitted pathogens, facilitates the establishment of an expanding pool of HIV-infected CD4+ T cells in the mucosa by recruiting and activating target cells of the virus at the site of infection. In the absence of inflammation, CD4+ T cells rarely reside within the epithelium, in contrast to dendritic cells and Langerhans cells. HIV can be uptaken by these cells in the upper layers of the epithelium and transported to the subepithelial mucosa or up to the draining lymph nodes, where CD4+ T cells are abundant and infection can be easily established

CSW women have revealed that adaptive as well as innate cellular and secretory responses are associated with resistance to HIV infection. Whether these correlates are a true hallmark of protection, or merely bystanders, needs further investigation in experimental systems or in prospectively followed clinical cohorts. Nevertheless, it has

been suggested that a silent immune response (immune quiescence) in the female genital mucosa is more beneficial for resistance to infection than a pro-inflammatory profile (Songok et al. 2012). To better understand the balance between pro- and anti-inflammatory responses to HIV, a detailed overview will be presented as

regards the immunological correlates of protection identified in HESN female CSW.

Innate Immunity

The first line of defense of the female genital tract is represented by a layer of mucus that covers the epithelium. The cervicovaginal mucus comprises many soluble components present in the secretions from various glands within the endometrium and oviducts, from epithelial and immune cells, as well as plasma transudate, which together constitute an extraordinary physical and immunological barrier against invading pathogens. Among these soluble components is indeed a plethora of molecules of different natures belonging to the innate arm of the immune system (Fig. 2). They possess a broad-spectrum antimicrobial activity, capable of targeting bacteria, fungi, and viruses by disrupting critical microbial structures, binding essential nutrients and rendering them unavailable to pathogens, and blocking cellular molecules targeted by pathogens. Gathering evidence supports an important role of these soluble factors in HIV infection, as they have been shown to inhibit HIV replication *in vitro*. Among these molecules are mucins, antiproteases, complement components, and antimicrobial peptides, such as defensins and cathelicidins. Serine and cysteine protease inhibitors, such as serpins, trappin-2 (also known as elafin), and cystatins, have been found in increased concentrations in cervicovaginal secretions from HESN CSW as compared with HIV-uninfected and HIV-infected control groups (Burgener et al. 2011). As antiproteases have anti-inflammatory properties, it is believed that they are an important component or driver of the reduced immune activation response that has been associated with resistance to HIV infection. On the other hand, increased levels of some soluble antimicrobial factors in cervicovaginal secretions have been correlated with genital infection and inflammation (e.g., SLPI and lactoferrin) as well as enhanced risk of HIV acquisition (e.g., LL-37 and alpha-defensins) (Levinson et al. 2009). These data indicate that the

mucosal immune defense against HIV is complex and likely an interplay of multiple mechanisms.

For this reason, as an important link between innate and adaptive immunity, the contribution of Toll-like receptors (TLR) and cytokines to the resistant phenotype displayed by female CSW has been investigated. Elevated levels of the chemokines RANTES and MIP-1alpha have been detected in the genital mucosa of these individuals, although the role of CCR5-binding chemokines in the transmission of HIV remains controversial. Recently, it was found that reduced expression of TLR7 and TLR8, which recognize HIV ssRNA genome, in the cervical mucosa of HESN CSW correlated with a reduction in the local expression of pro-inflammatory cytokines (Yao et al. 2014). Similarly, reduced concentrations of pro-inflammatory cytokines, such as TNF-alpha, IL-1alpha, and IFN-gamma, and the chemokines MIG and IP-10, which are involved in the recruitment of leukocytes to the mucosa, were detected in cervicovaginal secretions of HESN CSW compared to HIV-infected women (Lajoie et al. 2012).

Natural killer (NK) cells have been the most investigated innate cell subset in the resistance to HIV infection. Enhanced activity measured as increased expression of activation and degranulation markers on NK cells isolated from blood was shown to be associated with the HESN phenotype in a number of cohorts. However, no study has ever characterized the phenotype and function of these cells in the genital tract of HESN female CSW.

Adaptive Immunity

HIV-specific CD4+ helper and CD8+ cytotoxic T lymphocytes (CTLs) have been encountered in both the blood and genital tract of HESN female CSW. Late seroconversion of HIV-resistant CSW with preexisting HIV-specific CTL responses, possibly following a break from sex work, indicates that ongoing exposure to the virus may be required to maintain the resistant status (Kaul et al. 2001). This seroconversion happened in

the absence of detectable CTL escape mutations and was related to the waning of HIV-specific CTL responses, most likely as a result of the sex break. CD4⁺ T cells in HIV-resistant CSW from the same cohort were shown to have a lower activation profile but a much greater ability to proliferate in response to HIV antigens than HIV-infected women. Reduced activation of T helper cells and CTLs was found to be associated with increased frequency of circulating regulatory T cells in HESN (Card et al. 2009), indicating that regulatory T cells can contribute to a lower immune activation profile, although they may inhibit important anti-HIV responses as well. The detection of HIV-specific cytotoxic responses raised the question whether productive viral infection is required to elicit such a response. However, a different, eventually more efficient, ability of some antigen-presenting cells to “cross-present” exogenous antigens on HLA class I molecules, compared to individuals susceptible to HIV infection, may account for the presence of HIV-specific CTLs in resistant individuals.

Immune protection from HIV transmission has also been linked to the presence of HIV-specific mucosal IgA antibodies in a number of cohorts of HESN subjects exposed to HIV through sexual intercourse. Among these studies, it was reported that HIV-neutralizing IgA isolated from cervicovaginal secretions of CSW can block infection of peripheral blood cells *in vitro* as well as inhibit transcytosis of viral particles across a mucosal membrane (Devito et al. 2000). Because secretory IgA molecules in mucosal fluids do not activate complement factors, they may contribute to a favorable noninflammatory environment. These antibodies can protect from HIV transmission to the female genital mucosa by aggregating the virus before it reaches the epithelial layer, by blocking its passage across the epithelium, and by inhibiting virus spread between target cells in the subepithelial mucosa. On the other hand, the relevance of HIV-specific mucosal IgA as mechanism of protection has been questioned by reports on their absence or low levels of detection compared to those of IgG antibodies in cervicovaginal secretions of HESN

CSW and women in a serodiscordant relationship (Mestecky et al. 2011). According to this hypothesis, the presence of HIV-specific antibodies in mucosal secretions of HESN individuals would be a mere marker of exposure to the virus, as may be true for other mucosal HIV-specific as well as innate immune responses.

Genetic Influences of Mucosal Protection Against HIV Infection

Already in the beginning of the epidemic, it was noted that relatives of HIV-resistant female CSW (mothers, sisters) had a higher chance of being resistant than women in general (Fowke et al. 1996). A genetic component was therefore suspected, and it was hypothesized that production and maintenance of HIV-specific protective immune responses in the mucosa and low-level immune activation may depend on genetically determined factors. Likewise, single nucleotide polymorphism in noncoding regions of the interferon regulatory factor (IRF)-1 was found to correlate with reduced expression of IRF-1 and reduced responsiveness to IFN- γ in a cohort of HESN female CSW. Recent studies have shown that HIV-resistant women have a sharper, but transient, expression of this gene when induced by IFN- γ compared to prolonged activation in control groups (Sivro et al. 2013). Although the downstream effects of this response are currently unknown, these data may suggest that an immediate and potent innate immune response, without sustained immune activation, is beneficial for developing resistance to HIV infection.

A number of other haplotypes and genetic variants have been described to contribute to favorable immune responses to HIV and eventually account for resistance to infection in several cohorts of HESN, including killing inhibitory receptors on NK cells, human leukocyte antigens, intracellular restriction factors (e.g., APOBEC3G, TRIM5 α , etc.), chemokines, and their receptors. Nevertheless the role of the genetic background in shaping the local immunological

milieu in the genital mucosa of either HIV-resistant or HIV-infected women has been poorly addressed. Interestingly, a recent genome-wide analysis of approximately eight million genetic variants in 1,350 individuals of European ancestry among HIV-infected and HIV-uninfected found no association with the risk of HIV acquisition, with the exception of the CCR5 Δ 32 homozygosity, suggesting that the host genetic background by itself plays little role in determining the efficiency of transmission (McLaren et al. 2013).

Conclusion

Altogether current knowledge suggests that multiple factors can contribute to reduced susceptibility of HESN female CSW to HIV transmission via vaginal intercourse. This phenotype may be associated with the full prevention or an early block of the spread of HIV infection in the female genital mucosa due to a finely controlled inflammatory response and a low number of activated HIV target cells, a strong early innate response able to contain viral replication, and the development of HIV-specific responses effective against future infections. In this regard, a continuous exposure to the virus, which nature is yet to be defined, could be pivotal for the maintenance of some of these immune responses. Understanding how the immune system of HESN female CSW can prevent HIV transmission to the genital mucosa, which represents the main portal of entry of the virus globally, has important implications for the design of prophylactic strategies aimed at dampening the rate of acquisition by targeting the early immune response at the site of infection.

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HIV-1 Assembly Cofactors

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Definition

Despite the highly evolved and complex nature of HIV-1, it is still merely an obligate parasite. Thus, the HIV-1 replication cycle is completely dependent on host cellular proteins, lipids, and nucleic acids, herein defined as “cofactors.” HIV-1 advantageously usurps host cellular cofactors to facilitate a number of steps throughout the viral replication cycle. For example, HIV-1 fusion and entry are mediated by the major receptor, CD4, and coreceptors, CCR5 or CXCR4 (► [Fusion](#)). Upon entry into the cell, HIV-1 reverse transcription is primed by cellular tRNA^{Lys3}. Integration of the newly synthesized double-stranded viral DNA into the target cell genome is promoted by a cellular integrase-binding protein known as lens epithelium-derived growth factor (► [LEDGF/p75](#)). The focus of this chapter will be to describe host cellular cofactors required late in the HIV-1 replication cycle, specifically, during viral assembly, budding, and release.

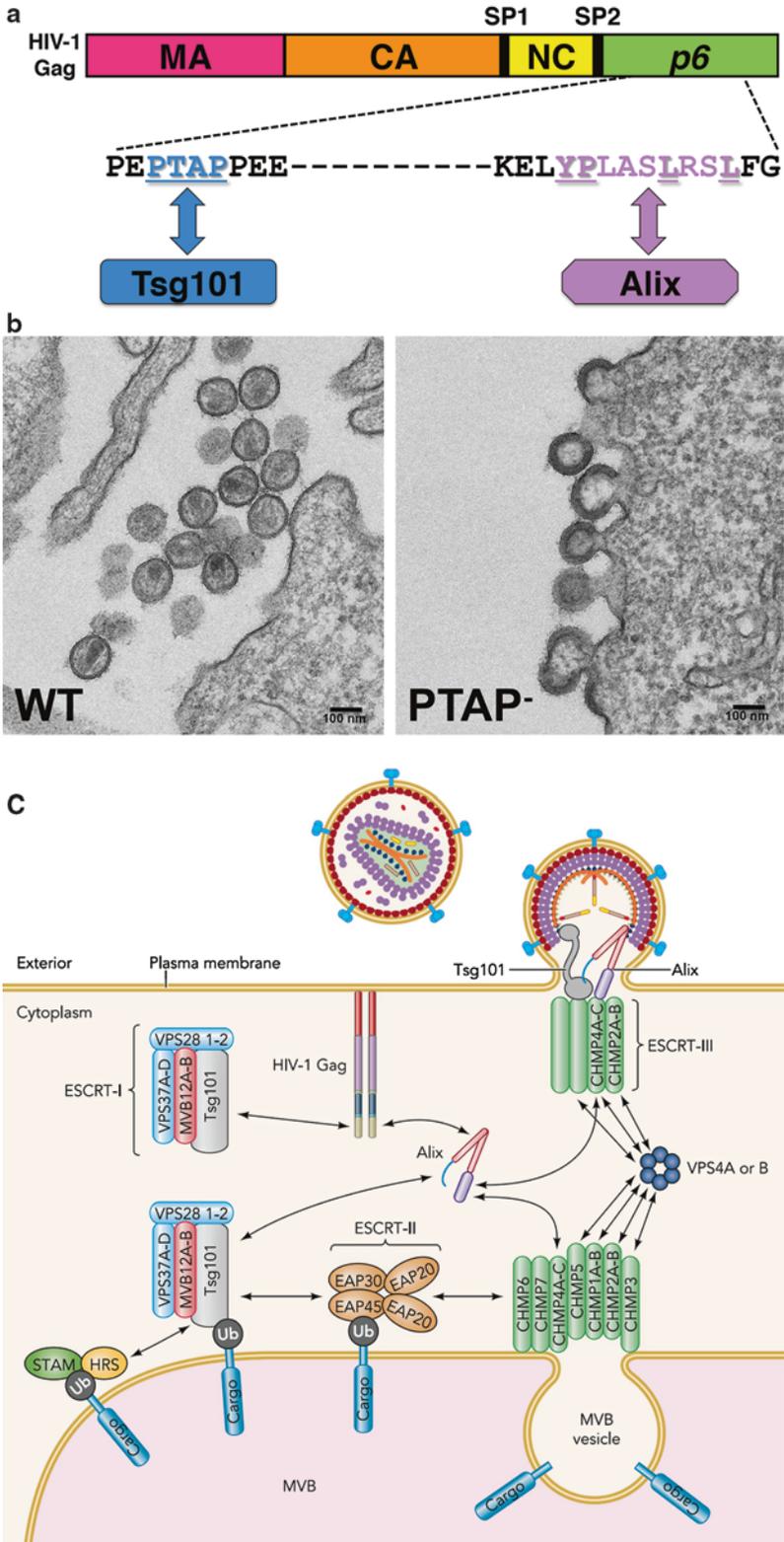
HIV-1 Gag Proteins Constitute the Virus Assembly Machine

The HIV-1 Gag polyprotein precursor, known as Pr55^{Gag}, is the primary viral determinant of particle assembly. Pr55^{Gag} itself is necessary and

sufficient to generate noninfectious viruslike particles (► [Assembly](#)). However, the complete process of virus assembly, budding, and maturation requires a highly regulated proteolytic processing cascade mediated by HIV-1 protease to generate mature Gag proteins and intervening spacer peptides (SPs), specifically matrix (MA), capsid (CA), spacer peptide 1 (SP1), nucleocapsid (NC), spacer peptide 2 (SP2), and p6 (Fig. 1a; ► [Maturation](#)). MA is responsible for Gag protein trafficking, membrane binding, and the incorporation of the viral envelope (Env) glycoproteins into virions. The N-terminus of MA is co-translationally modified by covalent attachment of a myristic acid moiety; this myristylation of MA is required for the binding of Gag to membrane. CA plays an important role in Gag multimerization and in the formation of the mature capsid core. NC, which contains two zinc fingerlike motifs flanked by highly basic sequences, is required for the encapsidation of viral genomic RNA into assembling particles. The p6 domain contains highly conserved peptide motifs, known as late domains, which are required for virus budding (Adamson and Freed 2007).

The Basic Steps in Virus Assembly and Release

The late stages of the HIV-1 replication cycle are characterized by mRNA export from the nucleus to the cytoplasm, a process dependent on the viral Rev protein. The Gag and Gag–Pol polyprotein precursors are subsequently translated in the cytosol and traffic to the sites of virus assembly on the inner leaflet of the plasma membrane. Once Gag and Gag–Pol associate with the membrane, Gag multimerization occurs, driven by CA–CA interactions as well as interactions between RNA and NC. The Env glycoproteins are incorporated into nascent virions during the assembly process. The final steps of particle budding and release are driven by p6 interactions with host cellular cofactors (Adamson and Freed 2007; Balasubramaniam and Freed 2011; ► [Budding](#)).



HIV-1 Assembly Cofactors, Fig. 1 Cofactors in HIV-1 assembly. (a) The HIV-1 Gag polyprotein consists of matrix (*MA*), capsid (*CA*), and p6 with intervening spacer peptides SP1 and SP2. The N-terminal p6 domain contains

HIV-1 Gag Targeting to the Plasma Membrane Requires Interactions with Cellular Lipid and Protein Cofactors

As indicated above, proper Gag trafficking and targeting to the sites of virus assembly are key steps in the HIV-1 replication cycle. Gag targeting to the plasma membrane is mediated by a highly basic patch of amino acid residues in the MA domain. This basic patch interacts with a specific plasma membrane lipid, phosphatidylinositol-(4,5)-bis-phosphate [PI(4,5)P₂]. Initial biochemical studies demonstrated that depletion of cellular PI(4,5)P₂ resulted in the mistargeting of Gag to intracellular compartments. Subsequent structural studies led to the proposal that binding of MA to PI(4,5)P₂ induces a conformational change at the N-terminus of MA that triggers exposure of the myristate group. This so-called myristyl switch occurs concomitantly with Gag multimerization, which together are required for efficient membrane binding (Ghanam et al.). Thus, PI(4,5)P₂ regulates Gag targeting to the plasma membrane, establishing this lipid as a key cellular cofactor for HIV-1 assembly (Ono 2009).

Membrane microdomains that are enriched in cholesterol and highly saturated lipids (often referred to as “lipid rafts”) play important roles both early and late in the HIV-1 replication cycle. In particular, lipid rafts serve as sites for virus assembly at the plasma membrane. Depletion of cholesterol from virus-producing cells impairs HIV-1 assembly by disrupting Gag–membrane association, and, as discussed below, cotargeting of Gag and Env to lipid rafts may increase the efficiency of Env incorporation into virions (Waheed and Freed 2009).

Apart from the abovementioned role for PI(4,5)P₂ and lipid rafts in Gag trafficking, the

pathway by which Gag reaches the plasma membrane is not well understood. It is widely accepted that Gag is synthesized in the cytosol and that the plasma membrane is the primary site for virus assembly (► [Assembly](#)). It has also been demonstrated by pulse–chase analyses that Gag becomes membrane associated within minutes of its synthesis. However, it is not clear whether Gag reaches the inner leaflet of the plasma membrane through passive diffusion or whether an active process involving the host cell cytoskeleton and/or vesicular trafficking machinery is used to promote Gag trafficking. Although it has been demonstrated that specific domains of Gag (e.g., NC) associate with cytoskeletal elements, divergent data can be found in the literature regarding the role for the cytoskeleton in Gag transport. Several recent studies have implicated host cell factors that function in vesicular trafficking in Gag–membrane association. These include the ADP ribosylation factors (Arfs) that associate with clathrin adaptor protein (AP) complexes to regulate protein and membrane trafficking in the cell and the SNARE [for SNAP (soluble NSF attachment protein) receptor] machinery that is required for movement and fusion of transport vesicles between membrane compartments in the cell. The AP complexes themselves have also been reported to modulate HIV-1 particle assembly. Kinesins (e.g., Kif3 and 4), P-body components (e.g., MOV10), Staufin, the ATPase ABCE1, and other host cell factors, as well as intracellular calcium levels, have also been implicated in various aspects of Gag transport. In some cases, a role for these factors could be linked to movement of the viral RNA, which, via association with the NC domain of Gag, regulates virus



HIV-1 Assembly Cofactors, Fig. 1 (continued) two highly conserved peptide motifs, proline–threonine–alanine–proline (*PTAP*) and tyrosine–proline–variable–leucine (*YPXnL*) which bind host cellular cofactors Tsg101 and Alix, respectively. **(b)** Representative electron micrographs showing HeLa cells expressing wild-type (*WT*) HIV-1 on the *left* and the *PTAP*-mutated HIV-1 on the *right*. The *PTAP*-mutated HIV-1 displays an accumulation of assembled virions arrested at the plasma

membrane (Adapted from Freed and Martin, *Fields Virology*, 5th Edition, with permission). **(c)** Host cellular ESCRT machinery is required for multivesicular body (*MVB*) biogenesis and HIV-1 budding. Key protein–protein interactions are denoted with *arrowheads*. *MVB* biogenesis is depicted on the *bottom* and HIV-1 budding on the *top*. Details provided in the text (Adapted from Balasubramaniam and Freed, *Physiology* 2011, with permission)

assembly (Balasubramaniam and Freed 2011). Clearly, more studies will be needed to dissect the pathway and mechanism by which Gag traffics to the inner leaflet of the plasma membrane.

HIV-1 Env Glycoprotein Trafficking and Incorporation

The HIV-1 Env glycoproteins are synthesized in the rough endoplasmic reticulum as a 160-kDa precursor protein known as gp160. gp160 oligomerizes in the ER and is transported to the Golgi apparatus. In the Golgi, furin or a furin-like protease cleaves gp160 into the surface glycoprotein subunit gp120 and transmembrane subunit gp41. Non-covalently associated gp120/gp41 complexes are transported to the plasma membrane where they form the functional Env complex, which consists of a heterotrimer of gp120 and gp41. The Env glycoproteins are heavily decorated with high-mannose oligosaccharide side chains in the ER; many of these side chains acquire complex modifications during transit through the secretory pathway. Indeed, >50% of the mass of gp120 is comprised of oligosaccharides. The cytoplasmic tail of gp41 contains recognition motifs for clathrin AP complexes. As a result, Env is rapidly internalized after it reaches the plasma membrane. Levels of Env at the plasma membrane are thus relatively low, limiting Env-mediated cell–cell fusion (syncytium formation) and detection by the host immune system.

Although the pathway by which Env reaches the plasma membrane is well understood (in contrast to the situation with Gag), many questions remain concerning the mechanism by which Env glycoprotein complexes are incorporated into virions. Data in the literature support four non-mutually exclusive models for Env incorporation. (1) The simplest “passive incorporation” model proposes that there is no interaction between HIV-1 Gag and Env; Env complexes are randomly incorporated into virions as a consequence of their presence at the plasma membrane. (2) Evidence that the gp41 cytoplasmic tail can interact with the MA domain of Gag favors a “direct Gag–Env interaction” model whereby MA

binding to the gp41 cytoplasmic tail allows for Env incorporation. (3) As mentioned above, lipid rafts are sites of virus assembly at the plasma membrane, suggesting a “Gag–Env cotargeting” model whereby colocalization of Gag and Env to lipid rafts or other plasma membrane microdomains enhances the efficiency of Env incorporation. (4) Finally, a variety of host cell proteins have been implicated as potential linkers that might bridge or otherwise promote Gag–Env interaction, leading to the “indirect Gag–Env interaction” model. Furthermore, the requirement for the gp41 cytoplasmic tail in Env incorporation has been shown to be markedly cell type dependent, consistent with a role for host cell machinery in Env recruitment into virions. Thus far, however, no host cell protein has been definitively established as a direct mediator of Env incorporation (Checkley et al. 2011).

Host Factors Incorporated Into HIV-1 Virions

As described above, various steps in the HIV-1 replication cycle require host cellular cofactors. For example, the process of reverse transcription, by which the single-stranded RNA genome that is packaged into virions is converted to double-stranded DNA, is primed by tRNA^{Lys3}. This tRNA is incorporated into virions during the assembly process via interactions with the primer binding site of the viral RNA and the NC domain of Gag. Lysyl-tRNA synthetase may also promote packaging of tRNA^{Lys3} into particles and placement onto the primer binding site. A number of membrane proteins are associated with the virion lipid bilayer, where they serve incompletely defined roles. Other host factors, such as cyclophilin A and cytoskeletal elements, can also be detected to varying levels in HIV-1 particles (Ott 2008). Cyclophilin A, which interacts directly with the CA domain of Gag, may function in viral counteraction of the innate host restriction factor TRIM5a (cross-reference with cyclophilin A). In other cases, incorporated proteins either play no discernible role in virus replication or perform functions that remain to be characterized.

HIV-1 Late Domains Mediate Virus Budding

All retroviral Gag proteins contain so-called “late” domains that play key roles in virus budding (► [Assembly](#) and ► [Budding](#)). These retroviral late domains are highly conserved peptide motifs that come in three varieties: proline–serine/threonine–alanine–proline (PS/TAP), tyrosine–proline–variable–leucine (YPXnL), and proline–proline–proline–tyrosine (PPPY). These peptide motifs are referred to as late domains because they function in the late stages of virus budding from an infected host cell. Equine infectious anemia virus (EIAV) contains a single YPXnL motif. A number of retroviruses, e.g., murine leukemia virus (MLV) and Rous sarcoma virus (RSV), contain PPPY motifs. In many cases, retroviruses encode multiple late domains. For examples, Mason–Pfeizer monkey virus (M-PMV) and human T-cell leukemia virus 1 (HTLV-1) contain two functionally redundant motifs, PPPY and PS/TAP. It is currently unclear why retroviruses often contain multiple late domains, but this feature presumably provides a functional redundancy that allows efficient budding under varying circumstances and in different cell types.

The HIV-1 p6 domain contains two highly conserved peptide motifs that promote the release of virions from host cells upon direct binding to host cell cofactors. Early studies observed that deletion of the p6 domain prevented release of assembled virus particles from the cell surface. Mutational analysis of the p6 domain mapped the defect in virus particle production to a highly conserved PTAP near the N-terminus of p6. Disruption of the PTAP motif yielded a striking phenotype when visualized by electron microscopy (Fig. 1b); cells expressing wild-type (WT) HIV-1 displayed numerous mature virions that had been released from the cell surface. In contrast, cells expressing a PTAP-mutated (PTAP-) HIV-1 displayed an accumulation of immature particles that failed to detach from the cell surface.

HIV-1 p6 also contains a secondary late domain – YPXnL – located downstream of PS/TAP. Although mutations in the YPXnL motif do not impair virus release as profoundly

as do mutations in PS/TAP, they do delay the kinetics of release and impose defects in Gag processing and virus replication. Altogether, the prevalence of these three retroviral late domains (PTAP, YPXnL, and PPPY) across divergent retroviruses demonstrates the fundamental role of these canonical peptide motifs in virus budding (Freed 2002; Fujii et al. 2007).

Retroviral Late Domains Directly Interact with Host Cell Cofactors: The ESCRT Proteins

A large number of studies have demonstrated that retroviral late domains promote virus budding and release by recruiting a cellular apparatus known as the endosomal sorting complex required for transport (ESCRT) machinery. Four ESCRT complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III) were initially described in yeast and then shown to be highly conserved across eukaryotes (Hurley et al. 2010). The ESCRT machinery plays a key role in the sorting of ubiquitinated cargo proteins to the lysosome (the vacuole in yeast) by delivering them into vesicles that bud into late endosomes to generate multivesicular bodies (MVBs). In addition to MVB sorting, a subset of ESCRT machinery is also required for abscission, the pinching off of the intercellular membranous bridge at the end of cytokinesis. The underlying parallels between these three biological functions – MVB sorting, cytokinesis, and virus budding – are their topological similarity, i.e., budding oriented away from the cytoplasm (Martin-Serrano and Neil 2011).

As depicted in Fig. 1c (bottom), ubiquitinated cargo proteins destined for the MVB pathway are recognized sequentially by the four ESCRTs. First, ESCRT-0 initiates the cascade upon STAM (signal-transducing adaptor molecule) binding to Hrs (hepatocyte growth factor-regulated substrate). This STAM/h complex recognizes and binds ubiquitinated cargo. Hrs contains a PSAP motif that binds the ESCRT-I subunit Tsg101. ESCRT-I is a heterotetramer comprised of Tsg101, Vps28, Vps37, and Mvb12. The

C-terminal domain of Vps28 subsequently interacts with the ESCRT-II complex. The mammalian ESCRT-II complex consists of two copies of EAP20 that bind asymmetrically to EAP30 and EAP45. Importantly, EAP45 binds ubiquitin via the GLUE (GRAM-like ubiquitin-binding in EAP45) domain. EAP20 recognizes the N-terminal domain of CHMP6, thereby recruiting ESCRT-III. The final membrane scission step is catalyzed by ESCRT-III, which assembles a ring-like constriction around the neck of the budding vesicle or virion. The energetics of membrane scission may also be favored by the catalytic activity of the ATPase Vps4, which, after pinching off is completed, is required for the release of the ESCRT components from the membrane (Hurley et al. 2010).

Tsg101 Is a Cellular Cofactor Required for HIV-1 Budding

As described above, multiple lines of evidence suggested that the highly conserved PS/TAP motif in HIV-1 p6 is required for efficient virus budding. Key studies from multiple groups in 2001 and 2002 determined that the PS/TAP motif of HIV-1 p6 binds Tsg101, essentially mimicking the interaction between Tsg101 and the PSAP motif of Hrs. siRNA-mediated depletion of Tsg101 from virus-producing cells resulted in profound defects in HIV-1 budding, recapitulating the previously observed effects of PS/TAP mutation (Fig. 1b). Support for the role of Tsg101 in HIV-1 budding also came from experiments showing that fusion of Tsg101 to Gag could rescue the budding defects induced by p6 mutation. Furthermore, expression of a dominant-negative Tsg101 fragment (TSG-5') potently inhibited HIV-1 release in a PTAP-dependent manner (Morita and Sundquist 2004).

Alix Serves a Secondary Role in HIV-1 Budding

Alix (apoptosis-linked gene 2 (ALG-2)-interacting protein X or AIP1) is another

ESCRT-related protein that serves as a cofactor in HIV-1 budding. The domain structure of Alix consists of an N-terminal Bro1 domain, a central V domain, and a C-terminal Pro-rich domain. The central domain of Alix is composed of two extended three-helix bundles that form elongated arms to fold back into a V-like conformation, hence the V-domain designation. Alix interacts with the YPXnL motif in p6 (Fig. 1a) via a highly conserved Phe in the V domain. Functional studies revealed that the Alix V domain itself could inhibit HIV-1 budding in a dominant-negative fashion (Fujii et al. 2007); this inhibitory activity was eliminated by mutation of the abovementioned Phe, demonstrating that V-domain-mediated inhibition required a direct p6–Alix interaction. Alix has been shown to interact with Gag not only via the YPXnL motif in p6 but also through the NC domain, an interaction that maps to the Bro1 domain of Alix and the NC zinc fingers. Mutation of the Alix binding site in p6 causes delayed virus release kinetics and impaired virus replication. A role for Alix in virus budding is also supported by the observation that the budding defect imposed by mutation of the PTAP motif can be rescued by overexpression of Alix.

Although ESCRT-II is required for the sorting of many cargo proteins into MVBs, it does not appear to be required for HIV-1 budding. How budding bypasses ESCRT-II remains an unresolved question. Interaction of Alix with Gag could provide a bridge between ESCRT-I and ESCRT-III since Alix harbors binding sites for both complexes (Fig. 1c). However, the small effect on budding of Alix depletion relative to that observed with Tsg101 knockdown suggests that Alix is not the primary host factor that allows HIV-1 to bridge between ESCRT-I and ESCRT-III. Further studies will be required to clarify this issue.

Discovery of Host Dependency Factors

As described above, a number of host factors – including proteins, nucleic acids, and lipids – have been demonstrated to play positive

roles in HIV-1 assembly and release. Several recent studies have used genome-wide siRNA screens to identify genes required for HIV-1 replication. While most of these analyses used viral infection as the readout and thus were not designed to identify factors required late in the virus replication cycle, one study (Brass et al. 2008) did incorporate an experimental arm aimed at identifying late-acting factors. Most of the factors identified in this study as being required for late steps in virus replication have not been validated or characterized; however, the genome-wide RNAi approach will no doubt be useful in the future for identifying host factors that play essential roles throughout the virus replication cycle.

BST-2/Tetherin Restricts Retrovirus Release

Although this chapter focuses on cellular machinery that plays a positive role in HIV-1 replication, it should be noted that mammalian cells have evolved a variety of mechanisms to restrict retroviral infection (Harris et al. 2012). These factors, which in some cases are interferon inducible, constitute a component of the innate cellular immune response to viral infection. An example of such a cellular restriction factor is BST-2/tetherin, a protein with an unusual topology comprised of an N-terminal cytoplasmic domain followed by a transmembrane anchor, an extracellular coiled-coil domain, and a C-terminal glycosylphosphatidylinositol (GPI) anchor. Expression of tetherin in the HIV-1 producer cell results in the retention of mature, released particles at the plasma membrane, apparently as a result of dimerization between the coiled-coil domains of tetherin molecules present on the plasma membrane and in the lipid bilayer of the viral envelope. The retention of particles at the plasma membrane greatly diminishes the number of cell-free virions that are produced from an infected cell. Since its discovery as an HIV-1 restriction factor, tetherin has been shown to impair the release of a number of enveloped viruses. In many cases, viruses have evolved strategies to counteract tetherin; in the

case of HIV-1, the viral accessory protein Vpu induces the internalization and degradation of tetherin, thereby neutralizing its activity. The antiviral activity of tetherin helps to illustrate the concept that mammalian cells are not passive vessels for retroviral infection but rather have developed an active, dynamic, and rapidly evolving set of cellular defense mechanisms that limit the assault by these viruses.

Conclusion

It is clear that HIV-1 and other retroviruses utilize host cellular cofactors to facilitate virus assembly and budding. To date, the best-described host cofactors for retrovirus assembly and release are members of the cellular ESCRT machinery. However, a myriad of additional, putative host cell cofactors have been implicated in the late stages of HIV-1 replication. These candidate protein cofactors may play functionally redundant roles across different retroviruses. Characterization of these cellular cofactors remains an exciting area of investigation. In particular, outstanding questions to be addressed include the following: (1) Do host cofactor(s) serve as an adaptor or scaffolding protein to facilitate Gag and Env interaction during Env incorporation? (2) What host cellular cofactors could provide a functional link between the ESCRT-I and ESCRT-III complexes during HIV-1 budding? (3) What are the differences in cell type and/or replication strategy that account for late domain redundancies among retroviruses? (4) And, ultimately, how can understanding of these key interactions between the virus and the cell be translated into the development of therapeutically effective inhibitors?

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HIV-1 Maturation

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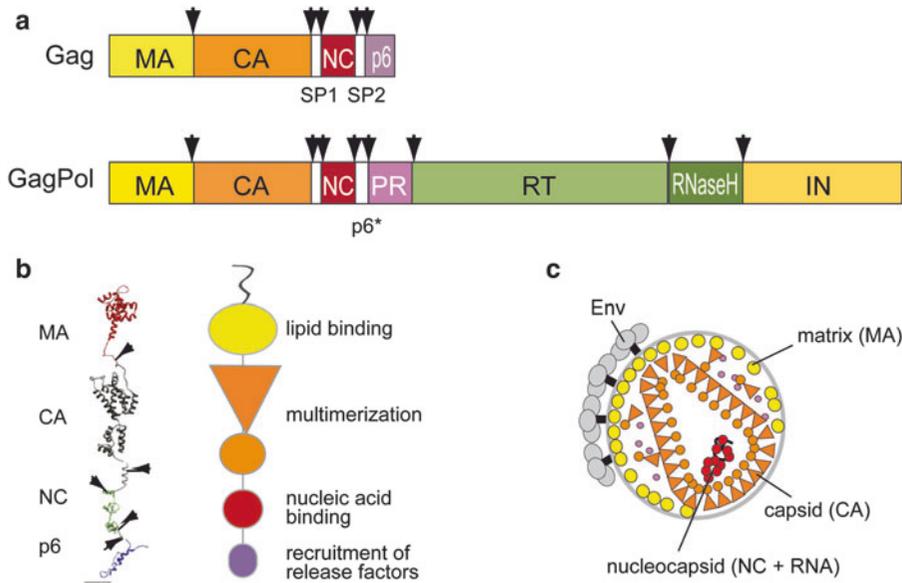
Definition

The term “virus maturation” in general refers to alterations of the architecture of a virus that occur after the particle with all its constituents has been formed. In the case of HIV-1 and other retroviruses, maturation involves cleavage of the main structural polyprotein, Gag, by the virus-encoded protease once the immature virion is released from

the cell. Gag proteolysis results in dramatic changes of the virus morphology that are essential for HIV-1 infectivity. Inhibitors of proteolytic maturation are commonly used as antiviral drugs in current regimens for treatment of HIV-infected patients.

HIV-1 Particle Maturation: What Is It and What Is It Good for?

Viruses may be regarded as “miniaturized” forms of life, reduced to the bare necessities required to ensure efficient replication. They employ various strategies of “genetic economy” to increase the amount of information encoded in a minimal length genome. An important genetic economy strategy is the production of polyproteins consisting of multiple functional domains. The polyprotein precursors can play a particular role in the replication cycle, whereas individual subunits produced by regulated proteolysis of the precursor may serve different purposes. This strategy is also used by HIV-1 and all other retroviruses. The HIV-1 genome is less than 10,000 nucleotides long and encodes only 15 proteins (Frankel and Young 1998). The inner structural proteins of the virus (Gag products) and the viral enzymes protease (PR), reverse transcriptase (RT), and integrase (IN) are produced as parts of the Gag and Gag-Pol polyproteins, respectively (Fig. 1a). Newly produced viruses are assembled from these precursors. As outlined below, the Gag polyprotein is a central organizer of productive HIV-1 assembly, and the presence of multiple functional domains within the molecule is crucial for the assembly process (Sundquist and Krausslich 2012) (► [Virus Assembly](#)). Released progeny virus particles containing Gag and Gag-Pol polyproteins as well as other essential virion components are still noninfectious and are designated “immature.” Cleavage of Gag and Gag-Pol into their individual functional subdomains by the virus-encoded PR leads to extensive rearrangements of the Gag subunits within the particle, activates the viral enzymes, and increases entry efficiency. This process is called “maturation,” and it is essential for HIV-1 infectivity.



HIV-1 Maturation, Fig. 1 The HIV-1 Gag and GagPol polyproteins. (a) Scheme of the HIV-1 Gag and GagPol polyproteins. (b) Model of the Gag polyprotein, derived by connecting structural models of individually folded domains (*right*; Modified from reference Briggs and

Krausslich (2011)) and functional domains of the poly-protein (*left*). (c) Gag subunits MA, CA, and NC as structural elements of the mature HIV-1 virion. Arrowheads in (a) and (b) indicate cleavage sites of HIV-1 PR

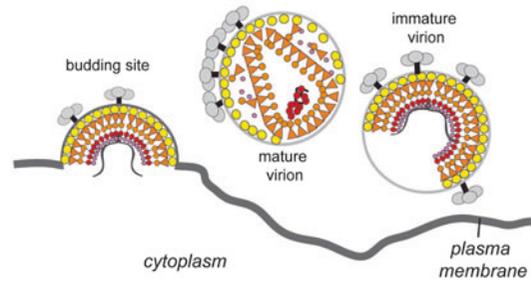
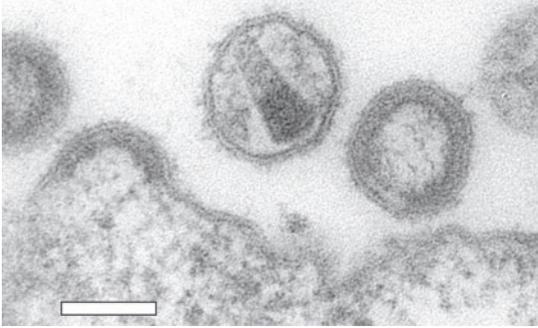
The Polyprotein Gag: A Multitasking Protein

HIV-1 particle morphogenesis is the final step of HIV-1 replication. Newly synthesized virion components assemble at the inner face of the plasma membrane of infected cells into spherical buds that are released by abscission of the surrounding lipid envelope from the host cell membrane (Sundquist and Krausslich 2012). The viral structural polyprotein Gag is the central orchestrator of these processes. Gag forms the inner protein shell of HIV-1; approximately 2,500 molecules represent ~50% of total virion mass. Gag traffics to the plasma membrane where it assembles into spherical particles that can be formed even in the absence of other HIV-1 proteins. In HIV-1-infected cells, Gag brings further components of the infectious virus, e.g., the viral Env glycoproteins, the Gag-Pol polyprotein, and the viral RNA genome, to the budding site (► [Budding](#)). It is also responsible for recruitment of the cellular ESCRT (endosomal sorting complex required for transport) machinery that catalyzes membrane fission

(Sundquist and Krausslich 2012). For a detailed description of these events, please see the entries on “► [Virus Assembly](#)” and “► [Budding](#).”

To organize this complex sequence of events, Gag needs to interact with various proteins, as well as with lipids and nucleic acids, in a temporally and spatially controlled manner. This is supported by the modular design of the polyprotein (Fig. 1b). Gag comprises a myristoylated lipid membrane binding domain (matrix, MA), a multimerization domain (capsid, CA), a nucleic acid binding domain (nucleocapsid, NC), and an adapter domain for the recruitment of other proteins (p6). In the polyprotein, these domains are linked by flexible hinge regions; two small spacer peptides, SP1 and SP2, separate the CA, NC, and p6 domains and provide additional flexibility (Fig. 1b). The interplay between these different domains regulates HIV-1 particle assembly (Sundquist and Krausslich 2012; Ganser-Pornillos et al. 2012).

Although particles formed in this manner contain all essential components of the virion, they are noninfectious. The generation of infectious



HIV-1 Maturation, Fig. 2 Stages of HIV-1 particle morphogenesis. The thin-section electron micrograph (*left*) and cartoon (*right*) show an HIV-1 budding site at the plasma membrane of a virus-producing human T-cell,

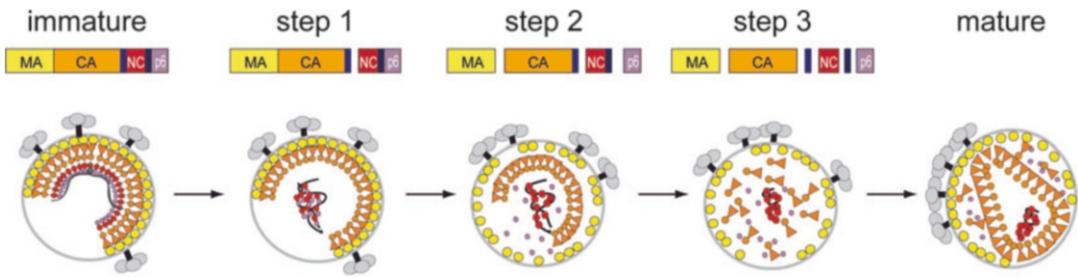
as well as an immature and a mature HIV-1 virion (electron micrograph: Sonja Welsch, Virology Heidelberg. Scale bar: 100 nm)

HIV-1 requires a further step, termed virus maturation, occurring concomitant with or after the release. During maturation, the viral PR incorporated in the particles as part of Gag-Pol cleaves Gag and Gag-Pol polyproteins into their mature subunits, accompanied by a dramatic alteration of virion morphology (Briggs and Krausslich 2011; Ganser-Pornillos et al. 2012). While immature particles display a truncated sphere consisting of a lattice of Gag polyproteins underneath their envelope, mature, infectious particles can be recognized in electron micrographs by a distinctive conical capsid encasing the viral genome (Fig. 2). In the mature particle, an MA layer lines the lipid envelope, and CA forms the characteristic conical capsid within which NC complexes and condenses the genomic RNA (Fig. 1c). It is evident that proteolysis needs to be tightly controlled: not only delayed but also premature processing is deleterious to HIV-1 infectivity. Surprisingly, it is still not clearly understood how maturation is triggered, however. Since each Gag-Pol molecule contains only a monomer of the dimeric enzyme PR, protein dimerization is clearly essential. However, other yet unknown factors may contribute to the initiation of the maturation process as well. Likewise, while it can be estimated that assembly and release of an HIV-1 particle occur within a period of 15–30 min, the duration of maturation has not been determined. The fact that mature particles are readily detected in the vicinity of the membrane of virus-producing cells, whereas

intermediates of morphological maturation are not observed by electron microscopy, suggests that the process is fairly rapid, but the determination of its kinetics has so far not been possible.

Mechanism and Regulation of HIV-1 Particle Maturation

Electron microscopy analyses of the mature and immature virion yielded detailed insights into the formation and architecture of HIV-1 particles and provided information about the mechanism of morphological maturation (Virus Structure). The basic element of both the immature Gag shell and the mature capsid is a CA hexamer. However, the structures of the “immature” and “mature” CA hexamer are not identical (Briggs and Krausslich 2011; Ganser-Pornillos et al. 2012). In the nascent virus bud and in the immature particle, Gag molecules are organized in parallel perpendicular to the membrane in a hexameric lattice with a spacing of 8 nm. A regular arrangement of hexamers would be flat, but small irregular defects in the Gag lattice permit the outward curvature of the nascent HIV-1 bud. While Gag-derived proteins can assemble into spherical particles *in vitro*, cryo-EM analysis of immature HIV-1 particles revealed that the Gag shell is not a closed sphere but covers only $\sim 2/3$ of the lipid envelope and that the degree of shell closure varies between individual virus particles. This finding suggested



HIV-1 Maturation, Fig. 3 Model of the HIV-1 maturation process. Stepwise cleavage of the Gag polyprotein (*top*) regulates the process of morphological rearrangements within the particle (*bottom*). See main text for details

that the membrane closure mediating virus abscission occurs in parallel to Gag assembly and not after completion of the Gag shell. Membrane fission is mediated by the cellular ESCRT machinery (► [Budding](#)), and the degree of completion of the Gag shell of an individual particle may thus depend on the relative kinetics of the polymerization of Gag and ESCRT. Structural analyses of the mature capsid indicated a buckminsterfullerene-like architecture consisting of CA hexamers, with a small number of CA pentamers (Briggs and Krausslich 2011; Ganser-Pornillos et al. 2012). With a spacing of 9.6 nm, CA hexamers in the mature lattice are more loosely packed compared to the immature lattice. Furthermore, only about half of all CA molecules contained in the virion are used for assembly of the mature capsid. Both findings suggest that capsid maturation does not occur through condensation of the immature shell into a cone shape but rather involves disassembly of the immature structure followed by reassociation of CA molecules to form the mature lattice. Since this complex transition occurs within the confined and tightly packed space of the released virion at millimolar CA concentration, it requires precise regulation.

Numerous biochemical and structural analyses contributed to the current model describing the sequence of events in the HIV-1 maturation process (Virus Structure) (Briggs and Krausslich 2011; Sundquist and Krausslich 2012; Ganser-Pornillos et al. 2012). The necessary regulation of the complex morphological transitions is accomplished by an ordered sequence of cleavage events within Gag and Gag-Pol by the viral protease that releases the mature subunits in an

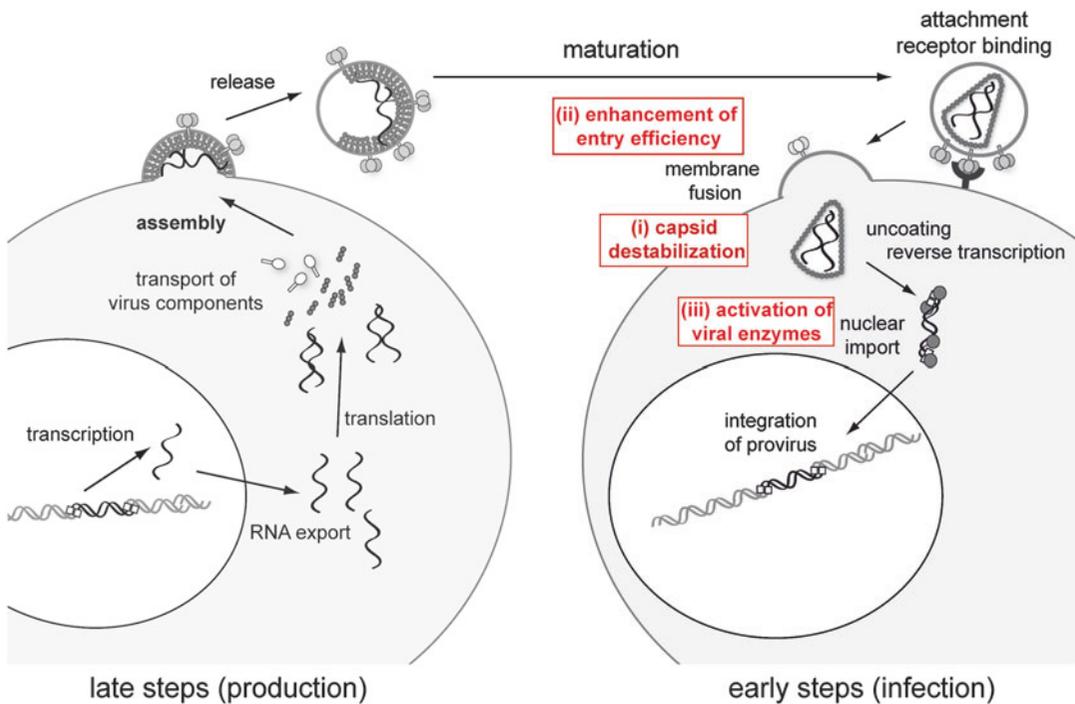
organized manner (Fig. 3). Different amino acid sequences at the individual processing sites and differential accessibility of the cleavage regions result in different rates of cleavage by PR. As a first step, proteolysis at the N-terminus of NC releases the viral genome-protein complex from the membrane-attached N-terminal portion of Gag. This allows initial condensation of the nucleoprotein complex, while the immature CA lattice remains unaltered (Fig. 3, step 1). Next, processing between MA and CA separates the CA lattice from the surrounding lipid membrane (Fig. 3, step 2). This separation is a prerequisite for dissociation of the immature CA lattice. If it is blocked by mutagenesis while all other cleavage events are allowed to proceed, CA retains the immature lattice structure. However, MA-CA separation is not sufficient for immature lattice dissociation, but cleavage at both ends of CA is required. An important regulatory function governing mature core formation is apparently provided by the spacer peptides SP1 and SP2 which are removed from the C-termini of CA and NC, respectively, during the final stages of maturation. The release of SP1 from CA allows CA multimer dissociation, thereby providing the building blocks for the mature core (Fig. 3, step 3). The role of SP2 is currently less well understood. Mutations that prevent the proteolytic cleavages within the C-terminal portion of Gag (NC-SP2-p6) severely diminish infectivity and disturb mature capsid formation, although mature CA protein is released. Based on available virological, biochemical, and structural data, it appears likely that a function of multiple ordered cleavages within the NC-SP2-p6 region may be to

ensure controlled condensation of the nucleoprotein complex in order to coordinate it with progression of the assembly of the surrounding conical capsid.

HIV-1 Maturation: The End or the Beginning?

HIV-1 maturation is required for the generation of functional viral enzymes PR, RT, and IN, contained in the immature virus in an inactive (or partially active) form as parts of the Gag-Pol precursor polyprotein. It is therefore usually displayed as the final step in graphic representations of the viral replication cycle (► [Overview Life Cycle](#)). Alternatively, maturation can be considered as an initial step, preparing the virus for cell entry. Mediated by proteolysis of the inner structural proteins, the particle switches from an “assembly mode” into an “infection mode” (Fig. 4). This solves a general problem

encountered by viruses, which has been described by A. Helenius as the “assembly-disassembly paradox.” Viral shells have to be assembled as stable structures in order to encase and protect the viral genome in an extracellular environment. However, in the newly infected host cell, this particulate structure needs to disassemble in order to release the infecting virus genome (“uncoating”) (► [Uncoating and Nuclear Import](#)). Since the producing cell and the newly infected cell are often of the same cell type and may be immediately adjacent, rapid alterations within the virion structure are necessary to mediate differential stability in these similar cellular environments. In the case of HIV-1, virus maturation leads to at least three functional changes important for infection: (i) destabilization of the inner protein structure, (ii) increased fusogenicity of the viral envelope, and (iii) activation of viral replication enzymes. Gag proteolysis transforms the protein shell of the particle from a mechanically stable into a metastable state ready for disassembly (Sundquist and



HIV-1 Maturation, Fig. 4 Maturation prepares HIV-1 for early replication events. Scheme of the late and early steps of the HIV-1 replication cycle. Red boxes indicate

functional consequences of maturation that affect the entry and postentry replication steps. See main text for details

Krausslich 2012). Biochemical studies revealed that the immature Gag shell remains stable upon removal of the surrounding lipid envelope by detergent treatment, while the mature capsid dissociates rapidly under these conditions. Moreover, physical measurements suggested that immature particles are more rigid than mature HIV-1 virions. Mutations in the viral CA protein that alter capsid stability in the test tube have been shown to affect HIV-1 infectivity. Interference with genome uncoating seems to block replication steps occurring after cell entry, in particular the reverse transcription of the viral genome. Both stabilization and destabilization of the capsid is detrimental for infectivity (► [Reverse Transcription](#)). Temporally controlled dissociation of the mature capsid thus apparently regulates events in the early postentry phase of HIV-1 replication. Vice versa, the progress of reverse transcription seems to be coupled to capsid uncoating: inhibition of reverse transcription has been reported to enhance the stability of entering capsids. The regulation of the uncoating process thus represents a main function of HIV-1 proteolytic maturation.

Interestingly, the inner structural maturation of the particle is also correlated to the entry competence of the virion. HIV-1 cell entry occurs by fusion of the viral lipid envelope with the membrane of the host cell (► [Fusion](#)), mediated by the viral Env glycoproteins on the virus surface. Immature HIV-1 particles generated by mutation of processing sites in Gag or by inhibition of PR display significantly reduced fusion efficiency, although these treatments do not affect Env incorporation or processing (Checkley et al. 2011). Recently, visualization of subviral details by super-resolution fluorescence microscopy provided evidence for a mechanism linking changes within the virion to its surface. In contrast to many other enveloped viruses, HIV-1 carries only a sparse number of Env molecules on its surface, which may be important for immune escape. Super-resolution microscopy revealed that in immature or partially mature virions, these molecules are distributed in several patches on the viral surface, while they cluster more closely together on the envelope of mature virions. Clusters of Env molecules contact patches of receptor molecules

on the cell surface, and Gag maturation-dependent Env clustering may thereby enhance the entry efficiency of the virus. This “inside-out signaling” strategy might ensure that only virus particles whose interior has sufficiently matured to allow efficient progression of early postentry steps are able to enter a new target cell.

Furthermore, proteolytic maturation is also essential for activation of viral enzymes RT and IN that mediate essential postentry replication steps. RT transforms the ssRNA virus genome into a double-stranded DNA copy (Catalyzed HIV-1 DNA Synthesis), whose covalent integration into the host cell genome is catalyzed by IN (► [Integration](#)). A monomer of each RT and IN is encoded as part of the Gag-Pol precursor protein. The active form of HIV-1 RT, however, is a heterodimer from the encoded RT monomer (p66) and p51, which is generated by PR-mediated cleavage of the p66 C-terminus. The active form of IN is a structured “intasome” complex, in which a dimer of IN dimers is bound to the ends of the viral genome (Engelman and Cherepanov 2012). Proteolytic release of the respective monomers from the precursor is required for proper formation of these active enzyme complexes.

HIV-1 Maturation as Target for Antiretroviral Therapy

HIV-1 maturation is crucial for viral infectivity, thereby representing a target for antiviral intervention. Since the maturation process required precise coordination, maturation inhibitors may either impair proteolysis at one specific site or inhibit viral proteolysis in general by targeting HIV-1 PR. Together with inhibitors of the viral reverse transcriptase, HIV-1 PR inhibitors (PIs) are the keystones of modern antiretroviral therapy (highly active antiretroviral therapy, HAART) (Anderson et al. 2009; Adamson 2012). PIs bind to the active site of HIV-1 PR and prevent the enzyme from cleaving Gag and Gag-Pol, thereby blocking morphological virus maturation. The infected cell releases virus particles with normal efficiency, but these particles remain immature

and are unable to infect a new target cell. The powerful antiviral effect of PIs illustrates the delicate balance of HIV-1 morphological maturation. In tissue culture inhibition experiments, even modest effects on Gag processing are correlated to a significant reduction of virus infectivity. A small proportion of uncleaved or partially cleaved Gag apparently suffices to disturb the ordered process of morphological maturation, thereby enhancing the inhibitory effect of PIs.

Mutagenesis studies revealed that even inhibition of cleavage at one of the five processing sites within Gag is sufficient to reduce or abolish virus infectivity. This is exploited by the maturation inhibitors bevirimat and PF-46396 (Salzwedel et al. 2007; Waheed and Freed 2012; Adamson 2012). These compounds do not affect the enzymatic activity of HIV-1 PR but rather act on its substrate. By binding to Gag in the vicinity of the processing site that separates CA and SP1, they specifically impair proteolysis at this position and prevent formation of the mature CA lattice. Although cleavage inhibition at the CA-SP1 site by bevirimat is incomplete and processing at the remaining sites in Gag and Gag-Pol is not impaired, HIV-1 infectivity is efficiently inhibited in tissue culture. The compound also proved to be effective in early clinical trials in HIV-1-infected patients. However, mutations in Gag that conferred bevirimat resistance *in vitro* could be selected under drug pressure. Unfortunately, these mutations were also detected in a significant proportion of viruses isolated from patients that had not been treated with bevirimat, indicating that these resistant isolates represent naturally occurring virus variants. The clinical development of the drug is halted, but compounds with a similar mode of action are being explored in pre-clinical studies.

The availability of a diverse panel of drugs targeting different steps of the viral replication cycle is a prerequisite for long-term successful antiretroviral treatment. It is therefore of interest to consider other aspects of HIV-1 maturation as potential targets for therapeutic intervention. First, the timing of proteolytic maturation with respect to assembly and release is crucial for HIV-1 morphogenesis. Accordingly, not only the inhibition

but also the stimulation of PR activity impairs HIV-1 replication in tissue culture. Premature or enhanced activation of HIV-1 PR, induced, for example, by certain mutations in Gag-Pol, results in intracellular processing of Gag and thus interferes with the assembly and release of immature virions. Interestingly, an increased level of intracellular Gag processing and reduced particle release can be observed in tissue culture experiments upon treatment with some of the therapeutically used non-nucleosidic HIV-1 RT inhibitors. However, drugs that exploit the principle of PR activation for the inhibition of HIV-1 infection have not yet been developed.

Second, a crucial step of virus maturation is the assembly of the conical capsid within the virus after the mature CA protein has been released. On theoretical grounds, this process appears to be an attractive target: interference with a small proportion of the interactions between subunits should disturb the arrangement of the complex higher-order multimer, and the interactions between HIV-1 CA molecules in the lattice are clearly virus specific. The fact that such an inhibitor would have to act within the released virus particle and thus needs to be incorporated during the budding process, while its molecular target, the mature CA protein, is not present at this stage, presents a conceptual difficulty for this strategy, however.

Conclusion

Proteolytic maturation of HIV-1 is essential for virus spread, and the development of effective PR inhibitors blocking maturation in the 1990s strongly contributed to the success of current highly active antiretroviral therapy. Maturation serves as an irreversible switch from the assembly mode to the infection mode and renders the virus particle competent for cell entry and replication. This process has to be tightly regulated in correspondence with virus assembly and release. While results obtained in recent years have provided detailed insights into the structural basis and mechanistic consequences of maturation, the mechanisms for controlling proteolysis are still

not understood. Interfering with the triggering events or disturbing the delicate balance of proteolysis would appear to be one of the most promising remaining targets for antiretroviral therapy.

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HIV-1 Mutational Escape from Host Immunity

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Definition

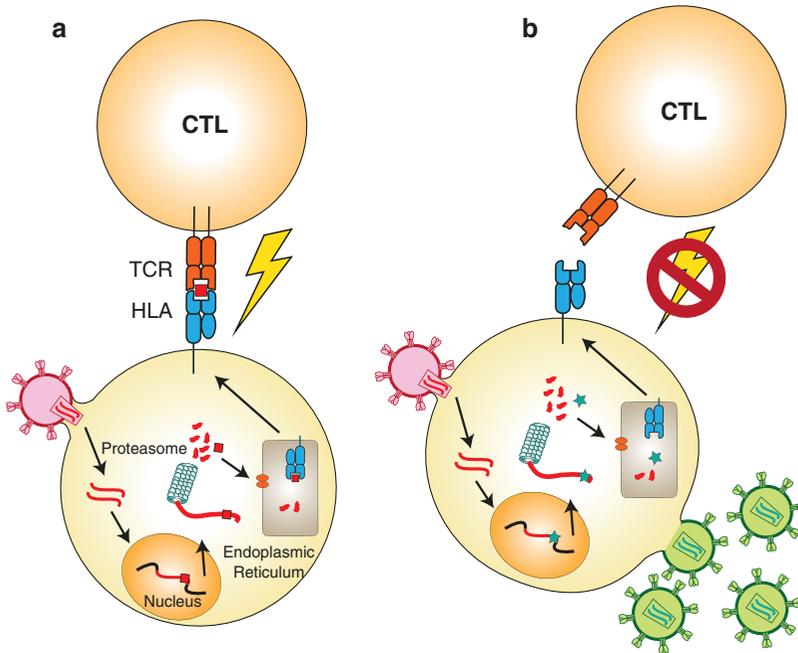
Within an infected individual, HIV-1 develops specific mutations within its genome that allow it to escape detection by host immune responses. As

such, host immunity represents a major selective force driving the evolution and diversification of HIV-1 at the individual and population levels. Here, we highlight HIV-1 mutational escape from adaptive, innate, and vaccine-induced immune responses as highly specific and reproducible processes beginning rapidly following HIV-1 infection. The potential biological implications of immune escape, including viral fitness costs and population-level HIV-1 adaptation to host immunity, are also summarized.

Escape from CD8⁺ Cytotoxic T Lymphocytes

CD8⁺ cytotoxic T lymphocytes (CTL) (► [HIV & SIV, CD8 T Cell Responses](#)) eliminate HIV-infected cells via the recognition of short, virus-derived peptide epitopes that are produced within the infected cell and presented at its surface by the highly polymorphic human leukocyte antigen (HLA) class I molecules (HLA-A, HLA-B, and HLA-C) (► [MHC Locus Variation](#)). HLA-restricted CTL play a major role in HIV-1 immune control. HIV-specific CTL first appear around acute-phase viremia decline and play an active role in its control to set-point levels (Goonetilleke et al. 2009). Experimental depletion of CD8⁺ lymphocytes in rhesus macaques results in their inability to control simian immunodeficiency virus (SIV) infection (Matano et al. 1998). Epidemiological links between host carriage of specific HLA class I alleles and HIV-1 disease progression have been demonstrated in natural history (Carrington and O'Brien 2003) and genome-wide association (Fellay et al. 2007) studies (► [Host Genetics and Genomics](#)). In particular, HLA-B*57 and HLA-B*27 are associated with lower viral loads and slower disease progression (Carrington and O'Brien 2003). Independent effects of HLA-C expression level on HIV-1 control have also been demonstrated (Apps et al. 2013).

That HLA-restricted CTL exert pressure on HIV-1 in vivo is also demonstrated by the virus' ability to escape this pressure via mutation. CTL escape was first described in 1991 when



HIV-1 Mutational Escape from Host Immunity, Fig. 1 CTL escape. (*Panel A*) HIV-1 proteins produced within an infected cell are processed into peptide epitopes by host cellular machinery and loaded onto HLA class I molecules for presentation at the cell surface. CTL eliminate infected cells via recognition of the viral peptide-HLA complex via their T-cell receptor (TCR). (*Panel B*) HIV-1 mutations can arise during the process of reverse

transcription that are then translated into protein. Here, a mutation abrogates the ability of the encoded viral epitope to bind HLA, allowing the infected cell to avoid CTL-mediated killing. Mutant progeny viruses are then released. CTL escape mutations may also hinder CTL-mediated killing of infected cells by interfering with viral antigen processing or by abrogating TCR-mediated recognition of the viral peptide-HLA complex (not shown)

researchers noted temporal shifts (and in some cases permanent loss of recognition) of HLA-B*08-restricted HIV-1 Gag epitopes targeted by patient-derived CTL over time, which coincided with the appearance of viral mutations within them (Phillips et al. 1991). Another key concept revealed by this study is the HLA-restricted nature of CTL escape, due to the requirement that epitopes be bound and presented by a specific HLA molecule for CTL recognition.

CTL escape mutations can be classified into three mechanistic categories. The most intuitive is escape via mutations that reduce or abrogate viral epitope binding to HLA, thereby impairing CTL recognition of infected cells (Fig. 1). Such mutations usually occur at HLA-specific epitope “anchor” residues – typically peptide positions two and/or C-terminus. A well-known example

is the B*27-associated R264K substitution selected at position 2 of the B*27-restricted KK10 epitope in Gag (Kelleher et al. 2001). Escape via abrogation of peptide-HLA binding represents a predominant CTL escape mechanism in vivo, with escape conferring an average (predicted) tenfold reduction in peptide-HLA binding affinity (Carlson et al. 2012a). CTL escape can also act upon processes that occur prior to, or following, peptide-HLA binding. For example, CTL escape mutations can inhibit epitope formation by interfering with their proper intracellular processing. The first such “antigen-processing (▶ [Antigen Processing](#)) escape mutation” to be mechanistically characterized was the B*57:03-restricted Gag-A146P substitution, occurring at the residue immediately upstream of the IW9 epitope, which acts via prevention of N-terminal aminopeptidase-mediated trimming

of this epitope (Draenert et al. 2004). Antigen-processing mutations can also occur within the epitope. For example, a substitution at position 5 of a B*07-restricted epitope in a cryptic Gag reading frame acted via introduction of a proteasomal cleavage site, yielding reduced epitope formation (Cardinaud et al. 2011). The final category of “T-cell receptor (TCR) escape mutations” retains the capability to bind HLA but reduces or abrogates recognition of the peptide-HLA complex by the TCR(s) expressed by the original selecting CTL. TCR escape mutations usually occur at central epitope positions. An example is the B*27-associated L268M substitution (selected at position 6 of the KK10 epitope) (Phillips et al. 1991). L268M-containing KK10 retains the ability to bind HLA-B*27 but abrogates its recognition by key B*27-restricted CTL clonotypes in the repertoire (Iglesias et al. 2011).

Despite substantial HIV-1 and host genetic variation, the mutational pathways of CTL escape are broadly predictable based on the HLA class I alleles expressed by the host. For example, three-quarters of HIV-1 subtype B-infected persons expressing the protective HLA-B*57 allele will select the T242N substitution in Gag (position 3 of the p24^{Gag} TW10 epitope) within weeks or months following infection (Leslie et al. 2004), while 50% will also select G248A at position 9 of this epitope later on (Carlson et al. 2012a). Together, these two mutations confer complete escape from B*57-restricted, TW10-specific CTL (Leslie et al. 2004). In contrast, among HLA-B*27-expressing persons, targeting of the immunodominant B*27-restricted p24^{Gag} KK10 epitope begins in early infection and is often sustained for years thereafter (Gao et al. 2005). KK10 escape begins via selection of the L268M mutation at epitope position 6 that abrogates its recognition by certain autologous B*27-restricted CTL (Iglesias et al. 2011), but complete escape from KK10-expressing CTL, via the R264K anchor residue escape at position 2 of the epitope, does not usually occur until years later (Kelleher et al. 2001). Notably, KK10 escape remains one of the few clear-cut examples where escape directly precedes loss of HIV-1 immune control (Goulder et al. 1997).

The predictable nature of CTL escape has allowed the identification of HLA-associated viral polymorphisms by statistical association. These studies, undertaken in cross-sectional datasets of linked HIV-1 and host HLA genotypes, identify viral polymorphisms significantly over- (or under-) represented among persons expressing a given HLA class I allele, identifying these as likely escape mutations (and their associated immunologically susceptible forms), respectively. The first such study, published in 2002, identified nearly 100 HLA-associated polymorphisms in HIV-1 reverse transcriptase in a cohort of ~400 patients, illustrating the extensive impact of CTL pressures on HIV-1 (Moore et al. 2002). In recognition of the potential confounding effects of viral lineage (or “founder”) effects in such analyses, more recent studies incorporate “phylogenetic corrections” (Bhattacharya et al. 2007), as well as statistical corrections for the confounding effects of linkage disequilibrium between HLA class I alleles and HIV-1 amino acid covariation.

Population-level studies have yielded comprehensive “immune escape maps” of the locations and mutational pathways of HLA-restricted CTL escape in HIV-1. These maps are most detailed for HIV-1 subtypes B (e.g., Carlson et al. 2012a) and C (e.g., Carlson et al. 2008). Population-level studies have also confirmed escape (and reversion, discussed later) as highly reproducible processes in context of host HLA. For example, the strongest HLA association in subtype B is the HLA-A*24:02-restricted Y135F escape mutation in Nef, where 81% of A*24:02-expressing persons harbor this substitution in chronic infection, compared to only 12% of persons who do not express an allele belonging to the A24 supertype (Carlson et al. 2012a). Such a strong statistical association (in this case, an odds ratio of ~30 and a p -value of 8×10^{-118} (Carlson et al. 2012a)) can only be achieved if the mutation is near-universally selected in persons harboring the HLA and reverts consistently in individuals lacking it (Fryer et al. 2012).

Escape is also highly HLA specific. When population-level analyses are undertaken at various HLA resolution levels (e.g., supertype, type, subtype), the majority (>60%) of HLA-

associated polymorphisms are identified as HLA subtype specific, while <10% are identified as shared across HLA supertypes (Carlson et al. 2012a). This high HLA specificity remains true even for closely related HLA alleles that present the same viral epitopes. For example, HLA-B*57:02, HLA-B*57:03, and HLA-B*58:01 all bind Gag-TW10, but they drive significantly different escape pathways within it (Carlson et al. 2012b). Escape pathways can also be complex and varied. Escape at a given viral site may occur along multiple pathways under pressure by a given HLA – for example, B*08-driven escape at Nef codon 94, position 5 of the B*08-restricted FL8 epitope, can occur via K94E, M, N, or Q (Brumme et al. 2007). A given HIV site may be under selection by various HLA alleles that select different, sometimes opposing, substitutions. For example, at Gag codon 147, HLA-A*25:01, HLA-B*13:02, and HLA-B*57:01 escape via selection of “L,” while B*14:02 and B*15:01 escape via selection of “I” (which also happens to be the subtype B consensus at this site) (Carlson et al. 2012a). Identification of HLA-associated polymorphisms has also aided the discovery of novel CTL epitopes, including those in cryptic HIV-1 reading frames (Berger et al. 2010).

HLA Class II-Driven Immune Escape

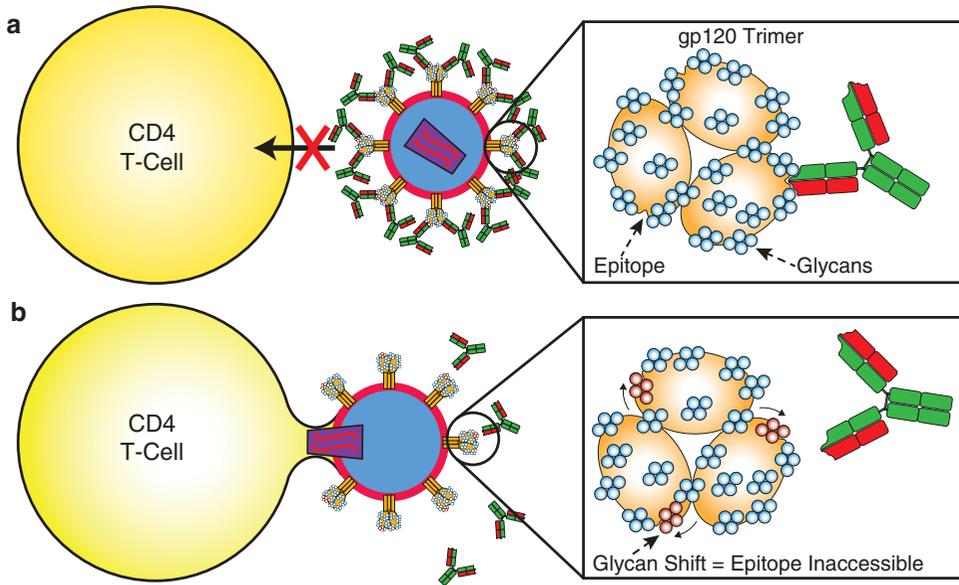
Effective antiviral immunity generally requires CD4⁺ T-lymphocyte help, but CD4⁺ responses rapidly become dysfunctional in HIV-1 infection, in part because of the specific elimination of virus-specific CD4⁺ T cells (► [HIV & SIV, CD4 T cell responses to](#)) (Douek et al. 2002). As such, the contribution of CD4⁺ T cells to HIV-1 control in vivo remains incompletely understood. Though some early studies supported the possibility of in vivo mutational escape from HIV-specific CD4⁺ T-cell responses Harcourt et al. (1998), others did not (Koeppel et al. 2006). Attempts to identify HLA class II-restricted viral polymorphisms by statistical association have yielded no strong evidence of their existence (Wright et al. 2012a).

Escape from Humoral (B-Cell) Immune Responses

HIV-1 envelope evolves rapidly within a host after infection and has diversified to an extraordinary extent at the population level (Gaschen et al. 2002) (► [HIV & SIV, B Cell Responses to](#)). Although CTL escape contributes to this process, the autologous neutralizing antibody (NAb) response is the major driver of envelope evolution. Beginning at approximately 3 months post-infection (Wei et al. 2003), HIV-infected individuals begin to develop antibodies capable of neutralizing their own virus (termed “autologous neutralizing antibodies”; NABs) Richman et al. (2003). However, unlike acute-phase HIV-specific CTL, autologous NABs do not appreciably contribute to virus containment, likely due to the rapid selection and outgrowth of neutralization-resistant escape mutants (Wei et al. 2003). Initial NAb escape exposes novel envelope epitopes against which subsequent waves of autologous NABs arise, driving further envelope evolution.

The fact that antibodies and virus coevolve in cycles of response and escape was first inferred via the ability of autologous sera to neutralize viral variants present in the infected individual 6 (or 12) months prior, but not those present at the time of serum sampling (Frost et al. 2005). Early studies of HIV-1 neutralization escape hinted at a variety of escape pathways including the accumulation of amino acid changes in envelope (Frost et al. 2005) (suggestive of escape through the selection of specific point mutations), changes in N-linked glycosylation patterns (Wei et al. 2003) (Fig. 2), and lengthening of certain hypervariable domains in gp120, notably V1/V2 (Rong et al. 2007). However, the identification of specific genetic events conferring NAb escape began only recently (e.g., the first specific identification of an envelope escape mutation conferring neutralization escape at the single antibody level was not achieved until 2009 (Rong et al. 2009)).

Unlike CTL epitopes whose (linear) sequences can be predicted from HLA-anchor residue motifs in HIV-1 sequences without knowledge of the



HIV-1 Mutational Escape from Host Immunity, Fig. 2 Neutralizing antibody escape. (Panel A) Neutralizing antibodies (NAb) bind to epitopes (shown as an indent) on the viral envelope, blocking their ability to infect target cells. (Panel B) Mutations in HIV envelope – in this case leading to changes in N-linked glycosylation patterns that block NAb access to the

epitope – confer NAb escape. NAb escape may also occur via point mutations, lengthening of certain gp120 hypervariable domains (notably V1/V2), cooperative interactions between different regions on a single or multiple members of the envelope trimer, or other mechanisms (not shown)

T-cell receptor sequence or structure, antibodies directly recognize three-dimensional epitopes whose sequences can span discontinuous sites on one or more members of the envelope trimer, rendering their locations difficult to predict based on viral sequence alone. Recent studies have therefore taken the approach of longitudinally characterizing envelope evolution while simultaneously attempting to isolate individual neutralizing antibodies (and/or the B-cell clonal lineages producing them; e.g., Moore et al. (2012)) in individual patients.

From these studies, a central role of immune-driven envelope evolution in driving autologous neutralization breadth is emerging. In one individual, initial autologous NABs were directed against epitopes in the first and second hypervariable loops of gp120 (V1/V2), and escape was achieved via point mutations in this region including one in V2 that created a putative N-linked glycosylation site conferring escape from two distinct monoclonal antibodies isolated from this patient (Rong

et al. 2009). In the second individual, escape from the initial NAB pool occurred via convergent evolutionary pathways (one involving changes in the V3–V5 gp120 outer domain and the other involving codependent changes in V1/V2 and gp41), whose lineage members subsequently oscillated in frequency (Rong et al. 2009). NAB escape via distinct evolutionary pathways within a single host was confirmed in an individual in whom escape in a V3-proximal epitope occurred along three divergent viral lineages, each featuring a unique amino acid change (Murphy et al. 2013). A study of three acutely infected individuals whose initial response was directed against different conformational epitopes in envelope, where each escaped along distinct pathways (Bar et al. 2012), also supports the strain- and host-specific nature of initial epitope targeting and autologous neutralization escape. That escape occurs via distinct mechanisms (e.g., point mutations, glycan shifts, and cooperative conformational changes between two domains) both within and among

hosts indicates that HIV-1 employs multiple mutational strategies to escape early autologous NAb (Rong et al. 2009). However, the extent to which neutralizing antibody epitopes – and their escape pathways – are shared across patients remains a key question. The observation that, compared to transmitted/founder viruses, chronic subtype C viruses are significantly enriched for a glycan at envelope codon 332 (whose presence can help trigger the evolution of broadly neutralizing antibodies against this key conserved region (Moore et al. 2012)) supports the idea of shared neutralization escape pathways.

In approximately 80% of infected individuals, this process of virus-NAb coevolution results in the continued production of NAb that remain largely specific to the individual's evolving virus. However in approximately 20% of individuals, this process leads to the emergence of antibodies that are capable of neutralizing a broad range of HIV-1 isolates across subtypes. Though individuals producing such “broadly neutralizing antibodies” do not likely derive clinical benefit from them (presumably because their own virus has already escaped) (Euler et al. 2010), the evolutionary mechanisms driving their development are of paramount interest as an effective preventative HIV-1 vaccine will likely require their elicitation (along with effective cellular responses). This discovery has led to the hypothesis that this process could be recapitulated via vaccination with specific transmitted/founder envelopes and their sequential escape variants (Liao et al. 2013), a strategy for which there is preliminary experimental support (Malherbe et al. 2011).

Non-neutralizing HIV-specific antibodies (► [Non-Neutralizing Antibody Responses and Protection Against HIV-1](#)) that mediate antibody-dependent cellular cytotoxicity (ADCC) through activation of effector cells bearing Fc receptors, notably natural killer (NK) cells, may also contribute to natural- and vaccine-induced HIV-1 immune control Wren and Kent (2011). Some evidence also supports ADCC antibodies, including those that do not possess neutralizing activity, as drivers of evolution within HIV-1 envelope and possibly other viral regions (Chung et al. 2011).

Escape from Innate Immune Responses: KIR-Driven HIV-1 Polymorphisms?

Innate immune responses (HIV & SIV, Innate Immune Responses to), in particular natural killer (NK) cells (► [Natural Killer Cells and their Role in Preventing HIV-1 Transmission](#); ► [NK Cells Responses to HIV](#)), may also directly drive immune escape. NK cells express cell-surface receptors belonging to the polymorphic killer cell immunoglobulin-like receptor (KIR) gene family, which comprise a variety of inhibitory and activating receptors that interact with HLA class I ligands on target cells. Engagement of activating KIR delivers a stimulatory signal, while engagement of inhibitory KIR delivers a tolerance signal. When the former overcome the latter, NK effector functions are initiated. Indeed, a major trigger for enhanced NK cell-mediated recognition of HIV-infected cells is the selective downregulation of their HLA-A and HLA-B (though not C) ligands by the viral Nef protein (Cohen et al. 1999), leading to a reduction in signaling through inhibitory KIR. Inhibitory KIRs bind their HLA class I ligands in an allotype-specific manner. For example, KIR3DL1 receptors interact with HLA-B molecules belonging to the Bw4 allotype (determined by amino acids 77–83 of the HLA coding region), notably those harboring isoleucine at position 80 (Bw4-80I), and to a lesser extent those harboring threonine at this position (Bw4-80T) (Cella et al. 1994). Some activating KIRs also recognize HLA class I in an allotype-specific manner, though generally at lower avidity than their inhibitory counterparts (Bashirova et al. 2011).

KIR, alone and in combination with their allotype-specific HLA ligands, may modulate HIV-1 susceptibility and pathogenesis. HIV-infected individuals expressing the activating KIR3DS1 allele in combination with HLA-Bw4-80I exhibit lower viral loads (Qi et al. 2006) and delayed clinical progression (Martin et al. 2002). Higher frequencies of KIR3DS1 homozygosity (Boulet et al. 2008) have been observed in HIV-1 exposed seronegative individuals, suggesting that activating KIR may also confer some level of protection against HIV-1

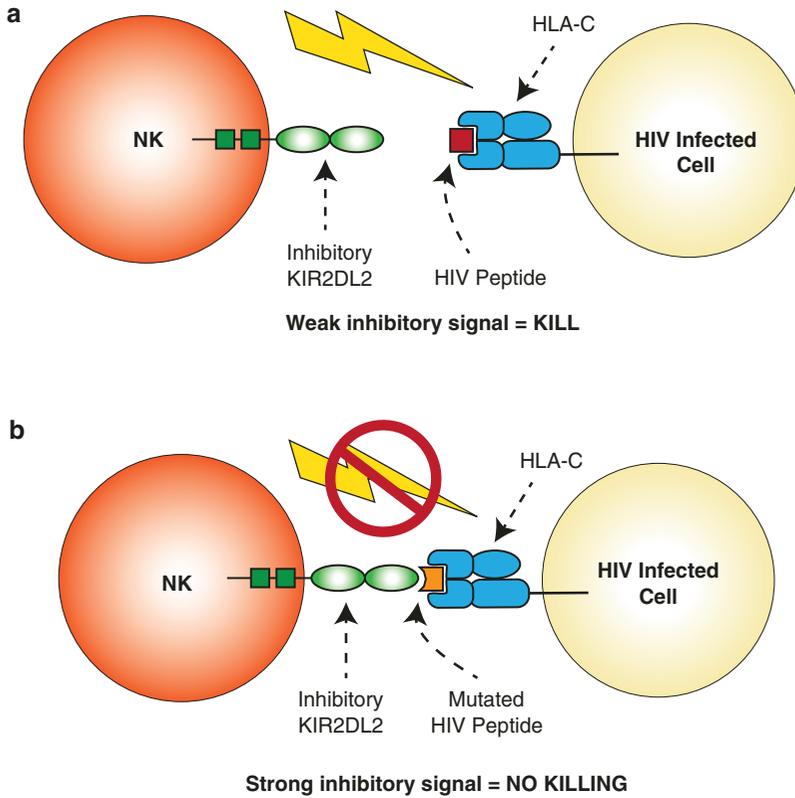
acquisition. Though protection via engagement of an activating KIR seems intuitive, the underlying mechanism remains unknown (KIR3DS1-expressing NK cells can suppress HIV-1 replication in Bw4-80I-expressing cells in vitro (Alter et al. 2007), but direct binding of KIR3DS1 to HLA-Bw4-80I has not been shown). KIR3DL1 alleles possessing a high-expression, high-inhibitory phenotype (termed KIR3DL1*h/*y) may also be protective. When present in combination with HLA-Bw4-80I alleles, notably HLA-B*57, KIR3DL1*h/*y alleles were associated with lower viral loads and conferred protection against HIV-1 disease progression (Martin et al. 2007). KIR3DL1*h/*y-HLA-B*57 co-expression may also protect against HIV-1 acquisition (Boulet et al. 2008). That highly inhibitory KIR receptor-ligand interactions can be protective seems counterintuitive, especially given that the opposing signals of activating KIR may also be protective. Nevertheless, the data support a role, albeit incompletely elucidated, of KIR in HIV-1 control.

KIR-associated immune pressures may also drive the selection of viral polymorphisms that allow infected cells to evade NK-mediated killing. To shed light on how such mutations could arise, we must first briefly revisit KIR-ligand binding. Though not antigen specific in the classical sense, KIR receptor-ligand interactions are nevertheless modulated in part by HLA polymorphism (through their allotype specificity) as well as the sequence of the HLA-bound peptide (Malnati et al. 1995). The idea that naturally arising HIV-1 variants could affect KIR-HLA binding was supported by reduced in vitro binding of KIR3DL1 to its HLA B*57:03 ligand in the presence of the TW10 epitope harboring a G-to-E substitution at position 9 (though this was not claimed to be an in vivo NK-driven escape mutation, as failure to engage KIR3DL1 would render infected cells more, not less, susceptible to NK-mediated killing (Brackenridge et al. 2011)). Rather, NK cell escape could theoretically be achieved via viral polymorphisms that reduce recognition by activating KIR or enhance recognition by inhibitory KIR. Toward the identification of such mutations, statistical association approaches

were applied to $N = 91$ linked KIR/HIV-1 sequences, yielding 22 KIR-associated viral polymorphisms. Two linked polymorphisms in Vpu (71M/71H), located in a region that overlaps the Env reading frame, were overrepresented among KIR2DL2-expressing persons, in particular those KIR2DL2⁺ individuals homozygous for HLA-C group 1 alleles (Alter et al. 2011) (consistent with the greater affinity of KIR2DL2 for HLA-C group 1 ligands (Moesta et al. 2008)). Researchers further showed in vitro that the presence of these polymorphisms enhanced the ability of the inhibitory KIR2DL2 to bind HIV-infected cells, that KIR2DL2⁺ NK cells failed to become activated in the presence of polymorphism-containing HIV-1, and that cells infected with polymorphism-containing HIV-1 were not inhibited by KIR2DL2⁺ NK cells (Alter et al. 2011). These findings suggest that immune pressure by an inhibitory KIR could select in vivo escape mutations conferring enhanced binding of the inhibitory receptor to HIV-infected cells, thereby allowing them to escape NK cell-mediated elimination (Fig. 3). The recent identification of an HLA-C*01:02-restricted p24^{Gag} peptide variant that bound KIR2DL2, that conferred functional inhibition of KIR2DL2-expressing NK cells in vitro (Fadda et al. 2012), provides theoretical support for this model.

Escape from Vaccine-Induced Antiviral Immunity

A challenge in designing vaccines against genetically diverse pathogens such as HIV-1 is the possibility that vaccine-induced immunity (► [Preventing HIV-1 Transmission Through Vaccine-Induced Immune Responses](#)) may protect against infection by strains similar to the vaccine immunogen(s), but not genetically divergent strains. Such “sieve effects” can be identified by retrospectively comparing HIV-1 sequences of vaccine vs. placebo trial participants who subsequently became infected (termed “breakthrough” sequences), to determine genetic differences between them (Gilbert et al. 2001). The idea that vaccine-induced immunity could induce a partial



HIV-1 Mutational Escape from Host Immunity, Fig. 3 KIR escape. Mutations in HIV-1, presumably within viral peptides presented by HLA on the infected cell surface, may impair NK cell-mediated recognition of HIV-infected cells, thereby conferring escape. Based on a putative KIR2DL2 escape mutation described by Alter et al. (2011), this figure illustrates how this process could occur. (Panel A) Weak interactions between the inhibitory KIR2DL2 on the NK cell surface and the viral peptide/HLA-C complex on the HIV-infected cell surface produce

weak inhibitory signals, leading to NK cell-mediated elimination of the infected cell. (Panel B) Viral escape mutations that enhance KIR2DL2-mediated NK cell recognition of the peptide/HLA-C complex on the HIV-infected cell produce strong inhibitory signals that protect the infected cell from NK cell-mediated killing. KIR escape could also theoretically occur via the selection of mutations that abrogate recognition by activating KIR (not shown)

barrier through which antigenically divergent HIV-1 strains could penetrate has been termed the “acquisition sieve effect,” while the related – yet mechanistically distinct – possibility that vaccine-induced immunity would fail to block infection but would instead drive the rapid outgrowth of escape variants has been termed “postinfection sieve effect” (Edlefsen et al. 2013). The latter is particularly relevant to CTL-based vaccines, as these are unlikely to block HIV-1 transmission. Notably, acquisition and postinfection sieve effects can be difficult to distinguish from one another, as both may occur

before HIV-1 RNA can be reliably detected in the blood, and/or may manifest themselves via the presence of identical immune-associated polymorphisms.

Analysis of vaccine trial data support sieve effects in HIV-1. Such effects were first suggested by the presence of atypical V3 amino acid motifs in HIV-1 *env* sequences from individuals vaccinated with recombinant HIV-1_{MN} gp120 (Berman et al. 1997). Recent comparisons of founder HIV-1 strains from vaccine and placebo recipients of the RV144 “Thai” vaccine trial identified differential amino acid frequencies at *env* V2 codons

169 and 181 between the two groups (Rolland et al. 2012), suggesting that the vaccine preferentially blocked viruses harboring specific substitutions at these positions. Rapid selection of CTL escape mutations by vaccine-induced cellular immune responses may also have occurred in the failed STEP vaccine trial (Buchbinder et al. 2008). Inferred T-cell epitope sequences within Gag/Pol/Nef (the regions contained within the vaccine) from infected vaccine recipients exhibited greater genetic distances to the immunogen sequence compared to those of infected placebo recipients, presumably as a result of extensive and rapid immune escape (Rolland et al. 2011). The lack of such differences for epitopes within other HIV-1 proteins also supported this conclusion. HIV-1 sequences from vaccine recipients also exhibited substitutions at Gag codon 84 more frequently than placebo recipients, identifying this as a putative signature site of HIV-1 evolution in response to vaccine-induced CTL responses (Rolland et al. 2011).

The implications of vaccine-induced immune responses on HIV-1 evolution are potentially profound. At the individual level, rapid vaccine-driven escape could accelerate disease progression (Betts et al. 2005), while the use of vaccines capable of blocking infection by only certain HIV-1 strains raises concerns regarding potential shifts in viral polymorphism frequencies and/or HIV-1 lineage distributions (and their clinical and pathogenic consequences) at the population level. That vaccine-induced immune responses (notably CTL) may target slightly different epitopes than those in natural infection Hertz et al. (2013) may further complicate this issue.

A Note on the Role of HIV-1 Accessory Proteins in Immune Evasion

Although beyond the scope of the present entry, certain HIV-1 proteins possess immune evasion functions that deserve brief mention. In particular, downregulation of HLA-A and HLA-B (but not HLA-C) from the cell surface by Nef Cohen et al. (1999) represents a major mechanism of

immune evasion by HLA-restricted CTL, as it reduces their ability to recognize infected cells. HIV-1 Nef, Vpu, and envelope (► [Nef/Env/Vpu/Tetherin](#)) also serve to remove CD4 from the infected cell surface (Chen et al. 1996). The recent observation that interaction of HIV-1 envelope with CD4 on the infected cell surface is required to expose certain ADCC epitopes suggests that cell-surface CD4 downregulation could represent an immune evasion strategy to reduce ADCC-mediated elimination of infected cells (Veillette et al. 2014).

Immune Escape Dynamics in Early Infection

Escape begins rapidly following HIV-1 infection. Recently, detailed studies of intra-host HIV-1 evolution using single-genome amplification (e.g., Salazar-Gonzalez et al. 2009) or next-generation sequencing (e.g., Henn et al. 2012) have advanced our understanding of HIV-1 transmission and escape dynamics. HIV-1 transmission (HIV-1 Transmission Dynamics; HIV-1 Transmission Selection) is characterized by a severe genetic bottleneck. An estimated 80% of heterosexual transmissions are productively initiated by a single transmitted/founder virus (Salazar-Gonzalez et al. 2009), while infection in persons who inject drugs is generally established by more than one closely related founder virus (Bar et al. 2010). In the days following infection, the transmitted/founder virus(es) undergoes rapid population growth and star-like diversification Herbeck et al. (2011), giving rise to a “quasispecies” swarm of related HIV-1 variants. This genetic pool becomes the evolutionary substrate upon which host immune responses exert pressure, driving the selection of escape mutations and the survival of viral lineages harboring them. The first CTL escape mutations appear during acute-phase viremia decline (Goonetilleke et al. 2009); the selection (and in some cases fixation) of CTL escape variants has been observed as early as 21 days postinfection in humans (Herbeck et al. 2011). Selection (and subsequent fixation) of the first NAb escape mutations also occurs

relatively rapidly, though on a slightly longer time course than CTL escape (Bar et al. 2012).

The evolutionary pathways along which these early mutations arise have recently been elucidated in detail. The conceptually straightforward pathway whereby the first selected escape mutation gradually outcompetes the original transmitted form is likely to be true for only a minority of cases (Goonetilleke et al. 2009). More commonly, the first escape variant tends to be followed by the emergence of numerous others, from which the “final” escape form is ultimately selected (Goonetilleke et al. 2009). This is likely because the initially appearing pool of low-frequency mutants often retains some ability to be targeted by existing (or *de novo*) CTL (Brackenridge et al. 2011). This drives the selection of more effective escape variants, often at HLA-anchor residues, that ultimately outcompete both transmitted founder and initial variants. For example, in a B*57:03-expressing individual, initial escape within the p24^{Gag} TW10 epitope occurred approximately 5 months postinfection via a transient, minority G-to-E mutation at position 9 (G248E) that retained the ability to bind B*57:03 and reduced CTL recognition only modestly (Brackenridge et al. 2011). By approximately 1.5 years postinfection, this mutation was outcompeted by variants expressing the canonical B*57-restricted G248A mutation at this position (along with T242N and V247I at epitope positions 3 and 8). Similarly, multiple amino acids often transiently appear in the regions under NAb pressure, from which the final neutralization mutant(s) ultimately emerges (Bar et al. 2012). Escape continues to occur (albeit at a slower rate (Koibuchi et al. 2005)) over the infection course.

Immune Escape as a Major Driver of HIV-1 Diversity

Immune escape is a major driver of HIV-1 diversity within individuals and populations. CTL escape accounts for a major proportion of within-host HIV-1 evolution in the first year of infection. For example, a study of seven newly infected individuals revealed that, 6 months

postinfection, between 9 and 18 positively selected substitutions were observed throughout the HIV-1 proteome (Herbeck et al. 2011). Another population-based study estimated that a minimum of 30% of substitutions in Gag/Pol and 60% in Nef were attributable to HLA pressures (Brumme et al. 2008). Escape is also widespread throughout the HIV-1 proteome. A recent statistical association study identified over 2,100 HLA-associated polymorphisms at ~35% of HIV-1's nonconserved codons (Carlson et al. 2012a), distributed somewhat unequally throughout the proteome. For example, Vpu exhibited evidence for HLA-mediated selection at one-quarter of its nonconserved sites, compared to ~70% of nonconserved sites in Nef (Carlson et al. 2012a). The status of HLA as the most important host genetic factor influencing HIV-1 diversity was recently confirmed via genome-wide association studies (Bartha et al. 2013). Similarly, HIV-1 Gag and Nef sites under HLA selection have diversified to the greatest extent over the past three decades of the North American epidemic, supporting a significant role of HLA in driving global HIV-1 diversification (Cotton et al. 2014).

Fitness Consequences of Escape

Upon transmission, some immune escape mutations selected in the previous host will revert to the original (usually subtype consensus) amino acid (Leslie et al. 2004). While some CTL escape mutations, for example, the B*57-associated Gag T242N, revert consistently and rapidly following transmission (Leslie et al. 2004), most revert more slowly (Crawford et al. 2007) and others rarely or not at all (Leslie et al. 2005). Reversion occurs because these mutations incur a fitness cost (► [Viral Fitness in Hosts](#)). Generally, escape mutations within conserved viral regions tend to be more fitness-costly, while escape in more variable regions tends to be fitness neutral (Troyer et al. 2009). An example of a fitness-costly mutation is the B*27-associated R264K substitution in the p24^{Gag} KK10 epitope, which essentially abolishes *in vitro* viral replication

when engineered alone into HIV-1_{NL4-3} (Schneidewind et al. 2007). Generally though, *in vitro* fitness costs of escape mutations observed *in vivo* tend to be subtler, often requiring multiple substitutions to reduce function. Alone, the B*57-driven Gag-T242N mutation reduces viral replicative capacity only modestly (Brockman et al. 2007), but dose-dependent replicative reductions are observed when it is present alongside other common B*57-driven mutations in p24^{Gag} (Crawford et al. 2009). Other examples of fitness-costly CTL escape mutations include B*13-associated mutations in Gag (Prado et al. 2009), Cw*05-driven mutations in integrase (Brockman et al. 2012), and B*35-associated mutations in Nef (Ueno et al. 2008). Fitness costs ranging from 0% to 24% have been observed for early envelope escape mutants, indicating that NAb escape can also be fitness-costly (Bar et al. 2012). Fitness costs of escape can be offset by the selection of compensatory mutations at secondary sites. Whereas most compensatory mutations occur in relatively close proximity to the primary escape site (e.g., S165N with A163G in B*5703-KF11 in p24^{Gag} (Crawford et al. 2007)), others, such as S173A with R264K in B*27-KK10 (Schneidewind et al. 2007), occur a substantial linear distance away, but may reside nearby in the folded protein structure.

In the case where escape can only occur at a functional and/or replicative cost, the virus' advantage gained via immune escape is offset in part by these costs, thus potentially conferring some residual biological benefit to the host in terms of lower viral loads. For example, the sustained protective effect of HLA-B*81 is believed to be due in part to selection of the fitness-costly Gag T186S escape mutation at position 7 of the immunodominant B*81-restricted TL9 epitope, which is difficult to compensate (Wright et al. 2012b). Relative clinical benefits of fitness-costly escape in HLA-mismatched individuals who have acquired HIV-1 with key Gag escape mutations have also been observed (Chopera et al. 2008). That HIV-1 sequences contain inherent determinants of pathogenesis is supported by the observation that set-point plasma viral load is to a certain extent "heritable" from

one infection to the next (Alizon et al. 2010) and that viral replication capacity correlates positively with viral load (and negatively with CD4⁺ T-cell count) at various infection stages (e.g., Prince et al. 2012). Indeed, acquisition of attenuated HIV-1, followed by further within-host selection of noncanonical fitness-costly escape mutations, is likely to explain a portion of HIV-1 elite control (Lobritz et al. 2011), a rare phenotype where individuals are able to spontaneously suppress plasma HIV-1 RNA to below limits of clinical detection without the need for antiretroviral therapy.

These observations have led to the idea that immune-mediated containment of HIV-1 replication to levels that slow disease progression and possibly reduce transmission might be achievable through the design of vaccines that stimulate CTL responses focused against critically conserved viral regions where escape can only occur at substantial fitness costs (Altfeld and Allen 2006). A related strategy would be to design immunogens featuring both susceptible and common escape variant forms – provided the latter retain the ability to bind the relevant HLA molecules – with the goal of generating broad, potent, variant-reactive CTL responses that, upon infection, will drive HIV-1 evolution down unconventional pathways not unlike those selected in elite controllers (Miura et al. 2009).

Population-Level Adaptation of HIV-1 to Host Immune Pressures

As HIV-1 genomes residing in an individual exhibit adaptations to its host's immunogenetic profile, then HIV-1 sequences circulating in a given host population exhibit adaptations that reflect the distinct immunogenetic profile of that population. This is often referred to as "population-level" adaptation of HIV-1. For example, >50% of HLA-associated polymorphisms identified in HIV-1 subtype B sequences in Mexico (Avila-Rios et al. 2009) and nearly two-thirds of those identified in Japan (Chikata et al. 2014) are distinct from those observed in subtype B-infected cohorts from Canada/USA/

Australia, because the former populations exhibit HLA alleles unique to those populations (e.g., B*39 in Mexico and B*67:01 in Japan). The frequencies of HLA-associated polymorphisms will similarly vary according to the frequencies of their restricting HLA alleles in the population. The B*51-associated I135X mutation in reverse transcriptase (at the C-terminus of the B*51-TI8 epitope, RT codons 128–135) provides an example. In an analysis of nine cohorts spanning five continents, HLA-B*51 and RT-I135X prevalence exhibited a strong positive correlation (Kawashima et al. 2009), indicating that the more frequent an HLA allele is in a population, the more frequent its associated adaptations will be observed in circulating HIV-1 sequences. Other host immune factors (e.g., variability in T-cell receptor genetics) may also play a role in population-specific HIV-1 adaptation. For example, a recent comparative study of HIV-1 subtype B cohorts in Japan versus Canada/USA/Australia identified numerous cases where the same HLA allele selected significantly different escape pathways across cohorts (Chikata et al. 2014), implying factors beyond HLA in driving these differences. HLA-driven escape pathways also differ across HIV-1 subtypes, presumably as a result of genetic differences in the viral backbone. For example, Gag-T242N is commonly selected by B*57 in HIV-1 subtypes B, C, and D, but rarely in subtype A1 (McKinnon et al. 2009).

The persistence of certain immune escape mutations following transmission to a new host has led to a related concern – namely, that escape mutations could gradually spread throughout the population (Goulder et al. 2001). Analogous to the negative impact of transmitted drug resistance mutations on treatment efficacy, acquisition of “escape mutant” HIV-1 by persons expressing the relevant HLA could undermine the ability of their CTL to control infection; as such, escape mutant spread could gradually undermine host antiviral immune potential (and potentially diminish the protective effects of certain HLA alleles as the epidemic progresses). Indeed, the S173A compensatory mutation has been shown to stabilize the B*27-associated R264K mutation in p24^{Gag} upon transmission (Schneidewind

et al. 2009), while the S165N compensatory mutation has been shown to stabilize B*57-associated mutations within the p24^{Gag} KF11 epitope (Crawford et al. 2007), supporting this concern.

The extent to which immune escape mutations are spreading in HIV-infected populations remains incompletely known, in part due to the scarcity of historic data. Nevertheless, it has been suggested that CTL epitopes in European HIV-1 sequences are being “lost” through mutational escape from HLA-B-mediated selective pressures (Schellens et al. 2011); similarly, higher viral polymorphism frequencies have been reported in modern compared to historic HIV-1 subtype B and F sequences in South America (Dilernia et al. 2008). The high frequency of the B*51-associated HIV-1 reverse transcriptase (RT) I135X mutation in Japan, a population where B*51 prevalence approaches 20%, is also suggestive of escape mutation accumulation (Kawashima et al. 2009) (though the possibility that the Japanese epidemic was founded by an HIV-1 sequence containing RT-I135X cannot be ruled out). A comparative study of historic (1979–1989) versus modern (2,000+) HIV-1 subtype B cohorts in North America revealed modest spread of CTL escape mutations over the study period which occurred alongside an approximate twofold increase in HIV-1 diversity during this time (Cotton et al. 2014). Despite limited evidence of escape mutation spread in North America, rates of spread may be higher in populations with high HIV-1 prevalence, older epidemics, differential transmission and dynamics and/or where host HLA diversity is relatively limited, and thus possess more immediate implications.

The gradual accumulation of CTL escape mutations in circulating HIV-1 sequences is paralleled by a similar phenomenon driven by humoral immunity. Two recent studies evaluating antibody neutralization resistance of historic versus modern HIV-1 envelope sequences suggest that HIV-1 is drifting toward a more neutralization-resistant phenotype over time (Bunnik et al. 2010). Furthermore, contemporary sera exhibited lower heterologous neutralizing activity than historic sera, consistent with a gradual undermining of humoral immunity as HIV-1 becomes increasingly

neutralization resistant (Bouvin-Pley et al. 2013). Taken together, evidence suggests that HIV-1 is becoming – albeit gradually – more “preadapted” to host immunity as immune escape mutations spread in circulation. Further studies are therefore warranted to explore the extent of HIV-1 adaptation to cellular and humoral immune pressures in different host populations as their respective epidemics increase in age and diversity, and the potential implications of this adaptation for natural (and vaccine-induced) immunity over time.

Conclusion

In conclusion, mutational escape from host immune responses represents a major selective force driving the evolution and diversification of HIV-1 within infected persons. By extension, immune escape is also responsible for the diversification of HIV-1 globally and for the continued evolution of the virus as the epidemic progresses. Continuing to advance our understanding of the dynamics and pathogenic implications of immune escape within individuals and populations – including how to recapitulate this process by vaccination as in the case of the generation of broadly neutralizing antibodies – will be paramount to achieving our ultimate goal of an effective HIV-1 vaccine.

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HIV-1 Pathogenesis in the Gut

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Definition

HIV-1 pathogenesis in the gut, considered to be a major driving force behind chronic immune activation and inflammation, is defined by significant immunological and structural abnormalities.

Early in infection, HIV-1 targets gut CD4 T cells resulting in high levels of viral replication and massive depletion of CD4 T cells which are important in maintaining intestinal homeostasis. Furthermore, HIV-1 disrupts innate immunity and is associated with significant gut epithelial barrier damage. The culmination of these dramatic changes in the gut is increased local inflammation, changes in the gut microbiome (dysbiosis) and the translocation of microbes and microbial products from the lumen into the gut tissue and ultimately into systemic circulation. Microbial translocation not only drives further gut mucosal immune activation and inflammation perpetuating the disruption in gut homeostasis, but is considered to be a major contributor to systemic immune activation and inflammation, even in the setting of viral suppression with effective anti-retroviral therapy (ART). Indeed, suppressive ART improves many features associated with gut pathogenesis, but in many instances does not fully reverse the breakdown in intestinal homeostasis. Recent efforts have focused on therapies aimed at restoring gut function by reducing inflammation, blocking microbial translocation or modifying the gut microbiome.

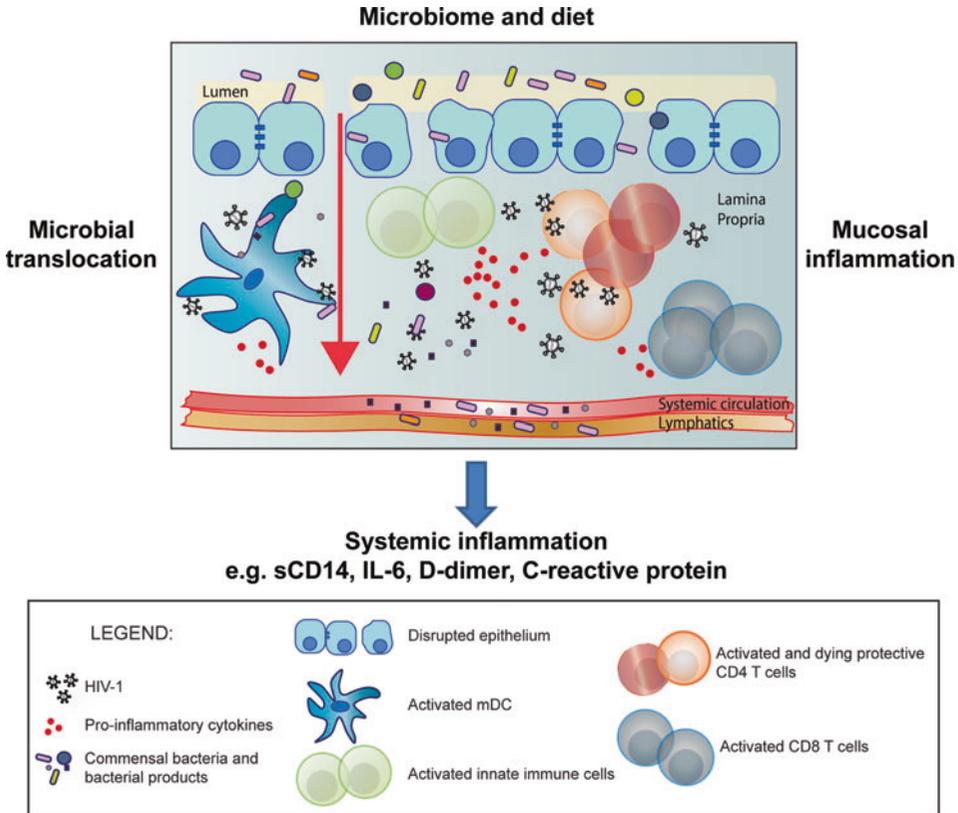
Overview

HIV-1 infection has long been known to induce a state of chronic immune activation, the extent of which predicts disease progression and CD4 T-cell loss in untreated infection and limits immune restoration following initiation of suppressive antiretroviral therapy (ART). Furthermore, chronic immune activation and inflammation during treated infection has been associated with numerous non-AIDS comorbidities and increased mortality, so understanding the mechanisms that drive chronic inflammation is a top priority in AIDS research. This state of chronic immune activation is likely multifactorial, but increasing evidence suggests that damage to the gut epithelial barrier and massive loss of CD4 T cells in the gut-associated lymphoid tissue (GALT) inflicted early in the course of infection may be a major source.

HIV-associated enteropathy accompanied by symptoms of diarrhea, weight loss, malnutrition, and malabsorption was observed in the earliest days of the epidemic. Over the ensuing decades of research in both HIV-infected humans and SIV-infected nonhuman primates, much more has become known about the mechanisms by which HIV-1 targets and destroys the mucosal immune system and thereby disrupts intestinal homeostasis. HIV-associated gastrointestinal (GI) tract pathology is characterized by early and high-level HIV-1 replication in GALT and by immunological and structural abnormalities which include alterations in both adaptive and innate mucosal immunity and substantial epithelial barrier disruption. These changes culminate in increased local inflammation, microbial translocation, and dysbiosis leading to chronic generalized immune activation, inflammation, and associated comorbidities (Fig. 1). Suppressive ART may improve, but in many instances does not fully reverse, this HIV-associated intestinal immune dysfunction.

HIV-1 Replication in GALT

HIV-1 is primarily a mucosally transmitted pathogen, with about 80% of cases linked to sexual transmission. Thus, the nature of HIV-1 strains that initially establish infection is of particular interest in vaccine development. In an infected individual, HIV-1 exists as a “swarm” of evolutionarily related viruses that vary by about 5% from each other. During sexual transmission, about 80% of the viruses that get established in the “recipient” host came from just one HIV-1 strain, suggesting an extreme genetic bottleneck. These “transmitted/founder” or TF virus strains have interesting biological characteristics compared to HIV-1 strains derived from chronic infection. In HIV-1 subtype C strains, which account for about 50% of HIV-1 infections worldwide, TF HIV-1 strains are characterized by more compact envelope glycoproteins that are more competent in fusing to target CD4+ T cells. However, compact envelope proteins were not observed in other HIV-1 strains such as subtype B strains, which are



HIV-1 Pathogenesis in the Gut, Fig. 1 HIV-1 targets and destroys the mucosal immune system and disrupts intestinal homeostasis. HIV-associated gastrointestinal tract pathology is characterized by early and high-level HIV-1 replication in the gut-associated lymphoid tissue and by immunological and structural abnormalities

including alterations in both adaptive and innate mucosal immunity and substantial epithelial barrier disruption. These changes culminate in increased local inflammation, microbial translocation, and dysbiosis leading to chronic generalized immune activation, inflammation, and associated comorbidities

the more prevalent strains circulating in North America and Europe. Nevertheless, HIV-1 subtype B TF strains were more infectious, and this was linked to higher envelope incorporation in virions (Parrish et al. 2013). Replicative fitness would be ideal for the successful initial establishment of HIV-1 infection.

TF HIV-1 strains could have emerged from initial expansion and amplification in the GALT, which harbor large numbers of activated CD4⁺ T cells. However, there is currently a dearth of knowledge on how TF HIV-1 strains replicate in mucosal tissues. To understand better the biology of TF HIV-1 strains, the Lamina Propria Aggregate Culture (LPAC) model has been developed. This model allows for the *in vitro* infection of

primary gut CD4⁺ T cells with various HIV-1 strains in the absence of exogenous mitogens (Dillon et al. 2012; Steele et al. 2014). Using this model, HIV-1 TF viruses were found to replicate to higher levels than counterpart chronic HIV-1 strains derived from the same patient. Of note, a critical property of TF HIV-1 strains is their almost exclusive usage of the CCR5 versus the CXCR4 co-receptor. In sharp contrast to blood and lymph node CD4⁺ T cells, mucosal CD4⁺ T cells express high levels of CCR5. Thus, the preponderance of CCR5-tropic HIV-1 strains in an infected individual may be explained by an initial selection process(es) in the GALT. Of note, it was proposed that HIV-1 may be particularly effective in replicating in the GALT through

the binding of the HIV-1 envelope protein to the integrin $\alpha 4\beta 7$, which facilitates the migration of lymphocytes to the GALT. However, subsequent studies revealed that most HIV-1 strains could not bind $\alpha 4\beta 7$ and blocking these interactions did not inhibit HIV-1 infection.

Intrinsic Immunity Against GALT HIV-1 Infection

The successful establishment of HIV-1 infection would require evasion strategies against host innate immune responses. During acute HIV-1 infection, a cytokine storm in plasma was observed. One of the early cytokines induced was interferon- α (IFN α), which can potently inhibit HIV-1 replication in culture and had modest effects in reducing HIV-1 viral loads when administered in human clinical trials. Of interest, HIV-1 TF strains were more resistant to IFN α compared to chronic counterpart strains, providing indirect evidence for the importance of innate immunity in the initial establishment of HIV-1 infection (Parrish et al. 2013). Type I interferons (a group of antiviral cytokines that include IFN α) regulate hundreds of genes. Among these interferon-stimulated genes (ISGs) are the retrovirus restriction factors, which can directly inhibit HIV-1 replication by interfering with specific steps in the viral life cycle (Malim and Bieniasz 2012). APOBEC3G is a cytidine deaminase that can get incorporated into virions and, in the next target cell, could inhibit reverse transcription and hypermutate reverse transcripts. Tetherin could restrict retrovirus release by “tethering” virion and cellular membranes together. HIV-1 encodes the Vif and Vpu proteins to counteract APOBEC3G and tetherin, respectively. However, these host-pathogen interactions may be subject to (1) the strength of the molecular interactions, as some Vif and Vpu alleles may be weaker, and (2) stoichiometric balance, which could be offset by type I interferon induction of the restriction factors. In fact, about 16% of TF HIV-1 strains harbor extensive hypermutation, indicative of APOBEC3G as a barrier to successful establishment of HIV-1 infection. Additional HIV-1

restriction factors, such as Mx2, IFITM, Schlafen11, and SAMHD1, have also been identified, but their relative contributions in the overall antiviral effect remain an active area of investigation.

Type I interferons consist of several cytokines that include the 12 IFN α subtypes and interferon-beta. These cytokines are released primarily by plasmacytoid dendritic cells (pDCs), which rapidly migrate to mucosal tissues during acute infection based on data in the SIV model. However, type I interferons expressed by pDCs following HIV-1 exposure appear to be dominated by weakly antiviral IFN α subtypes, suggesting a sub-optimal antiviral response (Harper et al. 2015). Another subset of DCs known as myeloid DCs (mDCs) may also play critical roles in early dissemination and spread. mDCs express lectin-type receptors such as DC-SIGN and Siglec-1 that can efficiently bind HIV-1 virions on the cell surface and facilitate *trans*-infection to CD4+ T cells. The formation of a “virological synapse” between DCs, CD4+ T cells, and HIV-1 virions significantly enhances the efficiency of HIV-1 replication compared to having CD4+ T cells alone. The migratory properties of mucosal mDCs could play critical roles in HIV-1 dissemination to various immune compartments. The role of DCs in HIV-1 mucosal immunity will be discussed in additional detail below.

HIV-1-Mediated GALT CD4+ T-Cell Death

A critical outcome of HIV-1 replication in the GALT is the extensive depletion of CD4+ T cells. The underlying mechanism was investigated using the human lymphoid aggregate culture (HLAC) model, which utilizes tonsil CD4 T cells (Doitsh et al. 2014). Infection of HLAC CD4+ T cells with CXCR4-tropic HIV-1 results in the predominant killing of abortively infected cells that have undergone premature reverse transcription. Incomplete HIV-1 reverse transcripts in the cytoplasm were sensed by a single-stranded DNA-binding protein known as IFI16, leading to the activation of caspase-1. This results in a highly

inflammatory form of death known as pyroptosis, characterized by the rapid release of cellular contents. A limitation of these studies with respect to modeling GALT CD4⁺ T-cell depletion was the use of CXCR4-tropic HIV-1 and tonsil CD4⁺ T cells. In the LPAC model, CCR5-tropic HIV-1 killed mucosal CD4⁺ T cells in a caspase-1-dependent manner (Steele et al. 2014). Pyroptosis may be a mechanism driving gut barrier dysfunction that leads to microbial translocation, but the identity of the pyroptosis-induced inflammatory mediators in the GALT remains unknown.

HIV-Associated Immunologic Changes in the GALT

In the GALT, both the innate and adaptive immune systems are important in maintaining immune homeostasis. During HIV-1 infection, most innate and adaptive immune cells are altered in frequency and/or function.

Adaptive Immunity

Progressive HIV-1 disease is characterized by a massive depletion of mucosal effector CD4⁺ T cells that occurs early in disease and includes decreased frequencies of IFN- γ -producing T helper cells (Th1), IL-17-producing cells (Th17), and IL-22-producing cells (Th22). Th17 and Th22 cells, subsets of T cells proposed to play roles in normal mucosal defense and homeostasis, were preferentially infected and killed in some studies, and their depletion is linked to HIV-1 pathogenesis. Mucosal CD4 T cells including Th17 cells are preserved in HIV-infected elite controllers who naturally maintain extremely low viral loads. Increased frequencies of activated and exhausted intestinal CD4 T cells have been observed in untreated acute and chronic HIV-1 infection. In the antiretroviral treatment setting, CD4 T-cell restoration is delayed and typically incomplete in the gut mucosa compared to peripheral blood. Correspondingly, reconstitution of Th17 cell numbers and function is variable and may be limited by defective gut T-cell homing. Long-term ART generally reversed Th22 depletion; however, interindividual heterogeneity was

observed and restoration was not associated with ART duration or blood CD4 T-cell count.

CD8 T-cell frequencies, including frequencies of activated CD8 T cells, are increased in rectal and colonic tissue of HIV-infected subjects and display a polyfunctional HIV-1-specific phenotype that is associated with lower HIV plasma viral load and higher blood CD4 count. However, the persistence of virus in the GI tract during the course of chronic infection suggests that effective CD8 T-cell responses may be delayed, absent, dysfunctional, or varied at different GI tract sites. With ART, frequencies of polyfunctional HIV-specific CD8 T cells diminish.

Regulatory T cells play an important role in modulating inflammation but may also suppress helpful immune responses in some settings. Despite overall decreased frequencies of intestinal CD4 T cells during untreated chronic HIV-1 infection, frequencies of colon and rectal mucosa CD4⁺ regulatory T cells (Tregs), either as a fraction of CD4 T cells or as an absolute number, were preserved in some studies (Angin et al. 2012; Shaw et al. 2011) suggesting these cells were not preferentially depleted. However, increased frequencies of Tregs as a percentage of rectal CD4 T cells and increased frequencies and absolute numbers of duodenal Tregs in untreated HIV-1 infection have also been noted. Duodenal Treg frequencies normalized with ART. These observed differences in Treg frequency may be related to the Treg quantification methods as well as tissue site. Rectal Tregs maintained their suppressive ability *in vitro* indicating that they may potentially contribute to reduced HIV-specific immune response *in vivo*. A decrease in the normal Th17/Treg ratio *in vivo* rectosigmoid biopsies of HIV-infected subjects was associated with markers of pathogenesis, including systemic CD8 T-cell activation and mucosal T-cell activation, and with plasma viral load.

Innate Immunity

Innate immune cells help to contain pathogens early in infection and may also initiate adaptive immunity. Recently identified subsets of innate lymphoid cells (ILCs) are considered to be “first responder” immune cells at mucosal sites and contain groups of cells important in maintaining

intestinal homeostasis. Conventional natural killer (NK) cells belong to group 1 ILCs and are traditionally known for their ability to kill infected cells. During chronic HIV-1 infection, gut NK cell subsets are depleted with some recovery following viral suppression with ART. Studies into group 2 and group 3 gut ILCs during HIV-1 infection remain limited. IL-22-producing ILC3s, cells important in maintaining epithelial barrier integrity, were increased during early (<7 months) HIV-1 infection and were considered to be a compensatory mechanism for the dramatic loss in IL-22-producing T cells (Th22). Preservation of ILC3 frequencies and IL-22-producing ILCs has also been observed in both untreated and in ART-treated subjects with chronic HIV infection.

Antigen-presenting cells (APCs), such as macrophages and DCs, play an important role in the elimination of pathogens and in the induction of adaptive immune responses against them. mDCs are professional APCs found throughout the intestinal tract that serve as a bridge between the innate and adaptive immune responses. In addition to their role in viral transmission (see above), gut mDCs contribute to HIV-associated mucosal pathogenesis. Chronic untreated HIV-1 infection is associated with altered colonic mDC function characterized by an activated, but dysregulated, mDC phenotype (Dillon et al. 2016). Increased mDC activation was associated with both mucosal and peripheral blood T-cell activation, thereby linking gut mDC activation to a marker of HIV-1 disease progression. Levels of indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme important in intestinal homeostasis via its regulation of Th17 and Treg development, were increased in mDCs from rectosigmoid biopsies of chronically infected HIV-1-infected subjects and linked to the inversion of Th17/Treg ratio. The impact of ART on intestinal mDC function is unknown. As detailed above, pDCs are the primary producers of type I interferons and are important in antiviral immunity. Increased numbers of activated colonic pDCs in untreated HIV-1-infected subjects are associated with levels of mDC activation, suggesting that common factors might be driving activation in both DC subsets (Dillon et al. 2016). In another study, high frequencies of ileum pDCs

are associated with plasma levels of IFN α and with mucosal CD8 T-cell activation in untreated HIV-1-infected subjects and did not normalize with ART (Lehmann et al. 2014). Unlike mDCs, pDCs are rarely found in intestinal tissue under steady state conditions; thus accumulation of activated pDCs during chronic HIV-1 infection is likely due to increased migration from the blood to the colon. Frequencies of gut phagocytes including macrophages and neutrophils are also altered during HIV-1 infection. Numbers of macrophages with impaired phagocytic ability increased during chronic HIV-1 infection but normalized after initiation of ART. Neutrophil infiltration was increased during infection and persisted despite effective ART.

Mucosal $\gamma\delta$ T cells play important roles in the earliest stages of immune responses against intestinal microbes. Intraepithelial $\gamma\delta$ T cells were increased in individuals with late-stage HIV-1 infection, and increased frequencies of $\gamma\delta$ T cells were also noted in rectosigmoid biopsies of HIV-infected subjects specifically associated with expansion of V δ 1 in conjunction with the contraction of V δ 2 $\gamma\delta$ T cells. Frequencies remained elevated despite ART. Mucosal-associated invariant T (MAIT) cells are recently identified innate-like mucosal-homing antimicrobial T cells. Reduced numbers of intestinal MAIT cells have been noted in the colon of untreated and treated HIV-infected subjects in one study, while other groups observed no significant loss of MAIT cells as a percentage of total T cells in rectal mucosa of chronically HIV-infected individuals.

HIV-Associated Epithelial Barrier Damage

HIV-1 infection is also characterized by significant intestinal epithelial cell (IEC) damage, including features of apoptosis, impaired regenerative and absorptive capacity, and increased permeability. Expression of various tight junction (TJ) proteins (e.g., claudin 1, claudin 2, ZO-1) is altered in early and chronic HIV infection. Exposure to HIV-1 directly increases epithelial barrier permeability in an epithelial mediated through

HIV-induced inflammatory cytokines (i.e., $\text{TNF}\alpha$) produced by epithelial cells and by disruption of TJ proteins. Extensive collagen deposition leading to fibrosis occurs rapidly in the GALT of HIV-infected persons and limits immune reconstitution during treatment.

Microbial Translocation

HIV-1-associated immunologic and structural changes in the gut mucosa allow for the movement of microbes and microbial products from the lumen into the lamina propria, draining lymph nodes and the systemic circulation. This process, termed microbial translocation (MT), occurs throughout all stages of HIV/SIV infection, starting as early as late acute stages of infection and persisting to AIDS. Levels of bacterial products (lipopolysaccharide (LPS), bacterial DNA) in the blood correlate with levels of T-cell activation, an independent predictor of HIV-1 disease progression. Plasma LPS levels in the first years of chronic HIV-1 infection predict HIV-1 disease progression. MT decreases but does not normalize after plasma viral load suppression during ART, and its persistence has been shown to predict mortality and other comorbidities such as dementia and has been linked to chronic monocyte and T-cell activation and to poor CD4 T-cell recovery. Multiple mechanisms have been postulated to underlie persistent MT after ART, including low-level viremia or persistently infected cells in the gut, failure of gut CD4 reconstitution and resulting epithelial barrier breakdown (especially due to loss of Th17 and Th22 cells), intestinal fibrosis that limits reconstitution, reduced T-cell homing to gut, and alterations or dysbiosis in the intestinal flora.

HIV-Associated Gut Dysbiosis

Intestinal mucosal and fecal microbiomes are altered in both composition and overall diversity during untreated and treated HIV-1 infection, defined as “dysbiosis,” although the nature of these changes vary depending on the patients

studied and type of analysis. HIV-associated dysbiosis has typically been characterized by increased abundances of commensal bacteria with pathogenic potential and decreased abundances of bacteria with known immunoregulatory properties. One study suggested that some of these changes may be due to sexual practices rather than to HIV-1 infection itself (Noguera-Julian et al. 2016). These HIV-altered bacterial communities were associated with increased mucosal mDC and T-cell activation, with mucosal inflammation, with MT, and with systemic T-cell activation demonstrating a link between dysbiosis and HIV-1-associated pathogenesis (Dillon et al. 2014). Higher proportions of *Lactobacillales*, an order of bacteria known to promote a healthy microflora and common in probiotics, were associated with markers predictive of better disease outcome. The normal relationship between diet and gut microbial composition is disrupted during untreated HIV infection. ART did not appear to restore a normal gut microbiome in all cases. Metagenomic and metabolomic studies to address the metabolic activity of these HIV-altered bacterial communities are in their infancy but thus far show an enrichment of genes involved in inflammatory pathways (e.g., LPS biosynthesis, bacterial translocation) in conjunction with a reduction of genes involved in amino acid metabolism and energy processes during HIV infection. Thus, gut dysbiosis may exacerbate chronic immune activation and provide targets for novel therapies.

Gut-Targeted Therapies

Given the major disruption of intestinal mucosal homeostasis associated with untreated as well as treated HIV infection, recent efforts have been focused on therapies aimed to restore mucosal function and limit systemic inflammation by reducing intestinal inflammation, blocking microbial translocation, or modifying the gut microbiome. Unfortunately, gut-targeted therapies in chronically infected individuals have thus far met with limited success. Mesalamine (5-aminosalicylic acid), an anti-inflammatory

agent used to treat ulcerative colitis, when given to HIV-infected individuals with limited CD4 restoration despite ART, had no obvious impact on mucosal or peripheral blood T-cell activation or on systemic markers of inflammation (Somsouk et al. 2014). In a similar patient population, rifaximin, a nonabsorbable antibiotic that decreases LPS in cirrhotics, had only minimal impact on markers of T-cell activation, MT, or systemic inflammation (Tenorio et al. 2015). Sevelamer, another agent meant to block MT by binding chylomicron-LPS complexes, likewise failed to reduce markers of MT, immune activation, or inflammation in a cohort of untreated, HIV-infected individuals (Sandler et al. 2014). Approaches to modify the gut microbiome in HIV-infected individuals on ART, including the use of probiotics, prebiotics, or their combination (“synbiotics”), have had better success, variably resulting in improvements in CD4 counts and decreases in some markers of microbial translocation, immune activation, inflammation, and coagulation. Larger controlled studies of this type are underway. As the underlying mechanisms of persistent gut dysfunction during ART are unraveled, novel therapeutics to target different pathways can be rationally developed.

Conclusion

HIV-1 pathogenesis in the gut, characterized by severe damage to the mucosal immune system and gut epithelial barrier, local inflammation, dysbiosis and microbial translocation, plays a significant role in driving generalized chronic immune activation and HIV-1 disease progression. Novel therapies to restore intestinal homeostasis in HIV-infected individuals are needed in order to reduce chronic inflammation and its adverse clinical consequences.

Cross-References

- ▶ Cellular and Soluble Immune Activation Markers in HIV-Infected Subjects
- ▶ Cellular Cofactors for HIV-1 Transcription

- ▶ Chronic Immune Activation in HIV
- ▶ Mucosal Immunity to HIV-1
- ▶ Mucosal Pathogenesis in SIV Infection

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HIV-1 Preexposure Prophylaxis

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Definition

► **Preexposure prophylaxis (PrEP)** is any medical or public health procedure used before exposure to the disease-causing agent; its purpose is to prevent rather than treat or cure a disease. The use of ► **antiretroviral** agents as PrEP is an experimental approach to HIV prevention that has recently been shown to successfully prevent the sexual transmission of HIV in several ► **clinical trials**. The challenge now is to determine how to translate these promising findings into a public health impact. Making the leap from clinical trials to real-world implementation of PrEP for HIV prevention presents a particular challenge because it requires multiple biomedical, behavioral, and social interventions in combination to maximize their synergy as appropriate for each unique epidemic setting. In real-world settings, particular effort will be needed to ensure high adherence, which has been shown to be challenging even in controlled clinical trial settings. Despite the numerous challenges in implementing preexposure prophylaxis, it has, in conjunction with antiretroviral treatment for prevention, created newfound optimism in HIV prevention.

Recent Additions to the HIV Prevention Toolkit: Overview of the Evidence for the Effectiveness of Biomedical HIV Prevention Technologies

Since 2010 there have been five randomized trials that have provided compelling evidence that ► **antiretrovirals** (ARVs) can prevent sexual transmission of HIV. The first in a series of trials showing that ARVs can reduce HIV acquisition was the CAPRISA 004 tenofovir gel trial. This trial, conducted among 889 rural and urban South African women, showed that tenofovir gel used before and after sex reduced acquisition of HIV infection in women by 39% (95% confidence interval (CI): 6;60) overall, thereby providing the proof-of-concept that ARVs can prevent sexual transmission of HIV (Abdool Karim et al. 2010). Soon thereafter, the results of the iPREX trial were announced, which showed that the daily oral tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) combination (Truvada) reduced HIV incidence by 44% (95% CI 15;63) among 2,499 men or transgender women who have sex with men (Grant et al. 2010).

Further evidence for the effectiveness of daily oral preexposure prophylaxis (PrEP) in heterosexual men and women comes from results of the Partners PrEP trial (Baeten et al. 2012) and the Botswana TDF2 trial (Thigpen et al. 2012). The Partners PrEP trial, which included 4,758 HIV-discordant couples from Kenya and Uganda, showed that daily oral TDF and TDF/FTC reduced HIV incidence by 67% (95% CI 44; 81) and 75% (95% CI 55; 87), respectively, while the Botswana TDF2 trial, conducted among 1,200 heterosexual men and women from the general population, found that daily oral TDF-FTC reduced HIV incidence by 62% (95% CI 22;83).

The use of ARVs for treatment of HIV-infected patients (note: this is ARVs as treatment and not as PrEP) has also recently been shown in a randomized clinical trial to prevent onward transmission of HIV to their uninfected partners (Treatment as Prevention – TasP). The HPTN 052 trial, conducted among 1,763 ► **HIV-discordant couples** from nine countries, showed that HIV transmission was reduced by 96% (95% CI, 73;99.5)

when ART was initiated in patients with CD4 counts between 350 and 550 cells/mm³ (Cohen et al. 2011).

This series of scientific breakthroughs in HIV prevention, combined with the recent approval of the first antiretroviral drug (Truvada) as PrEP for reducing the risk of sexually acquired HIV infection by the US Food and Drug Administration (FDA), has made the use of antiretroviral drugs, as part of a comprehensive HIV prevention package, a reality and has created newfound hope that the epidemic can be stopped. This unprecedented opportunity to alter the course of this disease will depend on the extent to which regulators, health service providers, funders, and researchers are able to translate this new evidence into effective large-scale treatment and ARV prophylaxis programs.

Potential Impact of Implementing ARVs as PrEP for HIV Prevention

The provision of PrEP to avert new HIV infections needs to be weighed against the potential costs of providing lifelong antiretrovirals for individuals who may become infected with HIV. Several mathematical models have illustrated the potential impact of ► [oral and topical PrEP](#) on the epidemic trajectory. Up to 3.2 million new HIV infections could be averted in southern sub-Saharan Africa over 10 years by targeting PrEP (having 90% effectiveness) to those at highest behavioral risk (Abbas et al. 2007). Similarly, mathematical modeling has shown that in South Africa alone, over the next two decades, tenofovir gel could avert 1.3 million new HIV infections and over 800,000 deaths (Williams et al. 2011). Mathematical models have also shown that oral and topical PrEP is cost-effective.

Challenges in the Implementation of PrEP

Despite the potential impact of tenofovir or Truvada as PrEP, only the US FDA has officially approved Truvada as an HIV prevention option,

and guidelines for its use in ► [men who have sex with men](#) and in heterosexuals have been developed by the Centers for Disease Control and Prevention. Since Truvada has not been approved by medicine regulators in any other country, country-specific individual patient guidelines and programmatic public health guidelines on implementation of PrEP have not been developed.

Besides the regulatory hurdles and lack of country-specific guidelines, several other challenges could impact on the rapid implementation of PrEP. The concerns about PrEP implementation listed below are not always warranted criticisms but include the following.

The Challenges of Adherence

Adherence is a major challenge for PrEP and long-term, high adherence is essential for its success. Although the CAPRISA 004, Partners PrEP, and TDF2 studies (described in section “[Recent Additions to the HIV Prevention Toolkit](#)” above) have shown that antiretrovirals can be taken with high adherence, two other studies, the FEM-PrEP and the VOICE trials, which were conducted exclusively among women, were not able to demonstrate a protective effect against HIV due to suboptimal adherence. There is a strong correlation between effectiveness and adherence.

Given that adherence can be challenging, even in a clinical trial setting, high levels of adherence may be difficult to achieve in “real-world” settings where PrEP may be implemented in underdeveloped public health-care facilities without adequate attention to adherence support. Not only will poor adherence contribute to suboptimal protection, but it may also impact on drug resistance development.

Therefore, the integration of behavioral interventions into any PrEP implementation program will be essential, and the close links between adherence and effectiveness will need to be emphasized to all potential PrEP users to ensure the highest possible adherence in the real world.

It is worth noting that drug adherence was also one of the key concerns raised when ART first became available as treatment. Concern about poor adherence was actually used as an argument against the implementation of these life-saving

drugs in Africa. Experiences from implementing ► [ART treatment](#) has shown that high levels of adherence are achievable in a real-world setting, even in developing countries. Although high adherence to treatment of HIV-positive people is encouraging, this may not be readily applicable to adherence in healthy asymptomatic people. On the other hand, adherence may not turn out to be an issue as the product's effectiveness may serve as strong motivation for adherence. Regardless, adherence is likely to be a challenge that will require a concerted effort to overcome, and PrEP programs will need to include practical and proven adherence support programs.

PrEP May Undermine Future AIDS Treatment by Causing Drug Resistance

The risk of drug resistance from PrEP is markedly different from that observed when, for example, nevirapine is given to HIV-positive pregnant women, as those taking PrEP generally do not have circulating virus which can become drug resistant. However, the possibility of resistance is present in instances where PrEP is taken for several weeks inadvertently by those with unidentified HIV infection.

A model based on the South African epidemic has shown that after 10 years of ART and PrEP rollout, the number of new infections would have decreased by 38% and the drug resistance prevalence would have increased to 11.4%. This compares with levels of between 10% and 17% observed in high-income countries. Importantly, most of the resistance is predicted to be a consequence of ART rather than from PrEP (Abbas et al. 2011). The main issue regarding resistance, however, is whether the use of PrEP will compromise an individual's future ARV treatment options when they may require ART. At present, there are no data to answer this question.

A separate concern about resistance is the use of the same drugs (e.g., tenofovir) in therapy and prevention. Therapy failure is associated with the development of resistance and thereby the spread of resistant viruses which in turn may compromise the efficacy of the same drugs (or occasionally, the same class of drugs) used for prophylaxis.

Currently there are over 30 licensed drugs to treat HIV, including several cost-effective non-tenofovir containing first-line regimens. Some consideration about setting aside a class (or classes) of ARVs for use in prevention only is warranted.

PrEP Users May Reduce Their Use of Higher-Efficacy HIV Prevention Strategies Like Condoms

The current ► [oral and topical PrEP](#) strategies are only partially effective, ranging from 39% to 73%. Risk compensation (also referred to as behavioral disinhibition) is the selection of lower-efficacy interventions when higher-efficacy interventions are available and is a potential concern when implementing any new suboptimal HIV prevention strategy and is not specific to PrEP. Risk compensation could potentially undermine and even reverse the beneficial effects of PrEP, as shown by mathematical models on HIV epidemics in Botswana, Kenya, and southern India (Vissers et al. 2008). While a low-efficacy intervention may be reversed by risk compensation, current evidence from ► [medical male circumcision](#) implementation has found this concern to be baseless. An assessment of the real-world effect of the rollout of medical male circumcision in a community in South Africa has shown no evidence of risk compensation after 3 years (Auvert et al. 2011). A more important consideration is that some of the PrEP strategies specifically empower women, who have no other alternative HIV protection strategies. Even a low-efficacy product would be critically important to large numbers of young women in South Africa who are unable to ensure their partner's fidelity or condom use. Indeed, PrEP is most appropriate for the target populations where condom use is low or nonexistent.

Antiretrovirals Should Be Prioritized for AIDS Treatment

Some have argued that it would be unethical to divert ARVs that would have been used for treatment for prevention (Macklin et al. 2012), especially since only 54% of those in need of ART

were receiving it in 2011 (World Health Organization 2012). While it is a legitimate concern that eligible HIV-positive patients should be prioritized for ART for their own health and to save their lives, it is spurious to trade off treatment and prevention as if these drugs are being taken away from sick and dying patients to be given to healthy people. Treatment and prevention strategies are a continuum in their use of ARVs – both are needed in conjunction with each other to ensure ART provision is sustainable in the long term and to realize the quest to end the HIV epidemic.

One of the main reasons why so many people who are in need of treatment have not yet accessed is because many do not know their HIV status. Based on ten recent national population-based surveys in sub-Saharan Africa, less than 40% of people living with HIV know their HIV status (WHO et al. 2010). According to the 2008 South African National HIV survey, 74% of those most at risk of acquiring HIV infection were unaware of their HIV status. If self-denial, a common reason for not testing, is not overcome, it will severely limit the potential impact of these new HIV prevention approaches. Initiation of PrEP will require an assessment of HIV status and could therefore be used as an opportunity for not only scaling up PrEP to HIV-uninfected at-risk individuals but also for identifying those who need ART for treatment.

Is It Safe to Give ARV Drugs to Healthy Asymptomatic People?

Tenofovir has an excellent safety profile and low rates of adverse effects. However, tenofovir may reduce bone density and exacerbate existing renal impairment and has been associated with hepatic flares in chronic hepatitis B virus-infected individuals when the treatment is stopped (Nuesch et al. 2008). Emtricitabine has a similar safety profile as tenofovir, and adverse events occurring in clinical trials were generally of mild or moderate severity. While mild side effects are readily tolerated when medication is taken for therapeutic reasons, the same is not necessarily true when medication is taken by healthy asymptomatic individuals where even mild side effects may

compromise adherence. Ongoing drug safety surveillance will need to be a component of plans for large-scale rollout of ARVs for PrEP. Hepatitis B testing and vaccinations for susceptible individuals at high risk may also need to be considered.

Who Would Benefit Most and Criteria for Initiating and Terminating PrEP

When resources are scarce, it is necessary to rationalize and prioritize certain high-risk groups for access to interventions over others. Determining who would benefit most will vary from country to country, and while some groups, like MSMs and injection drug users (IDUs), are easily identifiable, identifying who should be prioritized in generalized epidemics is more complex. Nevertheless, before PrEP can be initiated, it will be important to establish the HIV status of the individuals, and ongoing HIV testing would be an important component of any PrEP rollout program.

The Efficacy: Effectiveness Gap – Implications for Implementation Programs and the Implementation Science Agenda

At present, there are no data available on the extent to which the outcomes achieved in the PrEP trials described earlier (section “[Recent Additions to the HIV Prevention Toolkit](#)”) can be translated into real-world effectiveness. This leap from trials to implementation, generally referred to as the efficacy-effectiveness gap, can be substantial, as seen in implementation of ► [prevention of mother-to-child transmission](#) (PMTCT) programs. For example, a PMTCT program in Cote d’Ivoire showed that 40% of HIV-positive pregnant women did not benefit from zidovudine to reduce mother-to-child transmission as only 60% returned to the clinic for their HIV test results.

Some of the biggest contributors to the efficacy-effectiveness gap anticipated in the PrEP field will be willingness to know and monitor HIV status, suboptimal adherence levels, the extent to which people continue with the other proven prevention interventions (behavior disinhibition), and extent to which the existing public

sector health services can facilitate uptake and maintain clients in long-term follow-up. The extent to which health services in countries most affected are sufficiently well managed to absorb the implementation of antiretrovirals for prevention, in a manner that provides high uptake, adherence, and follow-up, will determine its success or failure. One approach to maximize the chance of success is to integrate PrEP as a component of existing comprehensive HIV prevention programs and services.

The Cost of Implementing PrEP

The implementation of PrEP also faces substantial financial challenges. Besides the drug costs, the programmatic and laboratory monitoring costs, including HIV testing, hepatitis B virus testing, and renal function assessment prior to tenofovir-containing PrEP initiation and then at regular intervals, of yet unknown duration, are likely to be substantial. Unfortunately, HIV prevention programs in regions with the highest HIV burden are already substantially underfunded, and many highly effective prevention options such as condoms are not being used at the scale and intensity needed. A recent analysis shows that the per capita spending on health in high-HIV burden countries like Kenya and Uganda is \$17 and \$16, respectively, with 60.7% and 50.2% of the health expenditure being dedicated toward HIV (Amico et al. 2010). The efficiency of health spending will need to be dramatically improved in these countries if PrEP is to be successfully implemented.

While funds for PrEP may not be readily available at this point, it would be shortsighted to consider this in isolation. For PrEP, the long-term consequences of not implementing PrEP need to be considered, especially in women who may have no other effective options. While the unmet need to provide antiretroviral treatment to all those in need is large, the opportunity to prevent new HIV infections cannot be passed over. Additional funding resources will need to be raised to implement PrEP as part of combination HIV prevention programs to avoid an unsustainable future with ever-increasing numbers of people requiring lifelong antiretroviral therapy.

Consequences of Not Implementing PrEP for HIV Prevention

The challenges of implementing PrEP should not detract from the potential importance of these interventions. The realization of PrEP implementation as part of an overall prevention plan is essential. The emphasis should be both on treating patients who require the ARVs for their own needs and also for those who need it for prevention. As was the case in MTCT, most of the concerns about scale-up can only be addressed in scale-up programs and not in relatively small phase I and II trials.

The regulatory approval of Truvada for PrEP by the FDA provides an opportunity to undertake scale-up programs, but critically, these programs can be used as an opportunity to generate the evidence on how best to address the concerns and shortcomings of PrEP.

Initially, implementation programs could focus on providing PrEP to individuals at highest risk, e.g., MSM and young women in Africa and progressively scale-up PrEP as part of a comprehensive HIV prevention package. To address the issue of the partial efficacy of PrEP, the impact of PrEP implementation should be monitored. Monitoring should include, at minimum, an assessment of HIV incidence rates, PrEP uptake levels, adherence levels, and the impact of PrEP uptake on condom use. Given the importance of adherence, programmatic implementation needs to carefully assess the factors impacting on adherence. In particular, the gender power imbalances and impact on power relations in acquiring HIV need special attention. Resistance will also need to be closely monitored, both from use of Truvada as part of treatment and prevention. Long-term safety will need to be monitored, with special attention to kidney, bone mineral, and hepatitis B-related safety concerns. Implementation of PrEP will address questions about who to prioritize, when to initiate, and when to terminate by monitoring who benefits maximally and the period of highest risk that benefits most from PrEP.

Other ► [clinical trial](#) research should also continue in parallel, with some consideration being given to the assessment of different dosing strategies (daily vs. intermittent) as well as a range of

formulations. Studies on alternative delivery mechanisms for PrEP such as gels and rings should also be pursued simultaneously.

Conclusion

PrEP has created newfound optimism in HIV prevention. ARVs increase options for HIV prevention, especially for specific high-risk populations such as young women in Africa. Despite the inherent challenges that lie ahead, implementation of ARVs for prevention is imperative and will be part of the solution to realizing the goal of finally turning the tide on the HIV epidemic.

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models of AIDS has proven valuable for understanding the nature of protective immunity against HIV transmission (► [HIV-1 Transmission Blocking Vaccines, How Feasible Are They?](#); ► [Models for HIV-1 Transmission, Non-Human Primate](#); Modeling Early HIV-1 Infection and Dissemination).

HIV-1 Prevention Using Live-Attenuated Vaccines

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Definition

Live, attenuated viral vaccines (LAVs) contain weakened forms of pathogenic microorganisms. Historically, LAVs have been the most effective interventions against viral infections. These vaccines produce a very mild version of natural infection, and they elicit immune responses that are often similar to those found in people recovering from pathogenic viral infections including strong cellular and antibody responses and long-lived immunity. Although safety concerns preclude the use of live-attenuated human immunodeficiency virus (HIV) in humans, LAVs have provided the most reliable protection from virus challenge in preclinical studies of AIDS vaccines. Thus, studying the protective mechanisms of attenuated primate lentiviruses in nonhuman primate (NHP)

Introduction

LAVs represent the first successful method of vaccination. In the eighteenth century, the British doctor Edward Jenner used cowpox virus, which produced mild disease in humans, to vaccinate against smallpox. This vaccination strategy was based on the observation that milkmaids who were occupationally infected with cowpox rarely got smallpox. Later, cowpox-based small pox vaccines were replaced by vaccinia virus-based small pox vaccines. Vaccinia virus causes minimal or mild disease in humans but elicits strong immune responses that cross-protect against smallpox. Using the vaccinia virus-based vaccine, smallpox was successfully eradicated in the late 1970s. The first LAV produced in the laboratory dates from 1880, when Pasteur and colleagues produced an attenuated avian cholera bacillus vaccine. Subsequently, Pasteur employed attenuation of other pathogens such as anthrax and rabies to produce vaccines for animals and humans.

Based on these seminal studies, many successful vaccines have been developed using attenuated bacteria or viruses. LAVs have been administered to millions of people worldwide with minimal adverse effects; LAVs have eliminated smallpox from the world and polio from the Western hemisphere. LAVs are currently used to prevent many human diseases including influenza, measles, mumps, rubella, chickenpox, yellow fever, tuberculosis, and typhoid fever; LAVs have dramatically reduced the incidence of these diseases in geographic regions that achieve high levels of vaccination in their communities.

Despite the advantages of LAVs, there are safety concerns that include reversion of the vaccine strain to wild-type virulence. For example,

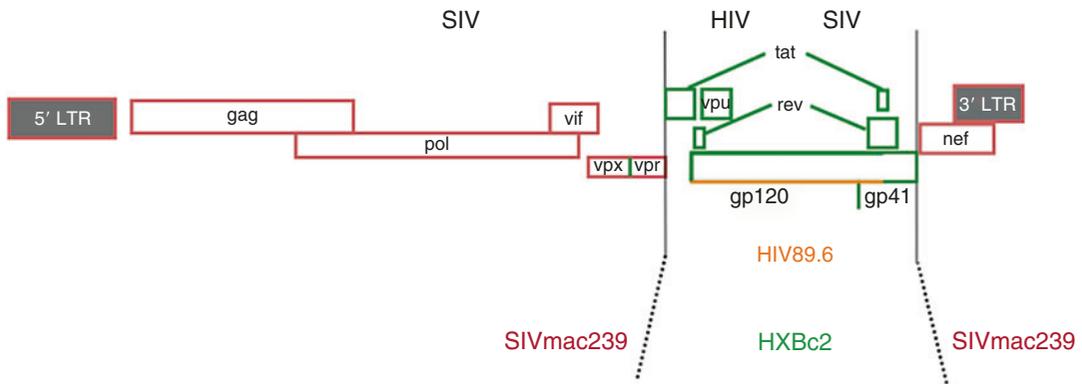
poliovirus revertants in the Sabin polio vaccine have caused local epidemics of polio. For this reason, LAVs are not used for infectious diseases that are uniformly fatal. Attenuated viruses used as vaccines have been derived from naturally arising nonpathogenic viral strains or by serial passage of a pathogenic virus through cells from an aberrant host. Current approaches to the development of attenuated viruses for new vaccines introduce specific mutations into the viral genome, resulting in attenuated viruses with molecular lesions (e.g., gene deletions, mutations in several genes) that will make the next-generation LAVs even safer than those currently in use. Although LAVs are relatively cheap and simple to produce, attenuated vaccines require refrigerated storage, an issue that makes them less useful in developing countries. Finally, individuals with impaired immunity cannot safely receive some LAVs due to the increased pathogenic potential of even attenuated virus strains in these individuals.

Live-Attenuated HIV Vaccines

While most licensed vaccines in use today are LAVs, safety concerns have limited development of live, attenuated HIV vaccines because the vaccine virus could revert to virulence or elicit autoimmune or malignant disease. However, experimentation with the live-attenuated AIDS vaccines has been done using simian immunodeficiency virus (SIV) and chimeric simian-human immunodeficiency virus (SHIV) infections in macaques (► [SIVmac Infection of Macaques, Immunopathogenesis of](#)). A large number of labs using a variety of attenuated SIV and SHIV vaccine strains over the course of more than 20 years have demonstrated that LAVs protect NHP from SIV challenge. Different strategies have been employed to produce attenuated AIDS viruses for immunization. Deletion of the *nef* gene from SIV results in a virus that grows in cell culture and replicates in monkeys but is largely controlled by immune responses and rarely causes AIDS. When animals infected with this attenuated virus were challenged with virulent SIV, they were protected

from the otherwise lethal infection (Daniel et al. 1992). In fact, immunization with SIV239 Δ *nef* or SIV239 Δ 3 has prevented infection in some experiments, while in others, it has led to a substantial reduction in plasma viral load during primary viremia and complete control of viral replication after months to years of infection (Wyand et al. 1996). LAVs are effective against mucosal and systemic routes of challenge, including intravenous, rectal, and vaginal challenge routes. The SIV Δ *nef* vaccine virus establishes a life-long infection with persistent low-level replication. In fact, SIV Δ *nef* can cause AIDS, especially when administered orally to infant monkeys (Baba et al. 1995) because persistent replication of the SIV 239 Δ *nef* vaccine virus generates variants that can replicate more efficiently than the original vaccine strain. Addition of deletions or mutations to the virus results in further attenuation but with a loss of vaccine efficacy (Girard et al. 2006). Indeed, NHP studies have shown that efficacy of AIDS LAVs inversely correlates with the level of attenuation of the virus used as the vaccine (Lohman et al. 1994; Wyand et al. 1996). LAVs that replicate only for a single cycle are not as immunogenic or protective and do not persist as do less attenuated LAVs (Alpert et al. 2010). These results highlight the challenge of making a safe and effective attenuated AIDS vaccine, and they also demonstrate that exposure to persistent antigen that occurs with effective AIDS LAVs is needed to generate reliable protection from challenge.

A human cohort that is infected with a naturally attenuated HIV mutant has provided extremely valuable information regarding the dangers of an LAV approach to AIDS vaccines (Zaunders et al. 2011). The Sydney Blood Bank Cohort (SBBC) is a group of people that were infected with an attenuated, *nef*, and LTR-deleted HIV-1 mutant through blood transfusion. These patients had particularly low levels of HIV-1 RNA and DNA in peripheral blood and survived without symptoms for more than 15 years. However, in three of five patients, there was ongoing low-level replication that allowed viral evolution. Thus, although *nef*-deleted



HIV-1 Prevention Using Live-Attenuated Vaccines, Fig. 1 SHIV 89.6 contains functional HIV-1 *vpv*, *tat*, *rev*, and *env* genes engineered to replace their SIV counterparts within the SIVmac239 proviral DNA. The virus was constructed using the *env* gene from HIV-1 HXBc2

except for the KpnI (nucleotide 5925) to BamHI (nucleotide 8053) fragment, which encodes the ectodomain of the gp120 and gp41 envelope glycoproteins. This *env* fragment in SHIV 89.6 was derived from HIV-1 89.6 (Lu et al. 1996)

HIV-1 had been discussed as a candidate vaccine in humans due to the protective effect observed in the SIV/macaque animal model, the emergence of virulent strains in multiple people infected with a *nef*/LTR-defective HIV-1 strain reemphasizes the point that a live, attenuated HIV vaccine cannot be used safely in humans (Zaunders et al. 2011).

Other LAV approaches tested in NHP include a highly attenuated, antibody neutralization-sensitive mutant of SIV with complete deletion of the V1 and V2 regions of Env from SIVmac239 (SIV239 Δ V1-V2) (Johnson et al. 2002). Infection of NHP with this virus confers potent protection from intravenous challenge by pathogenic full-length SIVmac239 (Mansfield et al. 2008). Insertion of the HIV-1 Env and regulatory genes into an SIVmac239 backbone to produce SHIVs that replicate in NHP can be used to directly test the efficacy of HIV-1 envelope vaccines. Although these chimeric viruses are attenuated for pathogenicity, SHIVs can become pathogenic after serial passage in NHP. Attenuated SHIV viruses have been used as LAVs in NHP that are subsequently challenged with SIVmac239, a model system in which the Env is completely heterologous but the remaining viral proteins are matched (Genesca et al. 2012; Fig. 1). With this LAV vaccine/challenge system, it is possible to determine if non-envelope antigens alone can provide protection from pathogenic SIV challenge.

Mechanisms of Protection

A remarkable feature of SIV LAVs is the efficient control of SIV replication during the first few weeks after challenge in vaccinated animals that become infected. Studies using various LAVs in NHP challenged by mucosal or systemic routes have tested the roles of antiviral T cells or antibodies, innate antiviral immunity, and viral interference in LAV-mediated protection.

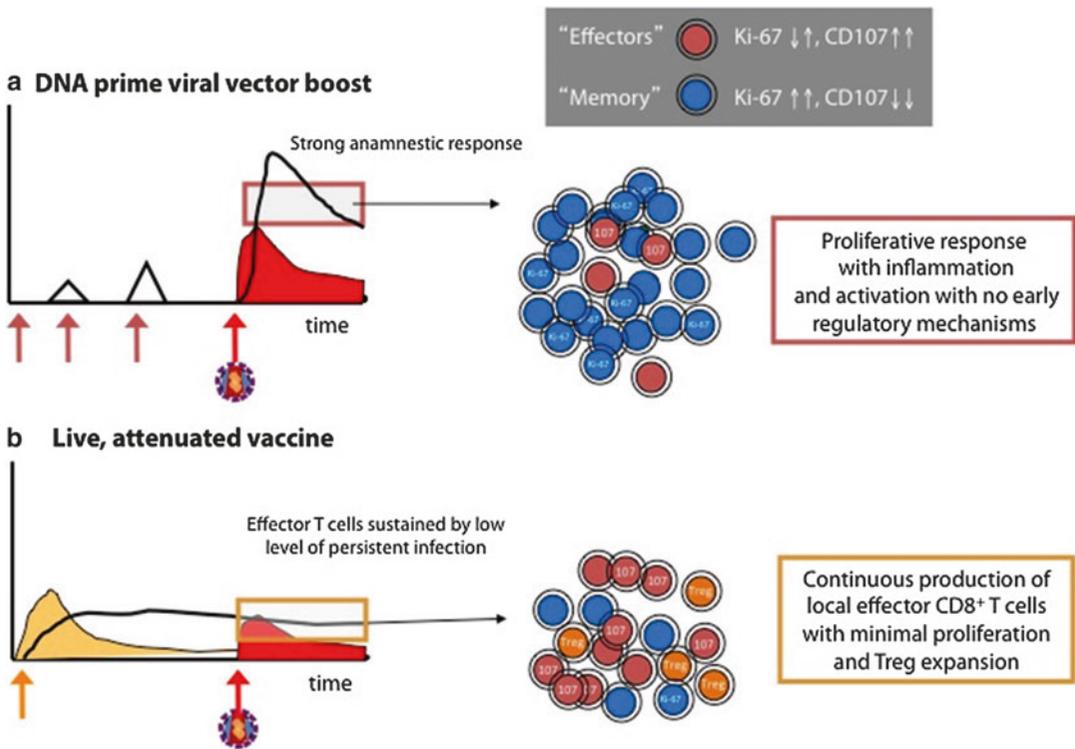
The protection generated in NHP by immunization with SIV/SHIV LAVs seems to be immune mediated, as a period of time must elapse after vaccination before protection develops. It is generally assumed that this interval provides the opportunity for protective immune responses to develop. Virus-specific CD4⁺ and CD8⁺ T cells and neutralizing Abs develop in animals vaccinated with live-attenuated SIV and people infected with attenuated HIV-1 strains (Rollman et al. 2007; ► HIV & SIV, B Cell Responses to; ► HIV & SIV, CD4⁺ T Cell Responses to; ► HIV & SIV, CD8⁺ T Cell Responses to). But the protection afforded by attenuated SIV cannot be transferred with immune plasma (Almond et al. 1997). In addition, nonimmune mechanisms have also been shown to facilitate the effectiveness of retroviral LAV vaccines (Rollman et al. 2007; Genesca et al. 2012; HIV & SIV, Innate Immune Responses to).

While the leading candidate DNA-based and viral vector-based HIV vaccines elicit robust cellular CD8⁺ responses in the peripheral blood (Koff 2006; ► [HIV & SIV, CD8⁺ T Cell Responses to](#)), vaccination with LAV SIV results in rapid development of SIV-specific effector cytotoxic T lymphocytes (CTLs), with robust degranulation and granzyme B release from tetramer-positive CD8⁺ T cells (Rollman et al. 2007). Antiviral CD8⁺ T cell responses have been consistently associated with protection in LAV studies in NHP, and the effector memory CTL elicited by LAV is present in mucosal surfaces of immunized animals (Genesca et al. 2008a). In fact, an attenuated SHIV vaccine that protects NHP from vaginal SIV challenge increases the total number of circulating effector memory CD8⁺ T cells while blunting the number of CD8⁺ T cells that are proliferating, activated, and/or undergoing apoptotic cell death. Simultaneously, terminal effector CD8⁺ T cells with multiple functions including high cytotoxic capacity are present in the vagina of these immunized animals on the day of SIV challenge (► [Mucosal Immunity](#); ► [Vaccine Induced Immune Responses, Preventing HIV-1 Transmission Through](#)). These antiviral effector CD8⁺ T cells elicited by attenuated SHIV protect from vaginal SIVmac239 challenge, as CD8⁺ T cell depletion abrogates protection in the vaccinated NHP (Genesca et al. 2008b).

In preclinical AIDS vaccine studies, many vaccine strategies have decreased challenge virus replication after immunized animals become infected, but few have blocked infection. In fact, LAVs provide superior protection from superinfection compared to all other approaches tested in NHP. This suggests that a persistently replicating vaccine virus that disseminates throughout the body and produces a low level of antigen continuously in the mucosal tissues has the potential to generate protective T cell immunity. A recent study of LAV-mediated protection against highly pathogenic intravenous SIV challenge in a large cohort of LAV-vaccinated rhesus macaques used several versions of attenuated SIV that varied in their level of attenuation, their degree of amino acid sequence homology with the wild-type

SIVmac239 challenge virus, or both (Fukazawa et al. 2012). The magnitude of SIV-specific CD4⁺ and CD8⁺ T cell responses in secondary lymphoid tissues, as well as the SIV suppression capacity of CD8⁺ memory T cells in these tissues were correlated with the degree of attenuation. Further, the degree of LAV-mediated protection against intravenous wild-type SIVmac239 challenge strongly correlated with the magnitude and nature of SIV-specific effector T cells in the lymph node but not with T cell responses in the blood or with other cellular, humoral, and innate immune parameters (Fukazawa et al. 2012).

There are many features of the CD8⁺ T cell response to persistently replicating LAVs that differ from the responses to other vaccine strategies that could explain the effectiveness of CD8⁺ T cell responses in these models. The key feature of LAVs that is responsible for these differences may be the chronic, low level of antigenic stimulation from residual vaccine replication in mucosal tissues. This antigen production generates and maintains a resident effector memory CD8⁺ T cell response at the portal of virus entry (Fig. 2; mucosal immunity; vaccine-induced immune responses, preventing HIV-1 transmission through). Further, after virus challenge, there is little expansion of the mucosal memory CD8⁺ T cells elicited by LAVs (Fig. 2a). The tissue environment after SIV challenge is actively maintained by a T-regulatory cell response (► [Regulatory T Cells](#)) that rapidly expands after SIV challenge to suppress immune activation and prevent T cell proliferation (Genesca et al. 2012; Fig. 2b; ► [Immune Activation and HIV Transmission](#)). After CD8⁺ T cell depletion of LAV-immunized animals, the highest levels of SIV replication are in the genital tract and genital lymph nodes, not in systemic lymph nodes or the GI tract as in control animals (Stone et al. 2009). These very high levels of viral replication in the genital tract suggest that, in the absence of antiviral CD8⁺ T cells, SIV replication in the genital tract is enhanced in LAV-immunized animals compared to unimmunized animals. In fact, SHIV-immunized animals have numerous SIV-specific CD4⁺ T cells in the vaginal mucosa on the day of challenge (Genesca et al. 2012), and they provide cellular



HIV-1 Prevention Using Live-Attenuated Vaccines, Fig. 2 Two patterns of CD8⁺ T cell response to vaccination against SIV. (a) Following a prime/boost vaccine with nonreplicating vectors, a strong anamnestic T cell response is observed post-challenge. (b) Following vaccination with live-attenuated SIV (or SHIV), an anamnestic response is not seen in the blood but it may be present in mucosal

tissue at the site of SIV challenge. In both panels, SIV challenge is indicated by the virion and red arrow and replication of challenge virus is denoted by red-filled curves. In panel (b), replication of attenuated vaccine virus is denoted by the yellow-filled curve (Adapted from (Genesca 2011))

targets that enhance viral replication in mucosal sites of entry in the absence of an effective immune response. Thus, the relative numbers of SIV target cells and vaccine-induced antiviral T cells in the vagina likely determine the outcome of vaginal SIV challenge in LAV-immunized animals (Genesca et al. 2012). The lack of immune activation and T cell division in response to challenge virus exposure coupled with an effector T cell response in the mucosal surface may explain the effectiveness of LAV (Genesca et al. 2012). This interpretation leads to the conclusion that the outcome of challenge results from a dynamic balance between SIV replication in abundant target cells and efficient killing of those infected target cells by antiviral CD8⁺ T cells.

Next-Generation LAVs

Advances in molecular virology and the identification of many viral genes associated with HIV virulence and immunogenicity have driven a new generation of LAVs (► [Overview: Life Cycle](#)), and this information has been used in an attempt to irrevocably attenuate HIV variants. These rationally attenuated viruses include deleterious mutations that alter replication fidelity, codon optimization, and/or susceptibility to control by microRNAs or zinc finger nucleases. While each of these approaches has recently garnered significant attention, further in vitro and animal testing is necessary before progressing to clinical trials (Lauring and Andino 2010). Finally, although in

early stages of development, self-replicating chimeric virus genomes that express lentivirus structural proteins and assemble into infectious particles that present lentivirus immunogens to the immune system in their native and functional conformation may be able to produce immune responses similar to first-generation LAVs, without the inherent safety issues (Jurgens et al. 2012).

Conclusion

The most important feature of LAVs is that they provide a high level of antigen exposure and, to some extent, they also have built-in adjuvanticity. Ideally, LAVs establish a mild infection. They can also be engineered to deliver a sufficiently high antigen load, while avoiding unwanted local inflammatory responses. These attributes are important because prolonged antigen exposure may be required for the maintenance of mucosal immune responses. A major challenge for the development of HIV LAV is the necessary balance between sufficient attenuation and vaccine immunogenicity (Lohman et al. 1994; Wyand et al. 1996). The production of a new generation of LAVs using molecular virology represents a controlled strategy for attenuation, and this approach is highly attractive for developing stable and safe LAVs.

The goal of conventional HIV vaccines is to elicit a strong and broad neutralizing antibody response that can limit infection upon HIV exposure and CD8⁺ T cell responses to clear the infection after transmission. To improve the efficacy of these vaccines against mucosally transmitted HIV infections, new strategies for directing immune responses into mucosal sites are needed. The first evidence that persistent vectors might have a superior ability to control pathogenic lentiviral infection came from analysis of live, attenuated SIV vaccines. Understanding and controlling the relationship between mucosal immune activation and protective adaptive immune responses may be a critical factor in developing an effective mucosal AIDS vaccine.

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HIV-1 Rev Expression and Functions

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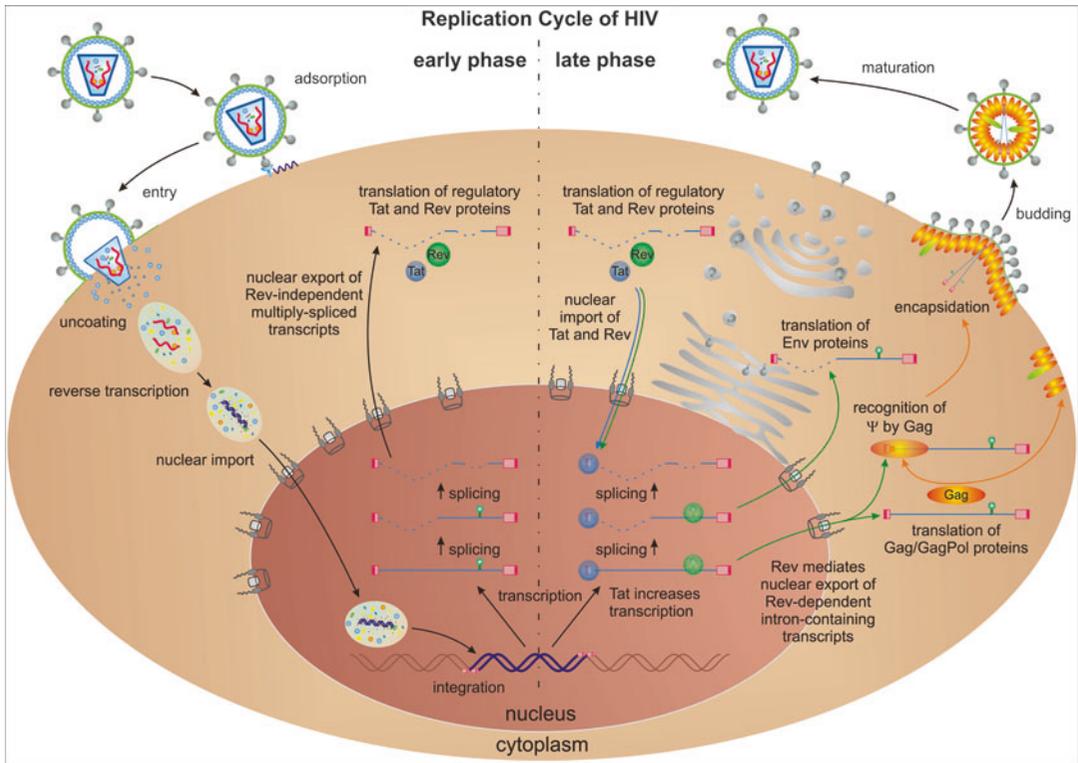
Definition

The viral Rev protein is essential for HIV replication, since it mediates nuclear export of intron-containing viral RNAs. In addition, Rev enhances translation and genomic RNA encapsidation and

prevents superinfection of target cells. The molecular mechanisms underlying these functions of Rev are discussed.

Introduction

Shortly after binding of the human immunodeficiency virus type 1 (HIV-1) to a target cell, the viral interior, containing a complex between (mostly) viral proteins and the genetic construction plan in the form of the viral, genomic RNA, is released from the viral particle into the cytoplasm. In the cytoplasm the genomic RNA is converted (reverse transcribed) to DNA by the viral reverse transcriptase protein (Fig. 1) (► [Reverse Transcriptase - Catalyzed HIV-1 DNA Synthesis](#)). The viral DNA is subsequently transported into the nucleus and integrated as provirus into the cellular DNA with the help of the viral integrase protein (Fig. 1) (► [Integration](#)). In the next step, the cellular transcription machinery generates a full-length RNA copy from the proviral DNA referred to as viral unspliced or genomic RNA/transcript. During and after transcription, some parts of the RNA, the introns, are removed in a process known as splicing (Fig. 1). Splicing of the genomic transcript is very complex and generates more than 40 different RNAs that can be grouped in (1) long (9 kilobases) unspliced, (2) intermediate (4 kilobases) spliced but intron-containing, and (3) short (2 kilobases) multiply spliced intron-less transcripts (Fig. 2). The exact nucleotide sequences of spliced RNAs of the same class, either still containing some introns or being intron-less, slightly differ because different splicing events generate RNAs that are grouped together. Multiply spliced transcripts have lost all their introns and are actively exported from the nucleus to the cytoplasm by cellular proteins. In the cytoplasm these RNAs are used as a construction plan to produce the viral proteins Tat, Nef, and Rev in a process called translation (Figs. 1 and 2) (► [“Nef/Env/Vpu/Tetherin”](#) and ► [“Tat Expression and Function”](#)). This section will focus on the function of the viral Rev (*regulator of expression of virion*) protein during the cellular multiplication of HIV-1 called HIV-1 replication cycle.



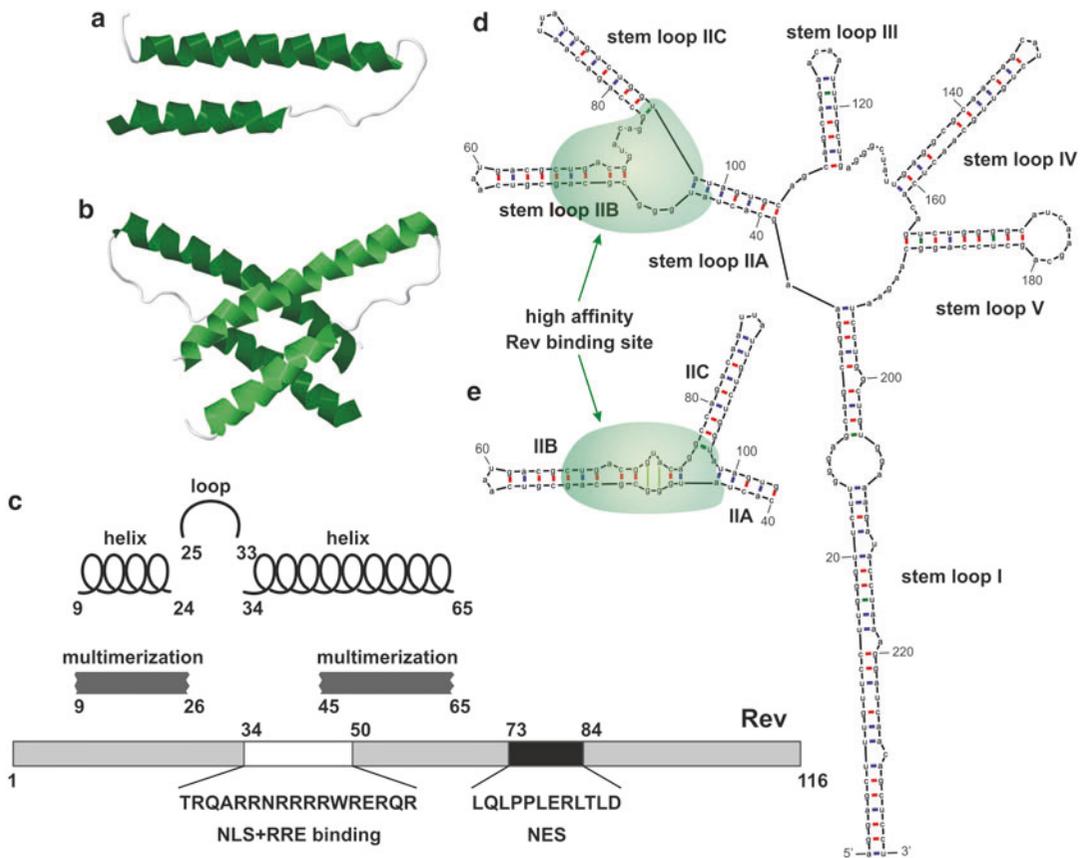
HIV-1 Rev Expression and Functions, Fig. 1 Replication cycle of the human immunodeficiency virus (*HIV*). Binding of the viral envelope protein (*Env*) on the surface of the viral particle to proteins in the plasma membrane surrounding the human cell (cytoplasm in *light brown*, nucleus in *dark brown*) allows fusion of the cellular and the viral membrane and release of viral components into the cytoplasm. After remodeling of the viral RNA-protein complex, the viral genomic RNA is converted into viral DNA in a process known as reverse transcription. The resulting DNA-protein complex is referred to as the pre-integration complex that is imported through the nuclear pores into the nucleus of the cell (*dark brown*). After integration of the viral DNA into the cellular chromosomes, the genomic information of the provirus is transcribed to RNA by the cellular transcription machinery. Complete removal of all RNA fragments called introns in a process known as splicing leads to multiply spliced RNAs that are exported to the cytoplasm where translation takes place. The resulting early proteins Tat and Rev are

imported into the nucleus. (The early protein Nef is not shown here.) In the beginning late phase of the replication cycle Tat interacts with an RNA structure called trans-activation response element (*TAR*) present in all viral transcripts leading to enhanced transcription. Rev interacts with an RNA structure called Rev-response element (*RRE*). This binding allows nuclear export of intron-containing viral RNA. Rev-mediated RNA export allows production of the viral proteins Env and Gag/GagPol. (Accessory proteins Vif, Vpr, and Vpu are also expressed in the late phase but are not shown here.) Gag targets the membrane of the cell and forms new viral particles. Furthermore, Gag binds the viral genomic RNA at an RNA structure called encapsidation signal or Psi (Ψ). This leads to the incorporation of two viral unspliced RNA molecules per viral particle. Maturation of the newly formed viral particle comprises cleavage of viral proteins and rearrangement of the interior of the particle. Mature particles are infectious and can start a new replication cycle (figure modified from Grewe and Überla 2010a)

Molecular Details of Rev and Its RNA Binding Site the Rev-Response Element (RRE)

HIV-1 Rev is a small ~18 kDa protein consisting of 116 amino acids. It can be subdivided in discrete but sometimes overlapping protein domains.

Domains essential for Rev’s activity are the nuclear localization signal (NLS), the RNA-binding domain (RBD), the multimerization domain (MD), and the nuclear export signal (NES) also called activation domain (Pollard and Malim 1998; Felber et al. 2007) (Fig. 3). Rev’s NES is enriched in the amino acid leucine. The sequence



HIV-1 Rev Expression and Functions, Fig. 3 HIV Rev and Rev-response element (*RRE*). (a and b) Structures of the HIV Rev protein monomer (a) and dimer (b) were modeled using the open-source software Jmol (<http://jmol.sourceforge.net>) with the data sets published by Dimattia et al. (a) and Daugherty et al. (b). Please note that very similar structures were obtained by two complementary approaches applied by these two working groups. The structure of amino acids 8/9–64/65 could be resolved and is shown. Remarkably, the NES is located downstream of this ordered sequence at the C-terminus and seems to be a flexible sequence following the second, longer helical structure. (c) A schematic representation of the domain structure of the whole Rev protein is shown. The amino acid sequence of the NES and the overlapping NLS- and RRE-binding domain are depicted in single letter code.

NLS, nuclear localization signal; NES, nuclear export signal; RRE, Rev-response element. (d) The RNA structure of the Rev-response element of HIV was generated using the open-source software mfold (<http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form>). The nucleotide sequence 7,124–7,357 of GenBank entry M15654.1 (HIV-1, isolate BH10) (<http://www.ncbi.nlm.nih.gov/nucleotide/M15654.1>) was used with standard settings of mfold. The structure obtained resembles those published earlier (Pollard and Malim 1998). (e) However, the high-affinity Rev binding site (marked in green) shows a distorted structure with two non-Watson-Crick base pairs (G47-A73 and G48-G71, indicated by bright green lines) which are important for Rev binding (Pollard and Malim 1998). The corrected RNA structure is shown in (e)

Rev specifically interacts with incompletely spliced and unspliced RNAs of HIV-1 due to its binding to an elaborated RNA structure referred to as Rev-response element (RRE) containing several discrete stem loop structures (Fig. 3). The RRE is only present in these transcripts, because of its localization in an intron that is lacking in multiply

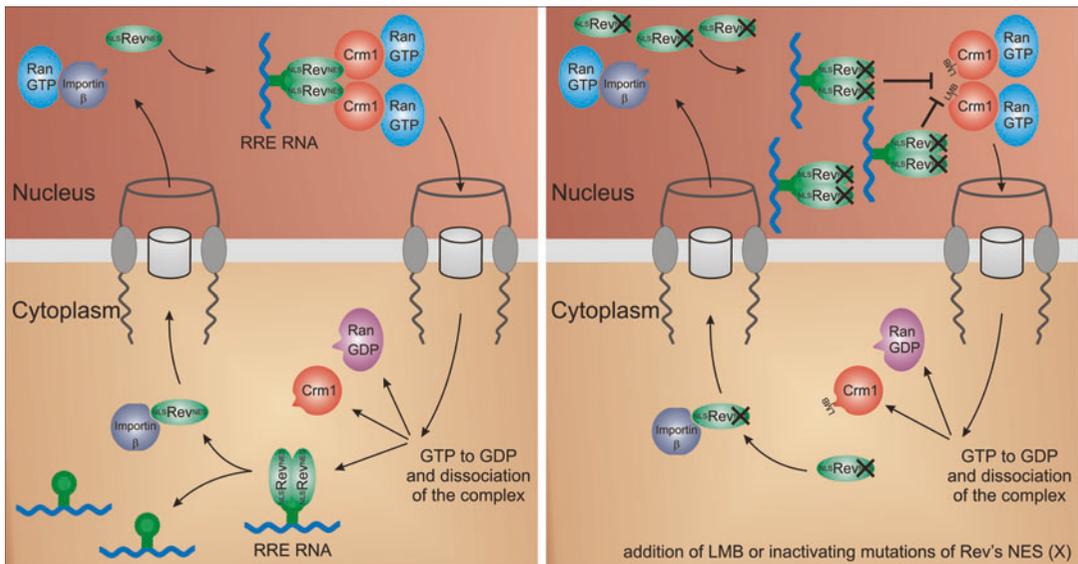
spliced RNAs (Figs. 1 and 2). An RNA stem loop structure is formed by consecutive base pairings of nucleotides on both sides of the stem connected by nucleotides of the same chain forming a loop. Rev especially associates with stem loop IIB of the RRE (Pollard and Malim 1998; Felber et al. 2007; Karn and Stoltzfus 2012) (Fig. 3).

Molecular Mechanism of Rev-Mediated Gene Expression

The nucleus is not freely accessible from the cytoplasm and vice versa, because it is surrounded by the nuclear membrane. Gateways exist in the form of nuclear pores. Regulated nuclear import and export is possible through these huge protein tunnels. However, intron-containing transcripts are not efficiently exported from the nucleus to the cytoplasm in the cell. Therefore, viral transcripts of HIV-1 that contain introns are retained in the nucleus and cannot be translated efficiently in the cytoplasm (for a detailed discussion, see “Additional Functions: Influence of Rev on Translation and Packaging” below) (Fig. 2). This poses a problem for the virus, because translation of these RNAs is

strictly necessary for the virus to complete its replication cycle. This retention mechanism is bypassed by the viral Rev protein. After translation of multiply spliced RNAs in the cytoplasm, Rev binds to the cellular protein importin-beta via its NLS. This leads to the transport of Rev through the nuclear pores into the nucleus (Pollard and Malim 1998; Karn and Stoltzfus 2012) (Fig. 4). Most Rev molecules (one Rev molecule is one Rev protein) are therefore localized in the nucleus and in dense nuclear structures called nucleoli. Recently, it was discovered that besides importin-beta also other nuclear import proteins can transport Rev such as the cellular protein transportin.

In the nucleus, most likely in the nucleoli, Rev associates with the RRE in unspliced and incompletely spliced, viral RNAs via interaction



HIV-1 Rev Expression and Functions, Fig. 4 Rev-mediated nuclear RNA export. *Left:* In the cytoplasm Rev’s NLS interacts with the cellular protein importin-beta leading to directed nuclear import. Association of the cellular protein Ran bound to GTP with importin-beta dissolves the protein complex, and free nuclear Rev is able to interact with the Rev-response element present in intron-containing viral RNAs. After oligomerization of Rev molecules at the RRE, the cellular protein Crm1 interacts with Rev’s NES and mediates nuclear export with the help of Ran-GTP. In the cytoplasm GTP is converted to GDP destroying the RNA-protein complex. Consequently, Rev

and the RRE-containing RNA are exported from the nucleus to the cytoplasm. Rev can reenter this export cycle by binding to importin-beta. *Right:* Mutation of Rev’s NES or inhibition of the association between Crm1 and Rev’s NES by the drug Leptomycin B (*LMB*) prevents Rev-mediated export of RRE-containing RNAs. Rev is imported into the nucleus by importin-beta and associates with the RRE, but a Crm1-mediated export is not possible. Consequently, in this setting Rev and the RRE-containing RNA cannot leave the nucleus. The Rev export cycle is now a nuclear dead-end road

between Rev's RNA-binding domain and the RRE stem loop IIB (Figs. 3 and 4). Subsequently, more Rev proteins assemble onto this complex because of Rev's multimerization domain. The NLS overlaps with the RNA-binding domain and with the multimerization domain (see Fig. 3). After multimerization of approximately 2–8 Rev monomers (one monomer is one single protein) on the RRE, the NLS is therefore believed to be masked by the RRE and by other Rev proteins. In contrast, the NES are accessible and associate with the cellular protein exportin-1 more often called Crm1 (Fig. 4). Analyses of Rev's structure revealed that two monomers interact in a V-shaped dimer (two Rev monomers bound to each other) (Fig. 3). The two disordered NES were proposed to project away from the lower end of this V-dimer enabling association with Crm1 (Daugherty et al. 2010; Dimattia et al. 2010; Hammarskjöld and Rekosh 2011). Since modeling of the RRE together with six single Rev proteins and six disordered NES was reminiscent of a jellyfish equipped with tentacles, this was called to be the jellyfish-like model and Crm1 is believed to bind to these "NES tentacles" (Hammarskjöld and Rekosh 2011; Karn and Stoltzfus 2012). In addition, Crm1 interacts with the cellular protein Ran that binds the small molecule GTP (guanosine triphosphate). Crm1 is the central protein of this protein-RNA complex (RRE-containing viral RNA, Rev, Crm1, and Ran-GTP) allowing the nuclear export through the nuclear pores into the cytoplasm (Fig. 4). In the cytoplasm Ran converts the small molecule GTP to GDP (guanosine diphosphate) thereby destroying the protein-RNA complex (Fig. 4). The components Rev, Crm1, and Ran are reimported into the nucleus. In contrast, the RRE-containing RNA stays in the cytoplasm where translation of unspliced RNA produces the viral proteins Gag and GagPol and translation of the incompletely spliced RNAs produces the viral proteins Env, Vif, Vpr, and Vpu (Pollard and Malim 1998; Felber et al. 2007; Karn and Stoltzfus 2012). The unspliced and spliced but still intron-containing RNAs of HIV-1 are therefore referred to as Rev-dependent transcripts. Gag, GagPol, and Env subsequently form new

viral particles able to infect new target cells (Fig. 1) (► [Virus Assembly](#)). Rev-mediated protein production is furthermore influenced by cellular protein partners interacting directly or indirectly with Rev or the viral RNA (► [“DDX3, Cofactors and RNA Export”](#)). These factors modulate the efficiency of Rev's activities, and their role in HIV-1 RNA transport was recently reviewed in detail (Groom et al. 2009; Williams et al. 2010).

The Rev-mediated nuclear export function is essential to complete the viral replication cycle. Prevention of the association between Rev and RRE (by destroying the *rev* gene and/or the RRE sequence) prevents efficient nuclear RNA export and consequently translation of Rev-dependent transcripts and production of viral proteins needed for the formation of new viral particles. Furthermore, changing the amino acid sequence of important domains like Rev's NLS and NES has the same consequences (Pollard and Malim 1998). For example, inactivating the NES or preventing association between Rev and the cellular nuclear export machinery with the drug Leptomycin B that directly binds to Crm1 sequesters Rev and the RRE-containing, Rev-dependent RNA in the nucleus. Production of Gag, GagPol, and Env, and therefore formation of new viral particles is impaired (Pollard and Malim 1998) (Figs. 2 and 4). Rev and the RRE are therefore essential components of the HIV-1 replication cycle.

Rev's hijacking of the cellular nuclear import and export vehicles in order to export intron-containing, viral RNA to the cytoplasm results in a timely regulated pattern of viral gene expression. The first viral proteins produced in an HIV-1-infected cell are Nef, Tat, and Rev encoded on multiply spliced transcripts. Proteins encoded on incompletely spliced and unspliced Rev-dependent RNAs are translated later in the replication cycle after Rev-mediated nuclear RNA export (see Fig. 2). This chronology is reinforced by the fact that a certain threshold level of the early, regulatory protein Rev is necessary before expression of the late genes from intron-containing RNAs is possible (Pollard and Malim 1998; Karn and Stoltzfus 2012).

Comparison to Other Retroviruses

HIV-1 as a lentivirus belongs to the family of the retroviruses (family of Retroviridae). All other lentiviruses (e.g., HIV-2, SIV, EIAV), some betaretroviruses (e.g., MMTV), and all deltaretroviruses (e.g., HTLV-1, HTLV-2, BLV) encode Rev-like proteins and contain an RRE-like structured RNA element. The overall mechanism of action is conserved between these viruses: Rev-like proteins are imported into the nucleus where they interact with the RRE-like RNA elements followed by a Crm1-dependent nuclear export leading to translation of Gag, (Gag)Pol, and Env. However, the molecular and mechanistic details vary. A prominent difference is the genetic localization of the RRE-like elements. Lentiviruses contain these elements in the *env* gene in the last third of the genome (see Fig. 2). Consequently, the RRE is present in incompletely spliced and unspliced transcripts. Most other retroviruses encode the RRE in the most 3' part of the genome near to or in the so-called 3' long-terminal repeat (LTR) (the "right end" of the genome, see Fig. 2). As a consequence these RRE-like elements are present in all RNA species irrespectively whether they have been spliced or not (Felber et al. 2007).

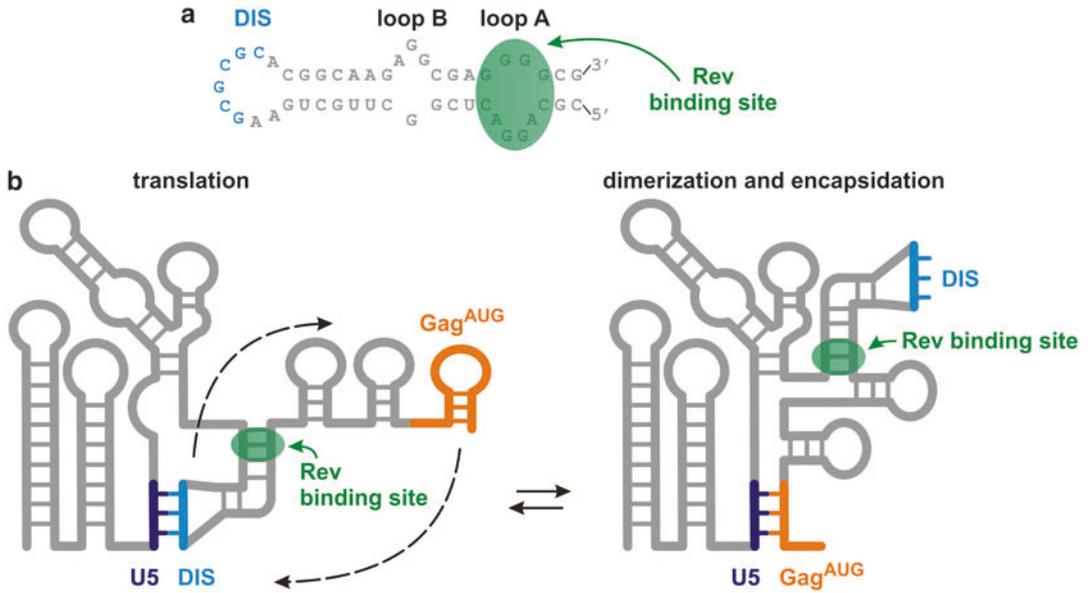
Besides the abovementioned complex retroviruses that encode regulatory (Tat and Rev for HIV) and accessory (Vif, Vpr, Vpu, and Nef for HIV) proteins, simple retroviruses only encode the viral proteins Gag, (Gag)Pol, and Env. Nuclear export of the unspliced RNAs is most often not well understood for these viruses. The best studied examples are those simple retroviruses that contain an RNA element named as constitutive transport element (CTE) like the betaretroviruses MPMV or SRV. The RNA structure of the CTE is directly bound by the cellular protein NXF1 (also called Tap). The heterodimer between the cellular proteins NXF1/NXT (Tap/p15) is known to mediate nuclear export of spliced cellular RNAs. Direct binding of these proteins to the viral unspliced RNA therefore allows export of these transcripts to the cytoplasm and subsequent translation. Similar to complex retroviruses (e.g., HIV-1), these simple retroviruses also hijack a

cellular nuclear export pathway but in this case not Crm1 but NXF1 is used. Interestingly, NXF1 is the main export pathway for cellular RNAs whereas Crm1 is the main export pathway for cellular proteins. Despite this difference the CTE can partially substitute for the Rev/RRE system. Deletion of *rev* and/or RRE can be compensated when a CTE is inserted into the 3'LTR region of the HIV-1 genome. However, these viruses show a reduced replication capacity (Felber et al. 2007).

Additional Functions: Influence of Rev on Translation and Packaging

It is well known that Rev-mediated nuclear export of intron-containing, viral RNAs allows production of the viral proteins Gag/GagPol and Env. When the HIV-1 Gag protein levels are measured, a 100–1,000-fold increased protein amount can be detected in the presence of a functional Rev/RRE nuclear export system. This strong enhancement of protein levels might not only be due to increased nuclear RNA export but could in addition also be based on a direct positive effect of Rev on translation of these RNAs in the cytoplasm. In studies analyzing the second mechanism, a small but significant effect of Rev on translation could be detected. When Rev can bind to the RNA, it increases the efficiency of translation independent of its nuclear export activity by up to 2.5-fold. However, it is not clear whether this effect is mediated by association between Rev and the RRE or between Rev and a second binding site present in the viral 5' untranslated region (5'UTR) (reviewed in Groom et al. 2009; Grewe and Überla 2010a) (see Fig. 5). The 5'UTR is the most "left" or 5' part of the RNA that is not used as template for protein production and is consequently referred to as being "untranslated."

Although a strong enhancement of protein production by Rev can be detected in all experimental settings, a discussion about the amount of intron-containing RNAs present in the cytoplasm in the absence of a functional Rev/RRE system is still ongoing. Whereas in some experiments unspliced and incompletely spliced RNA could not be detected in the cytoplasm at all, other experiments



HIV-1 Rev Expression and Functions, Fig. 5 RNA structure of the HIV genomic RNA 5'UTR. (a) The structure of the dimerization initiation site (DIS) stem loop of the HIV genomic RNA 5' untranslated region (5'UTR) adapted from Groom et al. 2009 (DOI 10.1099/vir.0.007963-0) is shown. The Rev binding site is depicted in green. The structure corresponds to nucleotide sequence 12–58 of GenBank entry M15654.1 (HIV-1, isolate BH10) (<http://www.ncbi.nlm.nih.gov/nuccore/M15654.1>). (b) Schematic representation of the dynamic

structure of the entire 5'UTR adapted from Lu et al. 2011 (DOI: 10.1126/science.1210460). The Rev binding site is depicted in green. It was suggested that depending on the structure of the 5'UTR, either the genomic RNA is translated or it dimerizes and associates with Gag for packaging. U5, nucleotide sequence in the U5 region of the LTR able to pair with the DIS loop; DIS, dimerization initiation site; Gag^{AUG}, stem loop comprising the AUG start codon of the Gag open reading frame

revealed a 3–40-fold reduction of the cytoplasmic RNA levels without Rev/RRE. Data obtained recently in carefully controlled experiments using sensitive detection methods confirmed the latter results. Therefore, Rev has a greater impact on the amount of Gag protein produced (~500-fold enhancement) than on the amount of cytoplasmic RNA (~20-fold enhancement). This discordance demonstrates that Rev's effect on protein amounts cannot solely be explained by its nuclear RNA export activity. Those RNAs that are exported from the nucleus to the cytoplasm by Rev are very efficiently translated resulting in a very strong increase in protein levels. In contrast, those transcripts exported in the absence of Rev are not efficiently translated. Almost no Gag proteins are produced from these Rev-independently exported RNAs (Fig. 2). Since Rev has only a small direct effect on translation in

the cytoplasm (see above), this implies that Rev-mediated nuclear export of Rev-dependent RNAs marks these transcripts for an efficient translation process in the cytoplasm. Correspondingly, it could be shown that Rev-dependent RNAs exported from the nucleus by Rev associate much more efficiently with the cellular translational machinery in the cytoplasm than Rev-dependent RNAs leaving the nucleus in the absence of a functional Rev/RRE system. Therefore, different export routes exist from the nucleus to the cytoplasm with distinct consequences for the exported RNA (Bolinger and Boris-Lawrie 2009; Groom et al. 2009; Grewe and Überla 2010a).

A similar observation was recently made while analyzing the viral encapsidation process in the presence and absence of Rev. Late in the replication cycle, the viral protein Gag forms viral

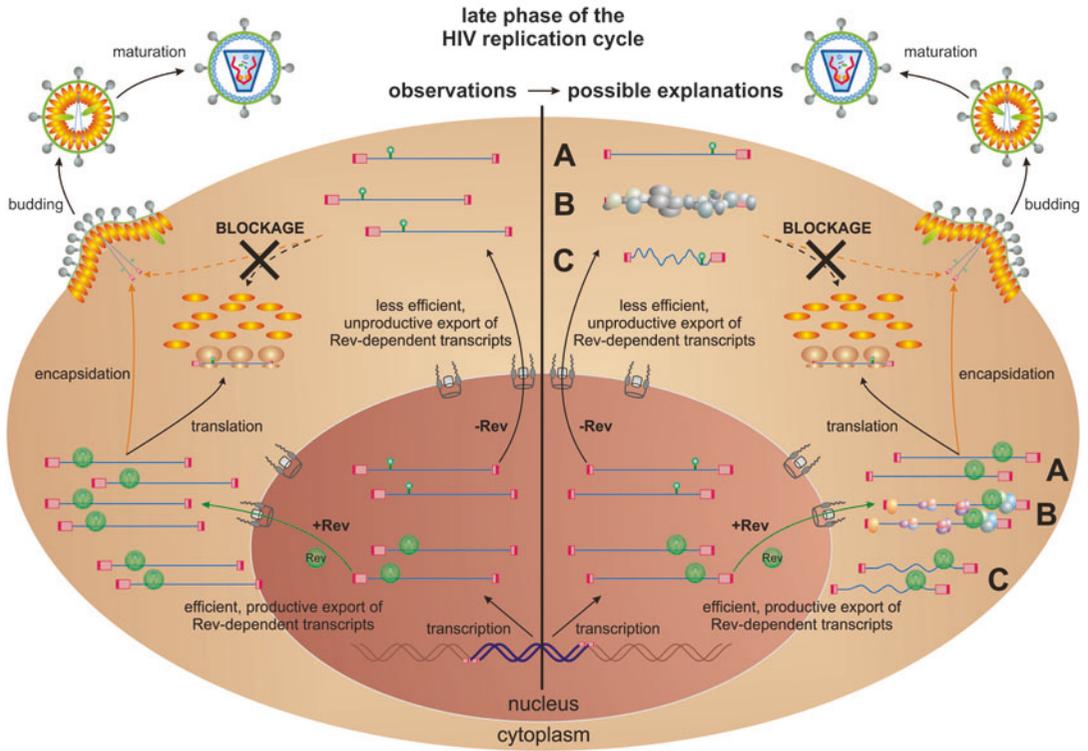
particles at the plasma membrane surrounding the infected target cell (see Fig. 1). Formation of infectious particles requires packaging or encapsidation of the viral construction plan in form of the genomic HIV-1 RNA which must be transferred to newly infected cells to start a new viral replication cycle. Incorporation of the unspliced, genomic RNA is mediated by a cytoplasmic interaction between the viral protein Gag and an RNA structure in the 5'UTR of this RNA referred to as encapsidation signal or Psi (Ψ) (see Figs. 1 and 5). After nuclear export mediated by Rev/RRE, the genomic RNA is very efficiently encapsidated into Gag particles. However, in the absence of Rev, cytoplasmic RNA could still be detected, but albeit high amounts of Gag protein were provided, the packaging efficiency of these RNAs was strongly diminished. Whereas the cytoplasmic amount of unspliced transcript decreased by only sixfold in the absence of Rev, the amount of genomic HIV-1 RNA in viral particles was reduced by more than 1,000-fold. Comparable to the situation observed for the translation efficiency, this shows that intron-containing, HIV RNA exported to the cytoplasm in the absence of Rev is blocked and can neither be utilized efficiently for translation nor encapsidation (Groom et al. 2009; Grewe and Überla 2010a) (Fig. 6).

In the absence of a functional Rev/RRE system, the following three mechanisms could prevent efficient translation and encapsidation of unspliced HIV-1 RNA in the cytoplasm: First, nuclear RNA export could result in an unproductive sub-cytoplasmic localization where translation/encapsidation is not possible (Fig. 6a). The cytoplasm seems to be composed of different sub-cytoplasmic regions comparable to districts in a huge crowded city. It seems possible that in some of these "districts," RNA usage is rather inefficient. Second, an interaction of the RNA with inhibitory cellular proteins (Fig. 6b) and, third, an inhibitory RNA structure (Fig. 6c) might also prevent cytoplasmic usage of the RNA after nuclear export. The RNA structure and inhibitory proteins would impair binding/function of the cellular translation

machinery and would block association of the genomic RNA with the viral protein Gag needed for packaging. A Rev-mediated export would prevent or resolve the negative RNA structure and binding of negative acting proteins. Furthermore, it could direct the exported unspliced RNA to a productive sub-cytoplasmic location where translation and encapsidation is possible (Groom et al. 2009; Grewe and Überla 2010a) (see Fig. 6).

Additional Functions: Nuclear RNA Export Influences the Functions of the Encoded Proteins

Nuclear export of RNA from nucleus to cytoplasm does not only influence the fate of the exported transcripts but can also have far-reaching consequences for the encoded proteins. HIV-1 is unable to replicate in mouse cells. One reason is that in these cells nuclear export of the unspliced HIV-1 RNA mediated by Rev/RRE allows production of high intracellular amounts of Gag protein but these proteins are not able to accumulate at the cellular plasma membrane. The formation of Gag particles is therefore reduced in these cells. Exchanging the Rev/RRE nuclear RNA export system by a CTE (RNA export element of some simple retroviruses – see above) allows production of intracellular Gag and additionally restores formation of Gag particles, because Gag proteins are efficiently targeted to the plasma membrane. Of note, the amino acid sequence of the encoded Gag proteins is identical in both settings. In human cells a similar finding was obtained when nuclear RNA export via Rev/RRE was compared with export mediated by the posttranscriptional regulatory element (PRE). This structured RNA element was discovered in the RNA of hepatitis B virus and promotes RNA stability and nuclear export. The PRE-mediated export allows high intracellular Gag protein levels but decreases the ability of these proteins to oligomerize (bind each other) and bud from the cell (form particles). In this case the Rev/RRE system was superior indicating



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HIV-1 Rev Expression and Functions, Fig. 6 Nuclear genomic RNA export in the presence and absence of Rev. *Left:* Effects observed in the absence and presence of a functional Rev/RRE system. In the presence of Rev nuclear export of Rev-dependent unspliced RNA is efficient and leads to high amounts of Gag/GagPol proteins. In the absence of Rev discordance between on the one hand cytoplasmic RNA levels and on the other hand translation and encapsidation efficiencies could be observed. Whereas cytoplasmic unspliced RNA levels were reduced approximately 20-fold, translation is reduced by a factor of approximately 500. Very similar, encapsidation efficiency is reduced by a factor of approximately 1,000. The dramatic reduction in translation and encapsidation cannot solely be explained by the reduced nuclear RNA export. Therefore, in the absence of Rev, unspliced RNA is less efficiently exported from the nucleus, and its utilization in translation and encapsidation is furthermore blocked in the

cytoplasm. *Right:* Possible explanations for the prevention of cytoplasmic translation and encapsidation in the absence of a functional Rev/RRE system. Reduced cytoplasmic genomic RNA levels in the absence of Rev are consistent with a pivotal role of Rev in nuclear export of viral intron-containing RNAs. However, the reasons for cytoplasmic blockage of translation and encapsidation of RNAs exported from the nucleus without Rev are undefined. Possible explanations are (A) an unproductive sub-cytoplasmic RNA localization, (B) association with inhibitory cellular factors, and/or (C) inhibitory RNA structures. All of these aspects could prevent cytoplasmic RNA utilization. A Rev-mediated export could lead to (A) a productive sub-cytoplasmic RNA localization, (B) removal of inhibitory factors or prevention of their association with the RNA, and/or (C) RNA structures enabling translation and encapsidation (figure modified from Grewe and Überla 2010a)

that this nuclear export strategy is optimized for HIV-1 replication in human cells. Together these findings clearly show that export from the nucleus to the cytoplasm of RNA can influence characteristics and functions of the proteins produced from these transcripts (Swanson and Malim 2006; Karn and Stoltzfus 2012).

Additional Functions: Prevention of Superinfection

Recently, a new inhibitory function of Rev very early in the replication cycle was discovered. Infection of a target cell with HIV-1 leads to the production of Rev. The presence of Rev in the cell

seems to inhibit infection of the same cell with a second HIV-1 viral particle. This phenomenon called prevention of superinfection is also attributed to the viral proteins Nef and Env. Rev specifically inhibits integration of the second viral DNA into the cellular DNA by inhibiting the nuclear import of the reverse transcribed viral DNA, preventing interaction of the HIV-1 integrase protein with the cellular protein LEDGF/p75 important for integrase's activity, and inactivating the integrase protein. These mechanisms are mediated by direct association of Rev with integrase and LEDGF/p75 (Grewe and Überla 2010b).

Open Questions Regarding Nuclear Events: What Defines an RNA as Rev Dependent? How and Where Does Nuclear Binding Between Rev and RNA Exactly Occur?

In the absence of a functional Rev/RRE system, Rev-dependent HIV-1 RNAs are retained in the nucleus, inefficiently exported to the cytoplasm, and partially degraded in the nucleus and cytoplasm. Furthermore, translation and packaging of cytoplasmic RNA is inefficient in the absence of Rev/RRE. In the presence of Rev, RRE-containing RNAs are efficiently exported from the nucleus to the cytoplasm, stabilized in both compartments, translated, and packaged.

As mentioned above Rev-dependent RNAs have to contain an RRE, because Rev has to bind to the RNA for nuclear export. Furthermore, all Rev-dependent HIV-1 RNAs contain introns. An intron is defined by nucleotide sequences at both ends in combination with intron-internal sequences. Particularly the 5' end ("left" end) is known to be important for Rev dependency, because it stabilizes the RNA. In the absence of an intact 5' splice site (splice donor, SD), the transcripts are quickly degraded and Rev is unable to stimulate the production of Gag/GagPol and Env. Inactivation of the regular 5' splice site can also lead to activation of cryptic splice sites destroying the sophisticated viral splicing pattern and ultimately prevents viral replication. It is

important to note that the stabilizing effect of the 5' splice site is independent of splicing itself. However, the efficiency of the splicing process also influences the degree of Rev responsiveness. Rev-dependent RNAs show an intermediate splicing efficiency. This seems to guarantee that association between Rev and the intron-contained RRE is possible before splicing is completed and all introns (including the RRE) are removed. Too strong splicing signals therefore abolish Rev's ability to promote protein production (Felber et al. 2007; Grewe and Überla 2010a).

Rev-dependent RNAs contain an unusually high amount of the nucleotide adenosine (A) and a surprisingly low amount of the nucleotide cytosine (C) and are therefore A rich and C low. Stretches of these A rich/C low sequences were shown to confer Rev dependency on formerly Rev-independent RNAs. These sequence fragments are called instability sequences (INS) or *cis*-acting repressive sequences (CRS). They act in an orientation-dependent manner. When they are added to Rev-independent RNAs in the normal (sense) orientation, protein production from the resulting RNA becomes dependent on a functional Rev/RRE system. Adding the sequences in the reverse (antisense) orientation only induces slight Rev dependency of protein production. Therefore, it was suggested that cellular proteins associate with these A-rich/C-low stretches and induce Rev dependency. Such a sequence-specific binding event can only take place in the normal (sense) orientation. In the current model, cellular proteins binding to INS/CRS in the nucleus shortly after transcription induce nuclear retention as well as RNA instability in nucleus and cytoplasm. Furthermore, these proteins are believed to transport newly generated transcripts away from splicing hot spots to subnuclear locations where Rev can interact with the RNA allowing nuclear export before the intron-containing RNA is completely spliced. However, up to now not much is known about the INS/CRS binding factors and their mechanisms of action. Those proteins identified to bind to INS/CRS so far are involved in multiple steps of cellular gene expression. It is unclear why such proteins may induce retention and degradation of HIV RNA whereas

other RNAs also bound by these proteins are not influenced in this way. Although the INS/CRS binding proteins should introduce Rev dependency, one study dealing with the cellular proteins p54nrb and PSF demonstrated that increasing the amount of PSF in the cell efficiently degraded intron-containing HIV RNAs. Rev could not induce protein production from Rev-dependent transcripts under these conditions. On the one hand, this supports the notion that CRS/INS binding factors induce RNA instability. On the other hand, it is not easy to combine these results with the model postulating a handover of the intron-containing HIV RNAs by CRS/INS binding factors from splicing sites to Rev for subsequent nuclear export. More detailed studies about the mechanisms of CRS/INS and their sequence-specific binding proteins leading to Rev dependency are therefore needed to verify the current model (Pollard and Malim 1998; Felber et al. 2007; Bolinger and Boris-Lawrie 2009; Grewe and Überla 2010a; Karn and Stoltzfus 2012).

As stated above Rev-dependent RNAs are A rich and C low. Artificial enrichment of the nucleotides guanosine (G) and cytidine (C) in these RNAs without changing the encoded protein sequence (which is possible because of the nature of the translation process and the degenerative genetic code) generates Rev-independent RNAs that allow production of proteins in the absence of a Rev/RRE system. This process is called codon optimization. It changes the RNA sequence but the encoded amino acid sequence remains unchanged. The production of Gag/GagPol that normally relies Rev/RRE is possible without Rev-mediated nuclear RNA export after codon optimization of the RNA. This once more underlines that the sequence composition of the HIV RNA is important for Rev dependency (Felber et al. 2007; Bolinger and Boris-Lawrie 2009; Grewe and Überla 2010a).

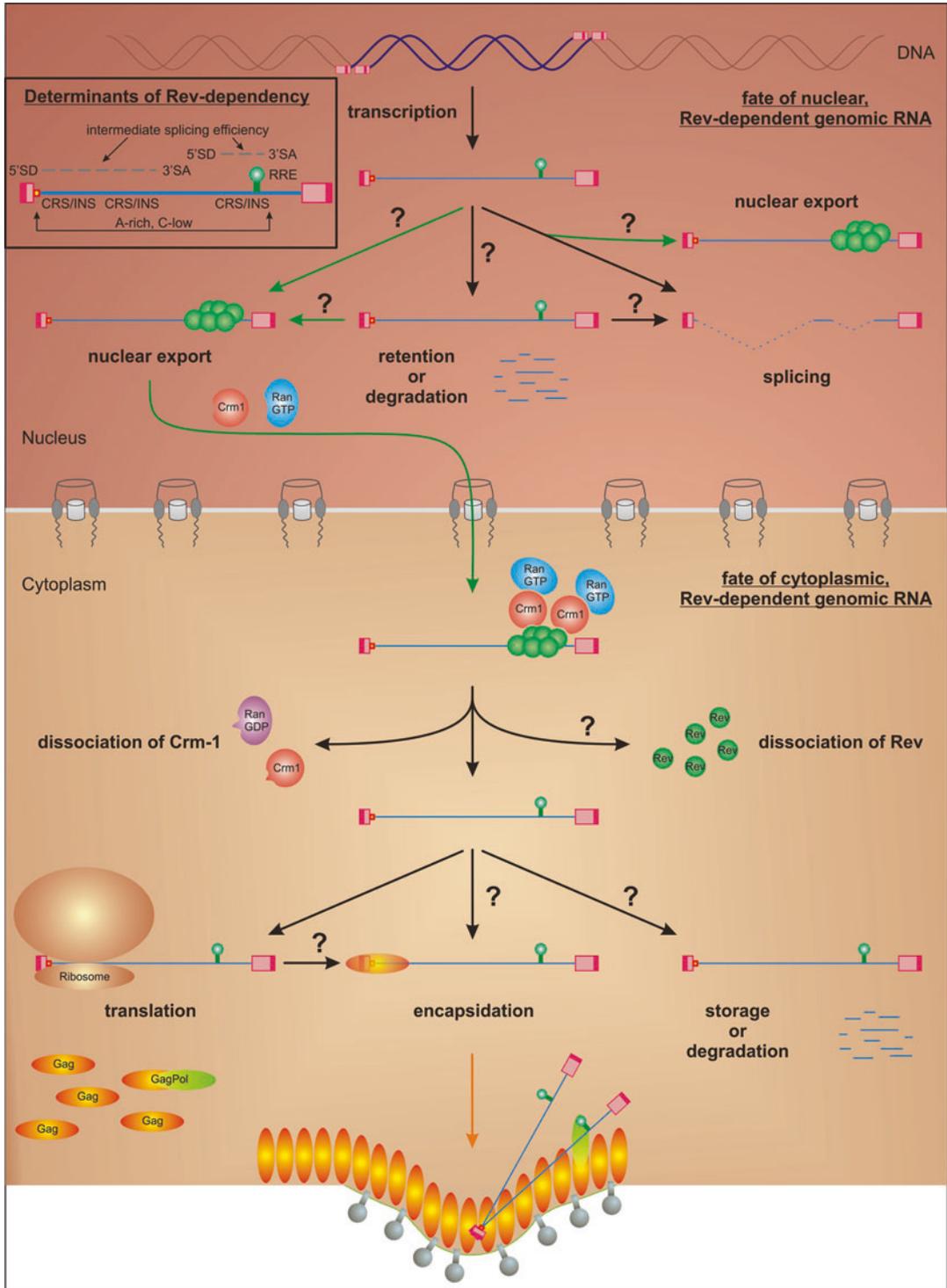
In an attempt to artificially induce Rev dependency, RNA encoding the green fluorescent protein (GFP) was changed from being GC rich to contain a high amount of A. Protein production from the resulting RNA was not possible until a 5' splice site and an RRE were added and the Rev

protein was provided in parallel. This directly proves that induction of Rev dependency is possible by increasing the A-content of an RNA. However, it is unlikely (but not impossible) that artificially increasing the amount of A in an HIV-1 unrelated RNA creates, by chance, a transcript that contains sequence-specific binding sites for cellular proteins comparable to the HIV-1 INS/CRS. These results once more challenge the model of CRS/INS-mediated Rev dependency (Felber et al. 2007; Grewe and Überla 2010a).

Intron-containing RNAs leaving the nucleus in the absence of Rev/RRE cannot be translated and encapsidated. This RNA export pathway is therefore unproductive and prevents further cytoplasmic utilization of the RNA. It was suggested that only nuclear export mediated by Rev and Crm1 is productive but exporting Rev-dependent RNAs via a CTE/Tap export route also allows translation and packaging. Direct association between RNA and Crm1 via Rev or RNA and Tap via CTE could result in a fast and therefore productive nuclear export. The nuclear mark deposited on RNA in the absence of Rev preventing cytoplasmic utilization as well as the detailed nuclear export pathway of these RNAs is not known up to today (Groom et al. 2009; Grewe and Überla 2010a).

Inhibiting the transcription process with the drug actinomycin D stops Rev-mediated nuclear RNA export implying that only freshly transcribed RNA can be exported by Rev. However, recently it was shown that actinomycin D not only stops transcription but also diminishes global Crm1-dependent nuclear protein export and should therefore reduce nucleocytoplasmic shuttling of Rev. Therefore, it is unclear whether newly synthesized or older RNA is a target for Rev-mediated export.

In summary, Rev-dependent protein production requires (1) a functional Rev/RRE system, (2) the presence of a 5' splice site, and (3) a high A-content of the RNA. In the case of an intron-containing RNA, (4) an intermediate splicing efficiency is important, too. However, not all details are known about how and where nuclear binding between Rev and RRE exactly occurs and which cellular factors are involved in this process. The



HIV-1 Rev Expression and Functions, Fig. 7 Multifaceted nuclear and cytoplasmic fate of Rev-dependent genomic HIV RNA. Inset *upper left*: The following

features seem to define RNA as Rev-dependent. Intronic transcripts need at least one splice donor (SD) site to stabilize the RNA. Furthermore, the splicing

reason for nuclear retention and RNA instability in the nucleus and cytoplasm of Rev-dependent RNAs in the absence of a Rev/RRE system is also incompletely understood. Similarly, the inhibitory mechanism preventing translation and packaging after nuclear RNA export in the absence of Rev is undefined. The identification of HIV-1 RNA-binding proteins, dynamic RNA structures, and RNA subcellular localizations is important to clarify these questions in the future (see Fig. 6).

Open Questions Regarding Cytoplasmic Events: What Exactly Happens After Rev-Mediated Export, Translation and/or Packaging?

Subsequent to Rev-mediated nuclear export, the protein-RNA complex composed of RRE-containing RNA/Rev/Crm1/Ran-GTP is dissociated in the cytoplasm. Disruption of the association between Crm1 and Ran is mediated by conversion of GTP to GDP (see Figs. 4 and 7). Dissociation of Rev and the RRE also occurs in the cytoplasm but how this process is regulated is not known. However, the NLS- and the RNA-binding domains in Rev overlap (see Fig. 3). Therefore, it is likely that binding of importin-beta to the NLS in the cytoplasm dissociates Rev from the RRE (see Fig. 4). Rev has a direct, positive but small effect on translation

independent of its nuclear RNA export activity (see above), but details on how Rev increases translational efficiency of RNA are lacking (Groom et al. 2009; Grewe and Überla 2010a).

After nuclear export the genomic, unspliced HIV-1 RNA is either translated or packaged by the Gag protein into viral particles (see Figs. 1 and 7). Translation of the genomic HIV-1 RNA is initiated in a Cap-dependent manner starting at the very 5' ("left") end of the RNA. This should destroy all RNA structures in the 5'UTR that are important for the interaction between Gag and the RNA. Therefore, this mode of translation should prevent subsequent encapsidation. On the other hand, binding of Gag to the 5'UTR of the RNA could prevent binding of the translational machinery and thus targets the RNA for encapsidation. In addition, it has been reported that translation of the genomic RNA is also possible by means of a putative IRES RNA structure in the 5'UTR. IRES-mediated translation might preserve the 5'UTR RNA structures important for packaging. Detailed information about how translation and packaging of the genomic, unspliced RNA of HIV-1 are timely and spatially regulated in the cell is lacking up to now (Fig. 7). Recently, two different, alternative RNA structures of the 5'UTR were proposed either to allow translation or packaging, but how and when these structures are generated is undefined (Fig. 5) (Balvay et al. 2007; Lever

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HIV-1 Rev Expression and Functions, Fig. 7 (continued) efficiency between splice donor and splice acceptor (SA) sites has to be of intermediate strength to allow interaction between Rev and RNA before completion of splicing. Furthermore, the RRE has to be present to allow this interaction. Intron-containing HIV transcripts are A rich/C low and contain CRS/INS. Details about how RNA sequence composition introduces Rev dependency are elusive up to now, but binding of cellular factors is likely to be essential. *Top*: Fate of nuclear genomic HIV RNA. After transcription the unspliced RNA is retained in the nucleus, degraded, exported, or spliced. How retention of intron-containing HIV RNA is mediated is not entirely clear. When after transcription and where exactly in the nucleus/nucleoli Rev interacts with the RRE-containing RNA is not known. Furthermore, it is unclear if Rev binds freshly transcribed RNA or RNA that has been

retained in the nucleus before. In addition, it is not known whether partial assembly of splicing components on the RNA influences Rev-mediated export. *Bottom*: Fate of cytoplasmic genomic HIV RNA. After nuclear export, conversion of GTP to GDP dissociates Crm1 and Ran from the Rev/RNA complex. Since the NLS- and the RNA-binding domains in Rev overlap, binding of importin-beta to Rev's NLS probably leads to dissociation of the Rev oligomers from the RNA. In the cytoplasm the unspliced viral RNA is translated, encapsidated, stored, or degraded. How translation and encapsidation are temporally and spatially regulated is not entirely known. At the end of the replication cycle, new viral particles are formed that contain two viral genomic RNA molecules. The exact details how and where dimerization of two RNAs and binding of Gag occurs have to be defined further

2007; Bolinger and Boris-Lawrie 2009; Lu et al. 2011).

Open Questions Regarding Rev's Action in HIV-Infected Patients: Does Rev/RRE Modulate HIV-1 Replication in Patients?

Substitution of the Rev/RRE system by a CTE in the genome of simian immunodeficiency virus (SIV), a lentivirus closely related to HIV-1, generates a live-attenuated virus in rhesus macaques that cannot efficiently replicate in the infected animals. The virus can be detected in the monkeys but they do not develop the acquired immunodeficiency syndrome AIDS. Although the CTE allows nuclear export of intron-containing SIV RNAs, it does not confer the same replicative capability to the virus as the normal Rev/RRE system. This demonstrates that the exact nature of the nuclear RNA export system can have dramatic effects in an infected animal and perhaps also in HIV-1-infected humans (Felber et al. 2007). HIV-1 sequence changes in infected patients might alter the activity of the Rev/RRE system and might consequently modulate replication capacity, latency, and pathogenesis of the virus.

Conclusions

At the end of the 1980s and the beginning of the 1990s, the key mechanism of Rev-mediated gene expression was discovered. The cellular protein Crm1 was found to export the complex formed between intron-containing HIV-1 RNA and Rev from the nucleus to the cytoplasm allowing HIV-1 late gene expression. This discovery led to the identification of Crm1 as the main nuclear export factor for proteins in human cells. Besides the nuclear export pathway mediated by NXF1/NXT (Tap/p15), it represents one of the two known, regulated routes through the nuclear pores from the nucleus to the cytoplasm. These findings could explain how intron-containing HIV-1 RNAs leave the nucleus for cytoplasmic translation and formation of infectious particles. Rev was therefore

identified as an essential component in the HIV-1 replication cycle. Despite this detailed knowledge about the nuclear export process, a lot of details are not well understood. Most intriguingly is the fact that the exact mechanisms mediating Rev dependency are undefined up to today. The identification of specific protein factors, RNA structures, or subcellular localizations that confer Rev dependency to HIV-1 transcripts will lead to general insights into the regulation of cellular gene expression and will intensify our understanding of HIV-1 replication. Since HIV-1 is the causative agent leading to the acquired immunodeficiency syndrome (AIDS), this detailed knowledge may in future lead to the discovery of new antiviral strategies targeting the essential Rev/RRE axis of HIV-1 gene expression.

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HIV-1 Transmission Blocking Microbicides

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Definition

Current available methods to prevent HIV infection, including condoms, male circumcision, and behavioral interventions, appear insufficient to control the epidemic. This may in part reflect the limited number of options for at-risk groups. Microbicides offer an important alternative

strategy for preventing HIV sexual transmission. An effective microbicide should be a safe, acceptable, and affordable product delivered topically as a gel, capsule, tablet, film, or intravaginal ring at the genital (vaginal, penile) and/or gastrointestinal (colorectal) mucosa to prevent, or at least significantly reduce, sexual transmission of HIV and possibly other sexually transmitted infections.

Introduction

Microbicides were originally conceived as affordable, over-the-counter products, not requiring a prescription, that offered protection against a broad spectrum of sexually transmitted diseases. First-generation candidate products included surfactants to disrupt the viral membrane, acid-buffering gels to inactivate HIV by lowering mucosal pH, and long-chain polyanionic compounds to nonspecifically block viral entry into cells. However, these approaches proved disappointing, showing lack of potency (Ramjee et al. 2010) or lack of safety when surfactant-based products were used several times a day (Hillier et al. 2005). These early setbacks drove the field to explore the potential of a new generation of candidate microbicides based on potent HIV-specific antiretroviral (ARV) drugs that inhibit key steps of the viral replication cycle. Viral replication is initiated by binding of the virion to specific receptors on a target cell triggering viral and cellular membrane fusion and then entry of the viral core into the cytoplasm of the cell. The viral RNA genome is then reverse-transcribed to generate a double-stranded DNA copy of its genome (known as the provirus), which then integrates within the host chromosomal DNA in the nucleus. Transcription of the provirus DNA is then triggered by the binding of host transcription factors generating splice and full-length version of its genomic RNA. The former is translated by the cellular machinery of the host cell making de novo viral proteins. These proteins are transported toward the cellular membrane together with full-length viral RNA for assembly of new virions that bud off from the host membrane as immature viral particles.

These immature virions rapidly mature into fully infectious particles following proteolytic cleavage of the Gag-Pol polyprotein by its own protease enzyme. Current ARV-based microbicides have been selected on the basis of their specific inhibitory activity against these different stages of viral replication: specifically viral attachment and entry, reverse transcription, ► [integration](#), and viral maturation. These approaches directly draw on experience acquired through the development of highly active antiretroviral therapy (HAART) and often use the same or very similar drugs.

Inhibition of Viral Attachment, Entry, and Fusion

Infection is initiated by binding of the viral envelope spikes (Envs) that comprise trimers of two glycoproteins (gp), gp120 and gp41, to the primary cellular receptor CD4 and subsequently one of two coreceptors, CCR5 or CXCR4, on susceptible target cells. This in turn triggers a series of conformational changes in Env that allow apposition and fusion of the viral and host cell membranes. The binding of gp120 to CD4 presents an important potential target to block the process of viral binding and/or fusion with target cells (Herrera and Shattock 2013). Binding of gp120 to CD4 can be inhibited by small proteins that mimic CD4, such as M48-U1, by drugs that block the conformational change in gp120 after binding to CD4, such as BMS-378806 and BMS-599793 (also known as DS003); by anionic dendrimers that provide a charge-based inhibition of gp120-CD4 interaction, such as SPL7013 (referred to as VivGel); and by affecting the level of CD4 expression on the cell surface with either down-modulators, such as cyclotriazadisulfonamide (CADA), or with CD4 aptamer-siRNA chimeras that knockdown CD4 gene expression. Alternative approaches include the use of lectins that recognize mannose moieties in *N*-linked glycans on gp120 and prevent Env binding and conformational change required for entry, these include lectins such as cyanovirin-*N* (CV-N) and griffithsin (GRFT) ++. In addition, the recent discovery of an increasing number of highly potent

broadly neutralizing antibodies (bnAbs) that bind to HIV-1 Env has stimulated programs to develop their potential use as microbicides. Nevertheless, large-scale production for many of these molecules needs to be addressed to ensure affordability when formulated as microbicides.

A second point of intervention during viral entry/► [fusion](#) process is the binding of Env to coreceptors, CCR5 or CXCR4. Epidemiological and genetic studies have shown that >90% of sexually transmitted infections worldwide are due to viruses exclusively using the CCR5 coreceptor (known as R5-tropic HIV) and in few instances dual-tropic (R5X4) tropic isolates able to use both CCR5 and CXCR4 (Keele 2010). The predominant role of R5 virus in HIV-1 transmission has driven the development of specific inhibitors of CCR5 binding as potential microbicides. Compounds in this category included derivatives of the chemokine RANTES, PSC-RANTES, and 5P12-RANTES and small molecule CCR5 inhibitors, such as CMP167 and maraviroc (currently used in HAART). A monoclonal antibody against CCR5, PRO140, that inhibits gp120-CCR5 binding also has potential for preventing transmission. In contrast only one small molecule inhibitor of CXCR4 has been explored for potential microbicide use and could be used in combination with a CCR5 inhibitor to prevent transmission of R5X4-viruses and the small proportion of transmitted viruses that exclusively use CXCR4.

The process of viral fusion presents a third point for interrupting the process of viral entry. Binding of gp120 to a coreceptor triggers a conformational reorganization in gp41 inducing the formation of a six-helix hairpin structure, known as six-helix bundle, that brings together viral and cellular membranes into close enough proximity for membrane mixing, fusion, and subsequent pore formation. The six-helix bundle is formed by folding in an antiparallel manner of the C-terminal region (HR2) and the N-terminal domain (HR1) of each gp41 in the Env trimer. Peptide analogs to gp41 HR1 and HR2 inhibit fusion by binding to HR2 or HR1 domains of gp41, respectively, and hence block the formation of the six-helix bundle. Several peptide fusion inhibitors (FIs) that mimic the HR2 sequence

represent potential candidates for use as microbicides, these including T20 (enfuvirtide/Fuzeon), currently used in HAART, C34, C52L, T1249, and L'644. Here issues relating to stability for use in a mucosal environment, where peptides may be rapidly degraded, and their relative cost for manufacture will need to be overcome to ensure their practical use. Here a cholesterol-derivatized version of C34 that shows increased antiviral potency, longer half-life, and persistent activity in the presence of biological fluids thanks to the cholesterol tag that acts as an anchor for L'644 to the cell membrane may prove advantageous. Broadly neutralizing antibodies that target fusion intermediates of gp41, including 2F5, 4E10, and 10E6, may also have potential use as candidate microbicides especially if formulated together with gp120 bnAbs.

Targets for Inhibition During Reverse Transcription

Following release of the viral capsid into the cellular cytoplasm through fusion pores, decapsidation, and uncoating of the viral RNA, the viral reverse transcriptase (RT) enzyme forms the reverse transcription complex by binding to one of the two copies on the viral genomic RNA and drives the synthesis of the proviral double-stranded DNA. Two strategies can be used to block this step, either by substrate competition or by directly targeting the RT enzyme itself (Shattock and Rosenberg 2012). Substrate competition can be mediated by a family of compounds known as nucleoside and nucleotide RT inhibitors (NRTIs) that mimic the natural deoxy-ribonucleotide triphosphates (dNTPs) required for DNA synthesis. These compounds are nucleosides or nucleotide 5'-monophosphates that, unlike natural dNTPs, lack the 3'-hydroxyl group necessary for the formation of a phosphodiester linkage with the next incoming nucleoside in the growing DNA strand, thus acting as premature chain terminators (Mehellou and De Clercq 2010). NRTIs were the first class of drugs developed for HIV therapy. The NRTI tenofovir (9-[(R)-2-(phosphonomethoxy) propyl] adenine

monohydrate /PMPA) is also the first candidate microbicide to have shown efficacy in a clinical trial (CAPRISA 004) when formulated as a microbicide gel (1% tenofovir). This trial indicated that when 1% tenofovir gel was used in a pericoital fashion (applied before and after intercourse), HIV infection was reduced by 39% after 30 months of follow-up (Abdool Karim et al. 2010). Interestingly, after 12 months, the efficiency was higher with a reduction of 50%, and in high adherers (gel used with >80% compliance to the protocol), it was reduced by 54%. However, no protection was observed in an alternative clinical trial performed by the Microbicide Trial Network (MTN, #10) (MTN-003/VOICE), with daily dosing independent of the timing of intercourse (MTN 2011; NIAID 2011), suggesting use close to the act of intercourse may be essential for protection. Currently a second placebo-controlled trial of pericoital use (FACTS 001), due to finish in 2014, has been initiated to confirm and extend the CAPRISA 004 results (CONRAD 2011). Tenofovir gel has also been tested for safety and acceptability as a rectal microbicide (Anton et al. 2012). Additional NRTI are being considered for combination with tenofovir, the most advanced being emtricitabine (FTC), already co-formulated for oral use as Truvada and now in development as a combination gel with tenofovir (Truvada gel) to increase potential efficacy.

Non-nucleoside RT inhibitors (NNRTIs) block reverse transcription by binding directly to the enzyme and inducing conformational changes of the active site of RT. This class of compounds includes structurally diverse inhibitors such as dapivirine (TMC120), UC781, MIV-150, MIV-160, MIV-170, MC1220, and IQP-0528. Dapivirine has shown high activity in preclinical and animal studies and has, therefore, progressed in the microbicide pipeline being formulated as a gel and in an intravaginal ring (IVR). These formulations are currently being tested in clinical trials (Herrera and Shattock 2013). IQP-0528 is one of the newly discovered NNRTIs and in contrast to most NNRTIs is active not only against HIV-1 but also against HIV-2. Importantly, the NNRTIs being developed as candidate microbicides are currently not used in HAART.

Targeting Viral Integration

Once proviral DNA is synthesized in the cytoplasm, the enzyme HIV-1 integrase (IN) catalyzes the processing, transfer, and integration of the proviral DNA into the cellular DNA in the cellular nucleus. Until recently, few IN inhibitors (INIs) have been developed as candidate microbicides; these include raltegravir (MK-0518) and elvitegravir, both of them currently used in HAART; and L-870,812 (Herrera and Shattock 2013). L-870,812 has already been tested in nonhuman primates showing activity in a pilot study.

Inhibition of Viral Maturation

Following integration, viral DNA is transcribed into messenger RNA, which is then translated into HIV proteins. These proteins together with two copies of HIV RNA genome assembled at the cell membrane, and new virions bud out of the infected cell in the form of immature viral particles that require posttranslational processing of precursor viral polyproteins (Pr55^{Gag} and Pr160^{GagPol}) for the budded virions to be infectious. The enzyme responsible for this processing is HIV protease (PR) and this enzyme presents another target for inhibition. Protease inhibitors were originally developed for HIV-1 therapy and remain an important class of drugs for treatment with ten PIs currently approved for clinical oral use. As virion maturation occurs late in the viral life cycle, after viral entry and integration, they would most likely be used in combination with other ARV acting on earlier stages in the viral life cycle. Here PR inhibitors would act as a second line of defense limiting the release of infectious virus not blocked by early acting ARV drugs in any combination. Currently, at least four PR inhibitors are being assessed in preclinical studies as potential candidate microbicides including saquinavir (SQV), darunavir (DRV), lopinavir (LPV), and ritonavir (RTV) (Herrera and Shattock 2012). Proof of concept that PR inhibitors can prevent infection in nonhuman primate models of transmission is likely needed to stimulate

further clinical development as viable microbicide candidates.

Candidate Microbicides with Multiple Mechanisms of Action

Interestingly, some compounds have been shown to inhibit more than one step in the viral replication cycle. These offer potential advantage through increasing the potential breadth and potency. Several NNRTIs such as IQP-0528, while designed to primarily inhibit reverse transcription, also block viral entry. ARV combinations have also been molecularly designed by generation of chimeric molecules containing, for example, an entry and a fusion inhibitor (Zhao et al. 2011). Other candidates with multiple mechanisms include the family of nucleocapsid inhibitors or zinc finger inhibitors (ZFIs) that primarily target the nucleocapsid protein (NCp7). NCp7 is a small protein with essential functions throughout the replication cycle. It is important for reverse transcription and integration and is required for dimerization and packaging of the viral genome late in the viral replication cycle. Its function is critically regulated by the binding of zinc to its two zinc-binding domains. ZFIs block this process and as a consequence block multiple stages in the viral life cycle. This class of compounds includes *S*-acyl-2-mercaptobenzamide thioesters (SAMT), four of which are being considered as candidate microbicides: SAMT-8, SAMT-19, SAMT-89, and SAMT-247 (Herrera and Shattock 2013).

A more conventional approach to targeting multiple stages within the viral replication cycle is the use of different ARV drugs in combination. This builds on the successful use of ARV combinations for HAART. Here combinations are likely to be used to increase the potency of individual compounds showing efficacy in clinical trials. Thus, a number of combination gels based on tenofovir are in early clinical development including the NRTU FTC and the CCR5 inhibitor maraviroc. Alternative combinations based on the vaginal ring platform (described below) have also been designed and at least one tested in a Phase I clinical trial (MTN-013/IPM 026).

Activity and Formulation of Microbicides for Mucosal Environments

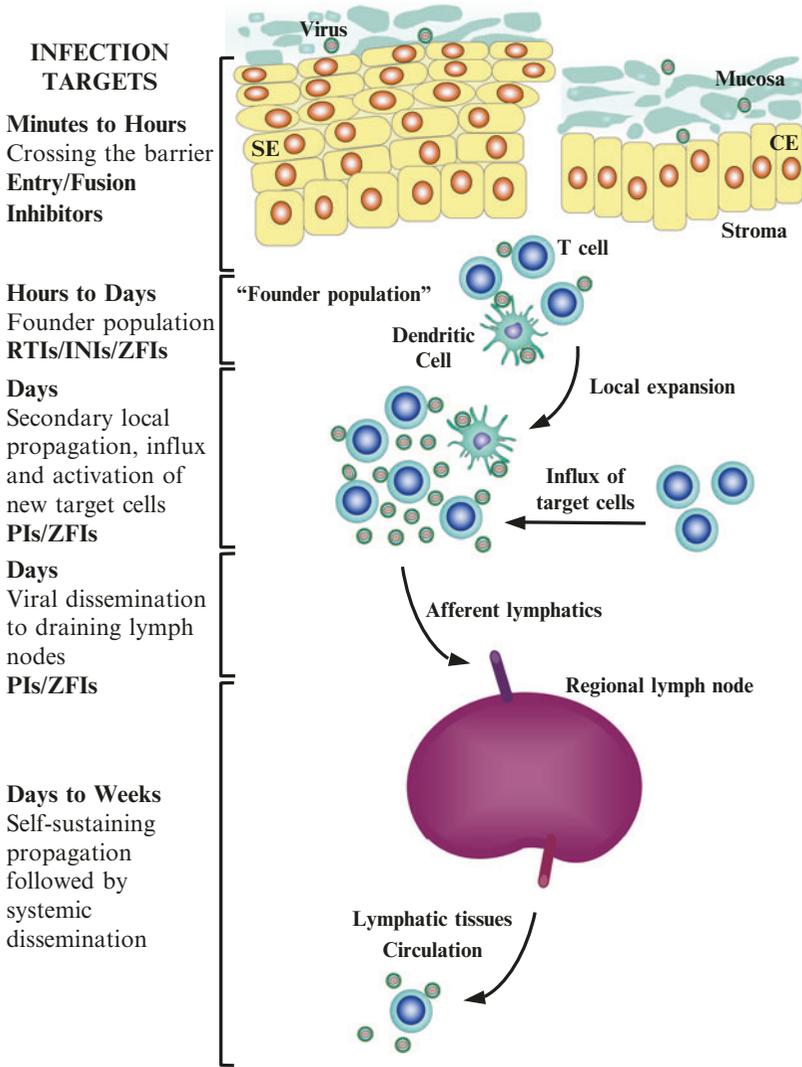
Microbicides are topical agents, and therefore, their mechanism of action inhibiting specific steps of the viral replication cycle needs to be considered within the context of mucosal transmission in the genital and colorectal tracts. Studies in animal models indicate that infection at the mucosal portal of entry is established relatively quickly after exposure to HIV. Indeed, transmission of simian immunodeficiency virus (SIV) in nonhuman primates has been shown to involve the virus crossing the mucosal epithelium in minutes to hours and establishment of an initial focus of infection or “founder population” in the underlying stroma which is originated by a single or a few viruses in the first hours after viral exposure (Li et al. 2009) (Fig. 1). Hence, entry/fusion inhibitors have a time frame of minutes/hours to act before the founder population is established; and this means that a topical inhibitory concentration needs to be present and maintained during this period of time. If production of infectious virus is not inhibited, local expansion will occur in the first few days following exposure (1–3 days), attracting new target cells to expand the initial focus of infection. Dissemination of infection continues with transport of infected cells to secondary lymphoid tissue via draining regional lymph nodes. Infected cells and newly produced virus are then disseminated throughout the systemic circulation (Shattock and Rosenberg 2012) (Fig. 1). Drugs acting post-viral entry will target local amplification and expansion. Specifically, RTIs, ZFIs, and INIs will block viral amplification within the founder population as they act before integration, while PIs and ZFIs, acting post-integration, will inhibit the local expansion phase that is reliant on secondary infection of surrounding CD4 cells and is probably enhanced by interaction with Langerhans cells and dendritic cells (DC) and influx of additional CD4⁺T cells (Shattock and Rosenberg 2012). One potential benefit of compounds acting later in the viral replication cycle, such as INIs and PIs, is that they might allow for postcoital dosing in addition to precoital application and in combination with

pre-integration inhibitors which might provide a much greater window of protection.

Ultimately the efficacy of any microbicide product will depend upon correct product use (adherence) as well as appropriate drug delivery. This is clearly illustrated by the CAPRISA 004 trial of tenofovir gel where volunteers were requested to use the drug in a pericoital fashion, “before and after sex.” Here efficacy of the gel was significantly affected by adherence to the regimen (Abdool Karim et al. 2010). In contrast, daily or coitally dependent dosing with the same gel provided no protection (Herrera and Shattock 2013), suggesting either poor adherence and/or insufficient drug delivery at the time of viral exposure. A range of additional delivery platforms is currently under development to increase choice and improve ease of use including tablets, films, and intravaginal rings (IVRs). The later approach (IVRs) is already used to deliver hormone replacement therapy and hormonal contraception. Microbicide IVRs are based on drug-impregnated rings that release hydrophobic ARVs for prolonged periods (up to 3 months), maintaining concentration sufficient to prevent HIV transmission. The most advanced IVR product contains the NNRTI dapivirine and is currently being assessed in two efficacy trials (MTN-020 Aspire and IMP027). Building on this platform combination, IVRs designed to deliver both hormonal contraception and ARV protection are in early clinical development.

ARV-Based Microbicides and Resistance

Many of the drugs (or drug classes) currently in the microbicide pipeline are already used in HAART regimens. This raises theoretical concerns that widespread use of ARVs as microbicides could induce resistance that would significantly reduced the efficacy of treatment or treatment options for those that became infected. This scenario would involve an individual using ARV-based microbicide even after becoming infected, and therefore, active replication could select for a resistant mutant to the drug contained in the microbicide. However, it is unclear whether



HIV-1 Transmission Blocking Microbicides, Fig. 1 Time line for HIV-1 inhibition of mucosal transmission. Viral infection in the cervicovaginal and colorectal tracts requires the virus present in the lumen to cross the epithelium reaching the stroma where a founder population is established within a few hours post-viral exposure. Entry

and fusion inhibitors prevent formation of initial foci of infection. RTIs, INIs, and ZFIs inhibit viral amplification within the founder population during the first hours/days postexposure, and PIs and ZFIs are candidates to inhibit local expansion and viral dissemination to draining lymph nodes (Adapted from Herrera and Shattock 2013)

topical ARV application, that does not involve high systemic levels of the drug, has the potential to induce resistance. Of importance, no evidence of resistance to tenofovir was seen in participants in CAPRISA 004 that became infected during the trial (Abdool Karim et al. 2010). Nevertheless, more data is needed to fully understand the potential of ARV microbicide use on the circulation of

resistant strains. Thus, for the foreseeable future, ARV-based microbicides will only be made available on prescription to individuals shown to be seronegative and with regular follow-up monitoring. Moving forward, appropriate design of drug combinations is likely to reduce or prevent transmission isolates resistant to any individual ARV class.

Microbicides in Context with Other Prevention Strategies

Microbicides were never intended to replace existing strategies known to impact on transmission rates, behavior change, condom use, and circumcision, but to supplement these approaches with an emphasis on increased availability and choice of prevention options for at-risk populations. Furthermore, microbicides represent one of a number of new biomedical prevention strategies that include oral preexposure prophylaxis (PrEP), treatment for prevention, and vaccines. Effective reduction in HIV incidence will likely depend on effective implementation of this growing number of prevention options, where combined approaches may have the biggest impact (Shattock et al. 2011).

Conclusion

The groundbreaking proof of concept that ARVs can provide safe topical protection against HIV sexual transmission, obtained with the CAPRISA 004 trial testing 1% tenofovir gel, was key for the field of microbicides driving further research to achieve higher levels of protection. The apparently contradictory results of the VOICE trial, testing daily use of tenofovir gel, underlined the clear importance of developing dosing strategies that promote product adherence. This is driving the development of new formulations and delivery strategies designed to increase user acceptability and adherence. In this respect, efficacy studies of the dapivirine intravaginal ring, designed to deliver sustained drug dosing, are likely to be key. Furthermore, better understanding of the mechanism of mucosal viral transmission and the impact of the mucosal environment on transmission will be critical to inform drug prioritization. It is anticipated that the use of ARV with increased potency, the development of ARV combinations that target different stages in the viral life cycle, and their delivery from sustained release platforms will deliver products with increasing efficacy.

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HIV-1 Transmission: Influence of Bodily Secretions

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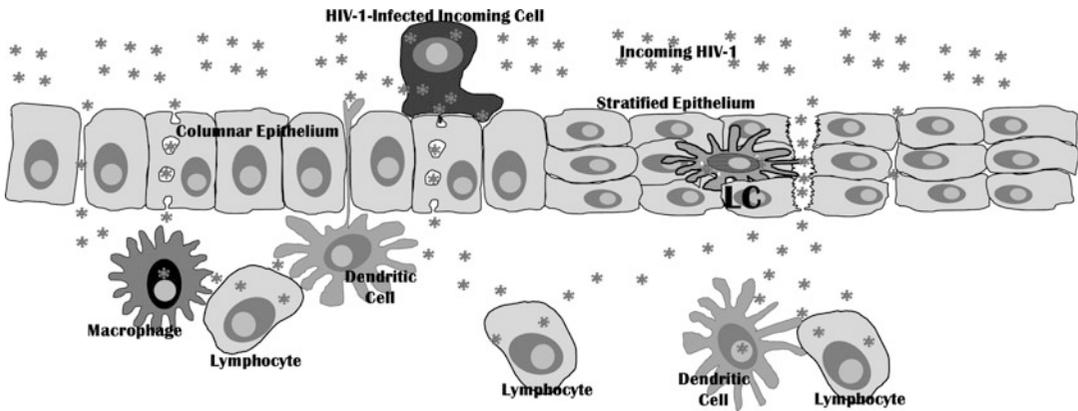
Definition

HIV-1 transmission via sexual intercourse or mother to child transmission (MTCT) requires the passage of virus across a mucosal barrier. The exceptions are exposure via direct injection of virus into the bloodstream through either the administration of contaminated blood or blood products or intravenous drug use (IVDU) with needle sharing. In all transmission cases, a distinctive group of viruses are transmitted and which exclusively utilize, in conjunction with CD4, the CCR5 chemokine receptor for entry (R5 viruses). In some individuals, the virus will evolve to utilize the CXCR4 chemokine receptor (R5/X4 and X4 variants) during disease course and where the switch has been associated with increased viral loads, accelerated CD4 declines, and faster rates of disease progression. Understanding what restricts the preferential transmission of R5 viruses and which host factors contribute to the R5 to X4 switch is poorly understood and remains one of the major questions to be answered concerning HIV-1 pathogenesis. Deciphering such mechanisms will undoubtedly aid in the development of the necessary tools required to prevent viral transmission and/or limit disease progression. The cell types HIV-1 interacts with or infects at mucosal surfaces will contribute to which virus variants establish infection. HIV-1 transmission across the numerous mucosal surfaces will be influenced by the array of host factors to be found within the bodily secretions to be found at these sites of exposure. This entry describes some of the factors to be found within these secretions and which have the potential to modulate HIV-1 infection as well as viral replication.

Cell Types Involved in HIV-1 Mucosal Transmission

HIV-1 transmission will be dependent on the cell types with which the virus interacts at the mucosal surface and will be greatly influenced by factors such as tissue morphology and cellular composition. Concerning sexual transmission, Langerhans cells (LCs) reside in stratified squamous epithelial of the vagina, ectocervix, glans penis, and outer foreskin and will represent the first cells to encounter HIV-1, unless the virus enters via tears in the mucosal wall. Underneath this layer, the dendritic cells (DCs) reside in the lamina propria and compose an additional cell type the virus can interact with. In more sensitive mucosal linings composed of a single columnar epithelium, as found in the rectal, endocervical, and inner foreskin, the LCs are absent but an abundance of DCs are to be found. Tissue macrophages also reside in mucosal tissues and can be one of the first cell types to be infected with HIV-1. There is still much debate as to whether the first cell types of infection are indeed one of these diverse lineages or whether CD4⁺ lymphocytes are the main targets of infection at time of transmission. What is striking is that CD4⁺ lymphocytes at mucosal surfaces can express high levels of the CXCR4 coreceptor, indicating that receptor expression patterns on lymphocytes at sites of infection are not determining selective R5 transmission over the R5/X4 or X4 variants. The complexity of cell types associated with HIV-1 transmission has been highlighted in Fig. 1.

A multitude of receptors expressed on the array of cell types described above can interact with the HIV-1 gp120 envelope molecule, including toll-like receptors (TLRs), C-type lectin receptors (such as DC-SIGN, MMR, BDCA-2, DCIR, DLEC, CLEC-1), as well as the asialoglycoprotein receptor which recognizes the polysaccharide patterns on pathogens and often characterized by their terminal mannose molecules which are uncommon on the surface of mammalian cells (reviewed Pollakis and Paxton 2012). LCs express langerin at the cell surface, a molecule which can interact with gp120; however, it has been shown that once bound, the



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Fig. 1 Depicts two types of mucosal tissue, *left* columnar epithelium as found in the anal mucosa, endocervical region, inner foreskin, and glands corona mucosa and *right* stratified epithelium mucosa as found in the vaginal interstitial region, ectocervical region, glans penis, outer

foreskin, and tonsil. The cell types residing within or below the different layers are shown. On the outer side and where HIV-1 encounters the mucosal barrier an array of bodily secretions can be present, including cervical, vaginal, seminal, and colorectal as well as saliva or breast milk

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virus is internalized and likely degraded within the Birbeck granules of the cell (de Witte et al. 2007). This can be envisaged as a protection pathway by the host where pathogens crossing the mucosa will be successfully captured by LCs and efficiently eliminated. Similarly other molecules expressed on LCs can capture HIV-1, such as galectin-3 (a β -galactoside-binding lectin) which can modulate HIV-1 capture and transmission. LCs possess a restricted susceptibility to infection due to the lack of CXCR4 expression at the cell surface, one explanation for the preferential transmission of R5 variants. On the other hand, DCs can capture HIV-1 via binding of gp120 to an array of C-type lectins, such as DC-SIGN, and efficiently transfer the virus to CD4⁺ lymphocytes, a mechanism known as *trans*-infection (Geijtenbeek et al. 2000). The model proposed is that HIV-1 can be captured by DCs at mucosal surfaces, either following transcytosis across the mucosal barrier or through tears and breaks in the mucosal lining and subsequently transferred to CD4⁺ lymphocytes residing below the mucosal epithelium or in localized lymph nodes. DC-SIGN can potently bind HIV-1 and cell types expressing this molecule have been shown to heighten HIV-1 infection and replication in other cell types. In addition to *trans*-infection, HIV-1 can infect DCs via the classical infection

pathway through binding CD4 and coreceptor; however, binding of virus to such molecules as DC-SIGN may facilitate enhancement to *cis*-infection. DCs have been shown to successfully capture R5, R5/X4, and X4 viruses and pass virus to CD4⁺ lymphocytes, indicating that this is not the selection pressure favoring preferential R5 virus transmission.

Macrophages are infected with HIV-1 through the classic infection pathway, and such cells derived from the genital mucosa have been shown to support HIV-1 infection and replication. In addition, peripheral monocytes have been shown to be infected with HIV-1 during acute infection (Centlivre et al. 2011). These cells can be infected with HIV-1 at mucosal surfaces and subsequently migrate to adjacent lymph nodes where they can disseminate the virus and establish infection, similar to DCs. In rhesus macaque models of SIV infection, it has been established that foci of infection can be found in plasmacytoid DCs (pDC) within the genital mucosa, indicating this to be a cell type involved early in infection. In addition to being infected, such stimulated cells create an inflammatory response where CD4⁺ lymphocytes are subsequently attracted to the infected site via upregulation of chemoattractants and which will be highly infectious for HIV-1. Various cell types defining the innate immune

system can therefore play a role in influencing HIV-1 transmission as well as subsequent disease progression. These cells cannot only capture HIV-1 and transfer virus to CD4⁺ lymphocytes, they also modulate immune responses at mucosal surfaces and therefore can influence immune mediated clearance of infection or protect against HIV-1 transmission in subsequent exposures.

HIV-1 mother-to-child transmission (MTCT) can occur via a number of routes, either in utero, intrapartum, or during the breastfeeding period and each at approximately equal frequencies. For each route of exposure, the virus has to cross a highly divergent mucosal barrier making each exposure route unique with variant transmission mechanisms at play. When considering in utero transmission, HIV-1 has to cross the placental trophoblast, which is different from the other routes of exposure, where virus will likely be ingested by the child and where HIV-1 has to cross the mucosal barrier of either the oral cavity or the gastrointestinal tract. The differing modes of MTCT transmission are also highly variable when considering the carrier fluids in which the virus will be present and the mucosal environment where transmission will occur, e.g., amniotic fluid, birth canal secretions, or breast milk. When considering transmission via breastfeeding, there are indications that functionally active CD4⁺ lymphocytes are taken up by the infant from the milk during the first days of life which could result in the transfer of HIV-1 infected CD4⁺ lymphocytes. Another possibility is the infection of infant cells at the site of exposure. Oral epithelial cells are susceptible to infection by either cell-free or cell-associated HIV-1 in vitro as well as tonsillar stromal cells and human oral keratinocytes, which have been shown to transfer HIV-1 to lymphocytes in vitro. Since gastric acid production in newborn infants is reduced, intact virus may reach the intestinal epithelial cells which can be infected by HIV-1. Similar to other mucosal transmission barriers, DCs below the epithelia may also have a role to play in viral capture and transfer, especially when coinfections or breaks in the mucosal lining are present. After the application of SIV to the tonsils of macaques, DCs were found to be positive for virus even though the virus was

primarily present in CD4⁺ lymphocytes. Tonsillar DCs have also been found to be positive for HIV-1 after in vitro exposure and have been shown to support infection of T cell lines *in trans*.

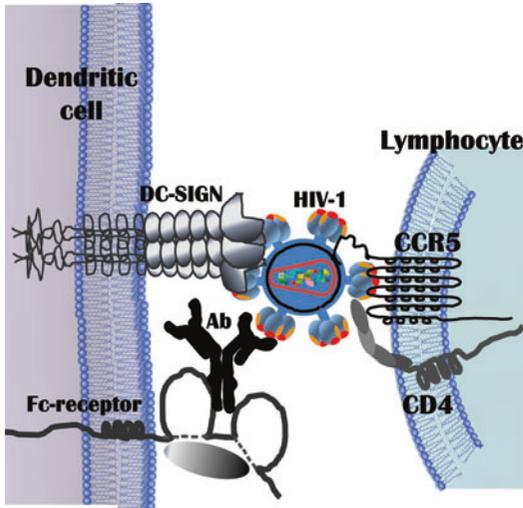
Influence of Antibodies at Mucosal Surfaces on HIV-1 Transmission

The current understanding is that antibodies present at mucosal sites will provide a level of protection against HIV-1 transmission. This has been determined by many monkey studies where animals can be protected by the application of antibodies to the mucosal surfaces or injected into the periphery and then challenging the animals with autologous or heterologous viral stocks at the mucosa. Whether this protection is achieved through virus neutralization or via other mechanism such as antibody-dependent cellular cytotoxicity or complement fixation is still very much in debate. The vaccine field has been spurred by the RV144 Thai vaccine trial where a modest 31% protection was observed in vaccine recipients and where subsequent analysis points to non-neutralizing HIV-1 antibodies responses directed against the V2 region of the gp120 molecule being the protective correlate. Confirmatory vaccine trials testing this hypothesis are currently under development and should indicate indeed whether such responses will be beneficial in curtailing HIV-1 infection. As way of caution, it should be noted that viruses coated with neutralizing antibodies can be more efficiently captured by cells expressing DC-SIGN and that the captured virus can be rendered subsequently infectious for CD4⁺ lymphocytes and as depicted in Fig. 2 (van Montfort et al. 2007). This would indicate that antibodies have the potential to heighten both HIV-1 replication in vivo and HIV-1 transmission. Further evidence for the involvement of DC-SIGN in supporting HIV-1 transmission stems from the findings that genetic polymorphisms within the gene coding for the C-type lectin have been associated with risk of infection, either through sexual intercourse or via MTCT.

A recent study has indicated that autologous neutralizing antibody responses are higher in

mothers and infants where HIV-1 transmission occurred and in comparison to matched non-transmitting controls (Baan et al. 2013). The effect

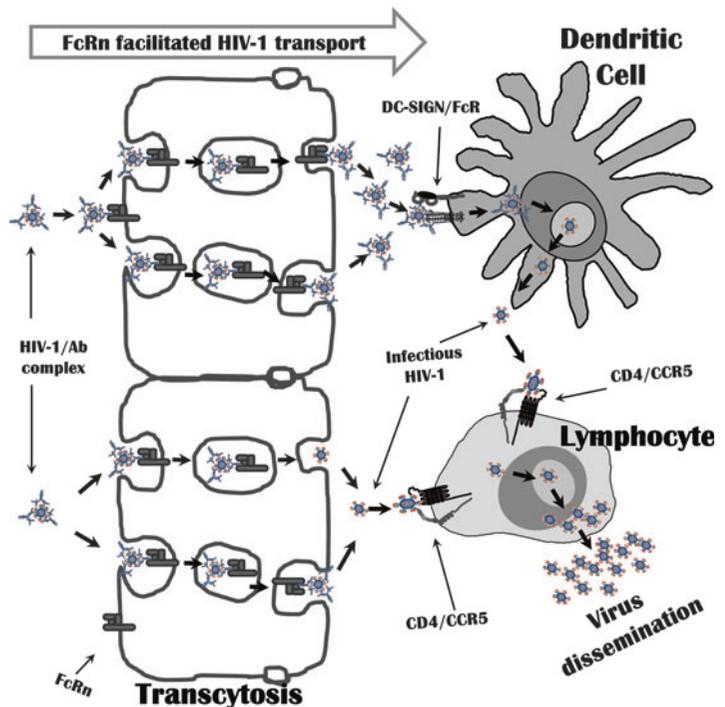
was only and seen for mother-infant pairs where in utero transmission had occurred and was not observed for either intrapartum or breastfeeding transmissions. An additional study has demonstrated that HIV-1 neutralizing antibodies have the capacity to enhance virus transcytosis across an epithelial barrier through interacting with the FcRn receptor, such as would be expected to be present within the trophoblastic layer within the placenta (Gupta et al. 2013). Furthermore, it has been shown that higher antibody levels in monkeys vaccinated against SIV associate with the number of transmitter/founder viruses to be found in the non-protected animals after rectal challenge (Gupta et al. 2014). The authors demonstrate that the sera from such monkeys can enhance viral transcytosis across a rectal model systems and where the FcRn molecule was shown to be expressed in the macaque rectal columnar tissue. These results collectively indicate a novel mechanism whereby immune complexes binding to FcRn may result in antibody-dependent enhancement to HIV-1 transfer across a mucosal barrier and as illustrated in Fig. 3. Vaccine strategies aimed at curtailing HIV-1 transmission may



HIV-1 Transmission: Influence of Bodily Secretions, Fig. 2 Schematic representation of HIV-1 interacting with DC-SIGN and presented to CD4+ lymphocytes. HIV-1 in the presence of gp120 binding antibodies can be captured by Fc receptors on DCs and provide for an enhanced capture and subsequent infection of CD4+ lymphocytes

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Fig. 3 Schematic representation of HIV-1 undergoing transcytosis across a mucosal or placental barrier and interacting with either DCs or CD4+ lymphocytes and where antibody-coated virus (neutralizing or non-neutralizing) can be captured by FcRn resulting in antibody-dependent enhancement to infection



therefore have to take into consideration which types of antibody responses are induced and indeed ascertain whether these responses can in fact be detrimental when considering HIV-1 transmission.

Breast Milk and Its Effect on HIV-1 Transmission

Human milk contains a variety of cell types including: CD4⁺ lymphocytes, CD8⁺ lymphocytes, macrophages, monocytes, DC's natural killer cells, as well as mammary epithelial cells, all of which can be either infected with HIV-1 or possess anti-HIV-1 activity and therefore contribute toward viral transmission. Mammary epithelial cells are among one of the most abundant cell types to be found in milk, and these cells are highly susceptible to infection with X4 viruses. The pool of cells therefore contributing to viral transmission is large and with the breastfed infants being exposed to both R5- and X4-using variants. Again the selective transmission of R5 viruses is surprising given the exposure to both variant phenotypes and the preferential expansion of CXCR4 expressing CD4⁺ lymphocytes in newborn infants. A number of molecules, in addition to CC chemokines, can be found in human milk and which possess anti-HIV-1 activity. Lactoferrin is one such molecule and which has been reported to block direct infection of CD4⁺ lymphocytes, but not to a particularly high degree. Furthermore, lactoferrin has been shown to inhibit the attachment of *E. coli* to intestinal cells and also exhibit broader antiviral activity against CMV, HSV-1, as well as fungi such as *C. albicans*. Lysozyme has also been shown to inhibit growth of HIV-1 on CD4⁺ lymphocytes as well as mediate the killing of both Gram-positive and Gram-negative bacteria. An array of cytokines and chemokines are expressed in human milk and all of which have the potential to activate cells at the mucosal surface as well as interfere with viral infectivity. Such cytokines expressed in milk are IL-1 β , IL-6, IL-8, IL-10, TNF- α , TGF- β , as well as the array of CC chemokines with the capacity to bind CCR5 and block R5 virus infectivity.

Aside to blocking CD4⁺ lymphocyte direct infection, human milk has been shown to inhibit DC-SIGN-mediated capture and transfer of HIV-1 to activated CD4⁺ cells (Naarding et al. 2005). In culture experiments, it was demonstrated that both R5 and X4 viruses are inhibited by high dilutions of milk and from binding studies, it was revealed that the factor in human milk interacts with DC-SIGN rather than HIV-1. The blocking effect was also shown to be mediated by immature as well as mature DCs, and the mechanism was at the level of the compound binding to the cells in a DC-SIGN-specific manner. Biochemical analysis revealed the responsible molecule(s) as large with the inhibitory effect being alleviated with antibodies that bound to Le^X sugar moieties, indicating that a glycoprotein was responsible. It was also shown that mothers possessed a large degree of variation through which their milk could block HIV-1 capture and transfer by DC-SIGN. Through protein purification and MALDI-TOF analysis, bile salt-stimulated lipase (BSSL) was identified as a molecule with DC-SIGN binding and HIV-1 inhibitory activity (Naarding et al. 2006). BSSL, otherwise known as carboxyl ester lipase, is a digestive enzyme secreted from exocrine pancreas and has broad substrate specificity. The molecule contributes to the hydrolysis of dietary mono-, di-, and triacylglycerols and is responsible for digestion of fat-soluble vitamin esters and cholesterol esters in the small intestine. In humans, BSSL is also expressed in the lactating mammary gland and is found at high levels in milk.

BSSL is a highly divergent molecule at both the protein and genomic level, with the variation stemming from the number of 11 amino acid repeats coded at the C terminus of the molecule. The number of repeats, each of which carries an O-linked glycosylation signal, varies from 7 to 24 and with 16 being the most frequent. BSSL forms a dimer, and it has been shown that mothers heterozygous for BSSL genes with a low (<16 copies) and high (>16 copies) repeat number provide milk samples with the strongest binding to DC-SIGN (Stax et al. 2011). Interestingly, BSSL forms a functional dimer; hence, structural differences in two variant sized monomers may

provide for the heightened strength to DC-SIGN binding. Whether differences can be identified between mothers who transmit HIV-1 to their infants via breastfeeding versus those who do not remains to be elucidated and is currently being tested. BSSL is also found in plasma but it is not known whether this form binds DC-SIGN. One interesting result has been the association between the different BSSL allele combinations and HIV-1 progression with certain genotypes associated with timing of disease onset. It has been shown that there is a link to the emergence of viruses with the X4 phenotype which is especially interesting in that BSSL has been shown to bind to the CXCR4 receptor (Stax et al. 2012). One other intriguing aspect to this study was the strong trend toward a specific BSSL genotype being overrepresented in an HIV-1 highly exposed but uninfected MSM (men who have sex with men). More and larger cohorts will need to be studied to identify the extent to which such BSSL genotypes can associate with the risk of HIV-1 infection.

Bodily Secretions and Their Effects on HIV-1 Transmission

Over the last years attention has turned to studying the effects that factors within bodily secretions have on influencing HIV-1 transmission. This is in part due to the fact that deciphering which molecules are involved and elucidating their mechanism of action will undoubtedly allow for a better understanding of which cell types and modes of infection support HIV-1 transmission. Any new HIV-1 prevention strategy (such as microbicides and vaccines) will have to prevent the multiple pathways of infection and work within the milieu of host molecules to be found at the different mucosal sites. A large number of extracellular factors are present at mucosal surfaces where HIV-1 transmission occurs, and the virus will be exposed to an array of bodily secretions (including saliva, vaginal secretion, seminal plasma, as well as colorectal mucus). All the described fluids will possess an array of molecules including cytokines, CC and CXC chemokines,

antibodies of variant subclasses (IgA and IgG), as well as an array of (glyco)proteins which have been shown to modulate HIV-1 infectivity and inhibit transmission. The expression of cytokines will have the effects of modulating viral expression within infected cells while CC and CXC chemokines will be able to restrict viral infectivity through the classic HIV-1 infection pathway (CD4 and coreceptor mediated). Numerous genotypes within the genes encoding for such factors are known to restrict HIV-1 transmission, and all contribute toward the overall infectiousness of an individual. Many of the compounds found in bodily secretions are shared or similar and will therefore provide the same function in influencing HIV-1 transmission, including such factors as lysozyme, while other factors will be restricted to the particular secretion in question. There are a large number of small peptides present in secretions which have been shown to possess anti-HIV-1 activity, which include the α - and β -defensin group of peptides which can exert their effects through either disrupting viral particles or altering target cells for infection. The peptide LL-37 cathelicidin is another small molecule which has been shown to possess anti-HIV-1 activity as well as induce expression of α -defensins from neutrophils, and it has been shown that levels of expression of both α -defensin and LL-37 in cervicovaginal lavage, although possessing anti-HIV-1 activity, associate with heightened HIV-1 acquisition within a group of commercial sex workers with bacterial coinfections. Another small molecule inhibitor which has received much attention is the peptide SEVI which has been identified in seminal plasma and which can enhance direct infection of CD4⁺ lymphocytes as well as the transfer of virus by cells expressing DC-SIGN to CD4⁺ lymphocytes (Munch et al. 2007). Small molecule inhibitors have also been identified which have the potential to bind SEVI and neutralize the enhancing effects. It has also been demonstrated that spermatozoa can capture HIV-1 through heparin sulfate expressed on their cell surface and in this way pass the virus to CD4⁺ lymphocytes as well as potentially LCs and DCs found at the mucosal surface.

SLPI is an extracellular innate factor with anti-HIV-1 activity which can be found in a variety of bodily fluids and which was first described in human milk. The molecule prevents HIV-1 from infecting macrophages through binding to the phospholipid-binding protein, annexin II, on the cell surface which is a cofactor required for infection. This would indicate that SLPI expression at mucosal surfaces may protect against HIV-1 infection of macrophages when such cells are exposed, such as within lesions generated from coinfections. A more recent report has indicated that SLPI-treated monocytes have the potential to down-modulate human CD4⁺ lymphocyte proliferation with obvious implications for reducing immune activation and inflammation at sites of exposure. Another group of molecules with the potential to inhibit HIV-1 activity belong to those expressed from the DMBT1 gene and encode for a number of molecules at mucosal surfaces with the capacity to modulate immune responses. One DMBT1 protein fragment has also been shown to bind to gp120 and interfere with viral agglutination.

Mucins, alternatively known as MUC proteins, comprise a large number of molecules to be found in bodily secretions and which have been implicated in interfering with HIV-1 infection and replication. MUC5B and MUC7 from human saliva have been shown to possess anti-HIV-1 activity through blocking direct infection of CD4⁺ lymphocytes via a mechanism whereby the carbohydrates aggregate with virus particles rendering them noninfectious (Habte et al. 2006). It has also been identified that MUC1 in human milk and MUC6 in seminal plasma can interfere with DC-SIGN-mediated viral capture by DCs and the subsequent transfer of virus to CD4⁺ lymphocytes (Reviewed Pollakis et al. 2011). All MUC glycoproteins have one common feature in that they carry fucose sugar containing epitopes which contributes to their receptor-binding characteristics. A large number of posttranslational modifying enzymes are known to play a role in the differential processing of such MUC molecules and which will in all likelihood modulate their receptor-binding properties. The FUT2 and FUT3 genes, encoding fucosyltransferases 2 and 3, are

involved in the synthesis of DC-SIGN-binding Lewis-type sugars, which are known to modify the binding of such glycoproteins to their receptors. Host variations to the posttranslational modification of MUC proteins may influence the HIV-1 inhibitory capacities of such molecules. As with BSSL, the MUC6 gene is highly polymorphic and is composed of a variant number of large repeats in the central region of the glycoprotein, with variation in the number of repeats having been associated with infection with the gut *Helicobacter pylori* bacteria. Clusterin, a molecule found in seminal plasma, has also been shown to bind DC-SIGN and prevent HIV-1 capture and transfer to CD4⁺ lymphocytes. These results point toward an array of molecules to be found in human secretions with the capacity to influence transmission of various pathogens.

MUC proteins also comprise a large part of vaginal secretions and have been shown to vary in composition between the different sites of the female reproductive tract, namely, the cervical mucus (CM) and the cervical vaginal mucus (CVM). Elegant studies have shown that microparticles will diffuse differently within each of these bodily fluids. It would be expected that these differences would have consequences on HIV-1 particle diffusion through the secretory products and thereby influence the likelihood of virus reaching or penetrating the mucosal surface. It has also been postulated that MUC proteins in bodily secretions can likely modulate antibody function. The IgG subclass has been shown to bind both CM and CVM, while the IgA subclass can interact with CM but not CVM (Shukair et al. 2013). Similarly MUC2 in the digestive tract has been shown to bind IgA through the secretory component of the antibody dimer, while an MUC-related protein (FcGBP), which interacts with MUC2, can bind to the IgG Fc region. Collectively, these results indicate that different Ab subclasses can function differently within the diverse array of bodily secretions. The complexities of these interactions need to be understood when considering how effective antibody responses will be when induced at different mucosal sites.

Genetic association is a powerful means to determine which factors are important for influencing HIV-1 transmission as well as disease progression, with the best examples being those identified for the CC chemokine and chemokine receptor axis. Similarly, restrictions within genes of the adaptive arm of the immune response, specifically those within the multi-histocompatibility complex class-I (MHC I) restricted cytotoxic T lymphocyte (CTL) responses (B27 and B57), have been associated with disease progression. The presumed mechanisms linking the MHC haplotypes with disease course can associate with the breadth of CTL epitopes being recognized by the MHC molecule or the strength of binding thereby influencing the capacity through which the induced responses kill HIV-1 infected cells and control infection. CTL responses at mucosal surfaces have been hypothesized to be associated with protection from infection in both commercial sex workers and HIV-1-exposed infants. Many host genetic restrictions have also been described in genes encoding for proteins involved in the innate arm of the immune response, including cytokines, cytokine receptors, TLR receptors, KIR receptors, and C-type lectins (such as DC-SIGN), and all which have been linked with either risk of HIV-1 infection or disease progression. These associations clearly indicate that the network of innate and adaptive immune responses at mucosal sites is paramount in influencing transmission. The findings that many of the molecules to be found at mucosal surfaces can bind these cells and influence HIV-1 capture, and presumably free antigen capture, in a host-restricted manner indicates that induced immune responses will likely vary between individuals and presumably modulate risk of HIV-1 infection.

Conclusions

The vast majority of HIV-1 transmissions require virus to cross a mucosal surface, with each route being composed of a multiple array of cell types and in the presence of a vast array of bodily secretions. These secretions will be composed of

a mixture of cytokines, chemokines, antibodies, antimicrobial compounds, as well as a range of high molecular weight glycoproteins. Many of these factors have been shown to possess an array of HIV-1 modulating activity and will in combination determine the susceptibility of each individual to infection. Vaccines, microbicides, and therapies aimed at preventing HIV-1 transmission will need to function in most of these environments in order to be successful and inhibit the many different routes of infection as well as viral variants present. Understanding the complexities of the host viral interactions occurring at each mucosal surface will need to be deciphered in order to ascertain how successful the different HIV-1 prevention strategies will be. The identification of molecules with HIV-1 inhibitory activity may also allow for the development of new and novel products with the capacity to restrict HIV-1 transmission.

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HIV-1 Virion Structure

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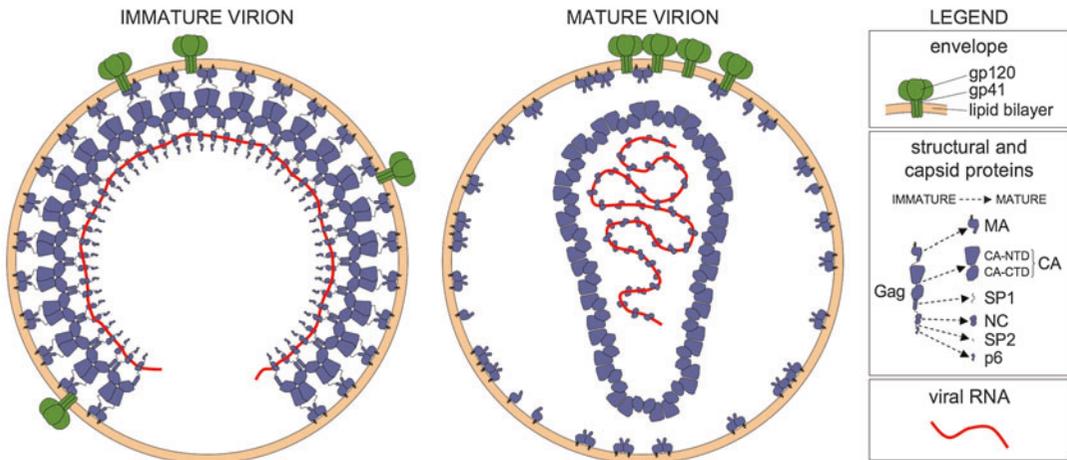
Definition

The HIV-1 particle, or virion, transmits the genetic information of the virus from one host cell to the next. There are two morphologically distinct forms of the virion, called “immature” and “mature.” The immature virion is the form that assembles and initiates budding at the plasma membrane of an infected host cell (► [Budding](#)). This form is not capable of transmitting infection. During or immediately after budding, the virion reorganizes into the mature form. The mature virion is fully infectious.

Overview

The HIV-1 virion is spherical in shape, with a diameter of about 100 nm (1/10,000 of a millimeter). The virion is delimited by an outer coat – the viral envelope – that surrounds and protects the internal components of the virus and contains the proteins that allow the virus to recognize new host cells. Inside the virion are viral structural proteins that assemble into a shell, or capsid, that further surrounds and organizes the viral genome. The HIV-1 genome consists of two strands of single-stranded, positive sense RNA molecules, each of which contains a complete set of viral genes. The function of the virion is to transmit the viral RNA from an infected cell (the “producer cell”) to a new host cell.

HIV-1 virions adopt two distinct morphological states (Ganser-Pornillos et al. 2008; Briggs and Kräusslich 2011; Sundquist and Kräusslich 2012) (see Fig. 1). The virion buds from a producer cell in its immature form, with a spherical capsid composed of the immature or precursor structural protein called Gag (also called p55 or



HIV-1 Virion Structure, Fig. 1 The HIV-1 virion adopts two distinct morphological states. Shown are schematic diagrams of the immature virion (*left*) and mature virion (*right*). The viral envelope comprises a spherical lipid bilayer shell (*orange*) and Env protein spikes (*green*). Inside the virion are the structural and capsid proteins (*charcoal gray*) and the viral RNA (*red*). In the immature virion, the precursor structural protein, Gag, forms a spherical shell that is tethered to both the viral envelope and the viral RNA. During

maturation, Gag is cleaved by the viral protease into three new proteins and several small peptides. The new proteins comprise the mature structural proteins, which reorganize the virion. The mature MA protein is derived from the amino-terminal domain of Gag and remains tethered to the viral envelope. The mature CA protein reassembles into a cone-shaped mature capsid. The mature NC proteins condense with the viral RNA to form a compact ribonucleoprotein complex within the mature capsid

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Pr55; Gag has a molecular weight of about 55 kDa). To become infectious, the immature virion undergoes a morphological change into the mature form. This process, termed “► [HIV-1 Maturation](#),” is triggered by site-specific proteolytic processing or cleavage of the precursor Gag protein by the viral protease. Gag cleavage by the protease does not lead to protein degradation but instead generates new structural proteins. The mature structural proteins include one called CA (also known as p24), which reassembles into a new, cone-shaped capsid. The mature capsid contains inside it the RNA genome and its associated enzymes. The mature capsid and its contents together constitute the virion “core” – this is the object that is introduced into the cytoplasm of new host cells at the start of a new round of replication (► [Uncoating and Nuclear Entry](#)).

The Viral Envelope

The viral envelope is composed of a lipid bilayer, which is derived from the plasma membrane of

the producer cell when the virion buds from the cell surface. Embedded within the envelope is the viral envelope protein, called Env. Env mediates recognition of and binding to the host receptor protein (CD4) and coreceptors (either CXCR4 or CCR5), which are displayed on the surface of immune cells such as macrophages and T cells. Receptor binding triggers a structural change in Env that facilitates fusion of the viral and cellular membranes and subsequent delivery of the virion core into the cytoplasm of the host cell (► [Fusion](#)). Env proteins are therefore essential for infectivity of the virion.

Env is composed of two proteins: a surface glycoprotein called gp120 (also known as SU) and a transmembrane protein called gp41 (also known as TM). These two proteins together form a single structural unit. Three of these units comprise the Env trimer. Each Env trimer forms a mushroom-shaped “spike,” which protrudes away from the surface of the viral envelope. The gp120 molecules make up the caps, whereas the gp41 molecules form the stem. Each gp41 protein contains a single transmembrane helix, which is

embedded within the membrane and passes through the entire lipid bilayer. The tails of the gp41 transmembrane helices are displayed on the inner surface of the viral envelope and interact with the structural proteins inside the virion.

Modern electron microscopy imaging studies have shown that the HIV-1 virion contains only 7–14 Env spikes (Liu et al. 2008; Zhu et al. 2008) (previous estimates have placed the number at around 70). Superresolution fluorescence microscopy studies have revealed that the spikes are distributed randomly on the surface of the immature virion and then coalesce into a single cluster upon maturation (► [HIV-1 Maturation](#); Chojnacki et al. 2012). Env clustering is important for efficient receptor binding and membrane fusion. The redistribution of Env is suggested to be triggered by a mechanism of “inside-out signaling” that involves interaction of the gp41 tails with the amino-terminal domain of the structural Gag protein. This ensures that only virions that have successfully generated the mature core are competent for membrane fusion.

The Immature Capsid

The immature capsid is a spherical protein shell composed of about 2,500 molecules of the structural Gag protein. The HIV-1 Gag molecule is composed of a series of structural domains and spacer peptides, which are demarcated by flexible linkers. The domains correspond to the mature proteins that are produced when Gag is proteolyzed during maturation and are called MA (matrix), CA (capsid), NC (nucleocapsid), and p6. The amino-terminal end of HIV-1 Gag (which is the MA domain) contains a covalently attached myristyl molecule, which is a saturated 14-carbon fatty acid chain.

The assembled Gag molecules (► [Virus Assembly](#)) are oriented with the MA domains positioned on the outer surface of the protein shell and juxtaposed with the inner surface of the viral envelope. The MA domain interacts with the viral envelope in three specific ways (Saad et al. 2006; Chojnacki et al. 2012): first, the domain binds to the membrane lipid,

phosphatidylinositol-4,5-bisphosphate; second, it inserts the myristyl fatty acid chain into the lipid bilayer; and third, it binds to the gp41 tails of the Env proteins.

The CA domain, together with the spacer peptide SP1, mediates the protein-protein interactions that stabilize the immature shell (Wright et al. 2007; Briggs et al. 2009; Bharat et al. 2012). The CA domain is further divided into two structural domains, called CA-NTD and CA-CTD, which are again connected by a flexible linker or hinge region. These three regions of Gag – CA-NTD, CA-CTD, and SP1 – form a three-layered paracrystalline lattice of hexameric (or sixfold rotationally symmetric) rings. The rings pack against each other side-by-side, like the hexagonal chambers of a beehive. The spacings between the rings have a characteristic value of 8 nm.

The NC domain is oriented toward the center of the virion. This domain contains two zinc knuckles, a small protein structural motif stabilized by a zinc ion, which bind to the viral RNA. Gag molecules within the immature virion are therefore tethered at both ends: the amino-terminal end (the MA domain) is tethered to the viral envelope, whereas the carboxyl-terminal region (the NC domain) is tethered to the viral RNA at the center of the virion.

The p6 domain is the carboxyl-terminal-most domain of HIV-1 Gag. This domain is unstructured and has no known structural roles within the immature virion. However, it is important for forming the virion because it hijacks proteins from the producer cell that are required for virus budding.

Analyses of immature virions by electron microscopy techniques have shown that the Gag shell is incompletely closed, with surprisingly large regions (up to 50% of the available area) devoid of ordered Gag molecules (Wright et al. 2007; Briggs et al. 2009; Keller et al. 2011). One likely explanation for this phenomenon is that Gag assembly (► [Virus Assembly](#)) and virus budding (► [Budding](#)) are in kinetic competition: by this model, incompletely closed shells are a consequence of virions being released from producer cells before complete Gag shells can be assembled (Carlson et al. 2010).

Virion Maturation

The viral protease cleaves HIV-1 Gag at specific sites to produce three new structural proteins: MA (also called p17), CA (p24, containing both the CA-NTD and CA-CTD domains), and NC (p7) (► [HIV-1 Maturation](#)). Proteolysis of Gag disassembles the immature shell, and the new mature proteins rearrange and reorganize the interior of the virion. The mature MA protein remains associated with the viral envelope, forming the “matrix” layer. A subset of the mature CA proteins reassembles into the mature capsid. Inside this new capsid, the mature NC proteins condense with the viral RNA, together with viral enzymatic proteins. As described above, maturation is also coupled to redistribution of the Env proteins. Altogether, the process of maturation reorganizes the entire virion into a particle that is now primed to infect a new host cell.

Proteolytic processing triggers a local refolding event at the amino-terminal end of CA-NTD: the first 13 amino acid residues of this domain change from an extended configuration (immature) into a beta-hairpin fold (mature) (von Schwedler et al. 1998; Tang et al. 2002). Similarly, processing at the CA-CTD/SP1 junction is suggested to induce a structural rearrangement of this segment from a putative alpha-helical bundle into an extended configuration (Wright et al. 2007). These structural changes are thought to comprise molecular switches that control or facilitate the immature-to-mature transition of protein-protein interactions involving the two CA domains. To a large extent, however, the mechanistic details (and in particular, the dynamics) of maturation are not yet well understood.

The Mature Capsid

The mature capsid shell has a similar basic arrangement as the immature shell – it is also composed of an array of hexagonal rings that pack side-by-side. The spacings between the rings are about 9 nm. Unlike the Gag shell, however, the CA shell also contains pentameric (or fivefold rotationally symmetric) rings.

The overall arrangement of the CA hexamers and pentamers that make up the mature capsid is mathematically described as a “fullerene cone” (Ganser et al. 1999). [Fullerene cones are related objects to buckyballs (buckminsterfullerenes) and nanotubes formed by elemental carbon. In fact, elemental carbon can be induced to assemble into fullerene cones (Krishnan et al. 1997).] The body of the HIV-1 capsid cone is composed of about 250 CA hexamers. In order to become a completely closed shell, the lattice incorporates exactly 12 CA pentamers. Unlike a buckyball, however, where the pentamers are distributed symmetrically at the vertices, the pentamers in a fullerene cone are distributed nonsymmetrically: there are seven pentamers at the broad end of the cone and five at the narrow end. As a consequence, the mature HIV-1 capsid is “globally” (taken as a whole) nonsymmetric, even though the CA molecules themselves come together “locally” in symmetric ways to form hexamers and pentamers.

The mature capsid of HIV-1 is not merely asymmetric – it is also pleiomorphic. There is variation in capsid size because each individual capsid can contain a different number of subunits. It is estimated that there are around 1,500 molecules of CA per capsid, which constitute only two-thirds of the available CA protein molecules within the virion. There is also variation in capsid shape, which arises from differences in the relative distribution of the 12 pentamers. It has been suggested that the pleiomorphic architecture of the capsid may reflect evolutionary warfare between the virus and its host (Ganser-Pomillos et al. 2011).

The architecture of the mature HIV-1 capsid is now understood in atomic detail. CA hexamers and pentamers are quasi-equivalent – that is, the two oligomers form by the use of the same sets of protein-protein interactions (Cardone et al. 2009; Pomillos et al. 2011). In both cases, the CA-NTD domains form rings with either sixfold or fivefold rotational symmetry. The CA-CTD domains form a “belt” around the CA-NTD rings and also connect the rings to each other.

An atomic resolution model of the HIV-1 CA fullerene cone, which was created by molecular dynamics and fitting of X-ray crystal structures of the hexameric and pentameric building blocks

into a low-resolution structure solved by electron microscopy, has been proposed (Zhao et al. 2013).

Comparison of the Immature and Mature Capsids

As is evident from the above descriptions, the immature and mature capsid lattices of share some similarities. This is not surprising, because in both lattices, the bulk of the protein-protein interactions are mediated by the two CA domains. However, the details of the interactions are quite different. In particular, the architectural roles of the two domains of CA are reversed: in the immature lattice, the hexameric rings are formed by CA-CTD, whereas in the mature lattice, these are formed by CA-NTD. Side-by-side connections between the hexamers are also different. The immature-to-mature transition of these protein-protein interactions is controlled, in part, by structural switches at either end of the CA polypeptide, which are triggered by the viral protease. Real-time visualization of these transitions is an important frontier in the field of HIV and retroviral structure.

The Viral RNA

The HIV-1 virion contains two molecules of the viral RNA. Each viral RNA molecule is approximately 10 kb (10,000 nucleotides) long. During maturation, the viral RNA condenses with the mature NC proteins into a compact ribonucleoprotein complex within the mature capsid. Early studies have shown that upon maturation, the viral RNA adopts a more stable structure (Fu and Rein 1993). To a large extent, however, the molecular details of how this occurs, the dynamics of viral RNA structure, and the molecular organization of the genome within the HIV-1 virion remain unknown.

Conclusion

The spherical HIV-1 virion consists of (from outside to inside) the viral envelope with embedded

Env proteins, the viral capsid, and the viral RNA with its associated factors. The virion initially assembles in its immature form, with a spherical capsid shell composed of the precursor structural Gag protein. During maturation, the Gag molecules are cleaved by the viral protease, giving rise to new structural proteins, which then reorganize the virion. Inside the virion, the viral RNA is repackaged in the mature capsid, which is a fullerene cone composed of the mature CA proteins. On the viral envelope, the Env protein spikes coalesce into a single cluster. Once maturation is complete, the virion is fully infectious.

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HIV-2 Diagnosis and Viral Load Measurements

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Definition

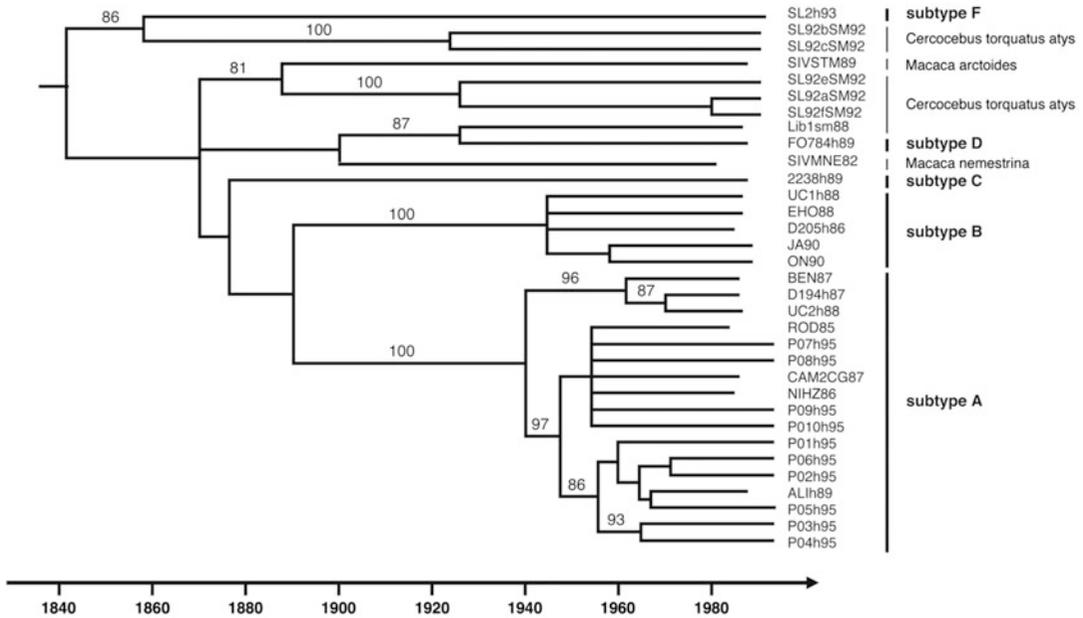
Detection and/or quantification of the presence of HIV-2 in biological materials, distinguishable from related lentiviruses, either directly as virus-

specific nucleic acid or protein or indirectly as immunological responses to infection. Viral load measurements relate specifically to genome quantification and infection dynamics, usually within already diagnosed individuals.

Background

Human immunodeficiency virus type 2 (HIV-2), a member of the Retroviridae, represents a related but distinct group of infections to HIV-1, the global agent of acquired immunodeficiency syndrome (AIDS). While HIV-2 bears similarity to HIV-1, it represents a separate genetic, virologic, and epidemiologic entity of viruses with marked differences in natural history and disease associations (► [HIV Prevention in the Correctional System](#)). Accurate, reliable diagnosis of HIV-2 infections is important not only from an individual perspective but when considering wider epidemiological and clinical consequences of HIV-2. Differences in HIV-2 biology impact on diagnostic approaches to detect HIV-2, either as monotypic infections or simultaneous dual HIV-1 and HIV-2 infections.

From the initial identification of HIV-2 in the mid-1980s, the disease course and relative pathogenicity of HIV-2 have been much debated although a clearer picture has emerged (► [Natural history and clinical features of HIV-2 infection](#)). HIV-2 is recognized as being less pathogenic than HIV-1, the majority of HIV-2 infections presenting as long-term nonprogressors (LTNP), with up to 80% of all HIV-2 infections falling into this category (de Silva et al. 2008). However, HIV-2 clearly can have severe consequences for a significant minority of individuals leading to profound immunological dysfunction, morbidity, and mortality. Hence, accurate diagnosis of HIV-2 infections is important to ensure blood donations remain HIV-2-free. Similar principles to HIV-1 diagnosis apply, including electron microscopy, direct virus isolation, detection of virus antigens, demonstration of virus-specific antibodies, or identification of components of the viral genome. However, only a proportion may be considered appropriate for HIV-2 diagnosis due to the differing



HIV-2 Diagnosis and Viral Load Measurements, Fig. 1 Phylogenetic relationship between members of the HIV-2/SIVsmm lineage of viruses. The timescale and phylogenetic relationship between different members of the HIV-2/SIVsm lineage are shown as depicted by Lemey et al. (2003) in a study of HIV-2 origins from

sooty mangabey-derived viruses. Different HIV-2 subtypes and sooty mangabey/maaque strains are indicated at the tips of the reconstructed phylogeny. The authors represent numbers at the nodes to indicate the percentage of bootstrap samples (of 1,000) in which the right cluster is supported and only values >80% are shown

biology of HIV-2 in vivo compared to HIV-1. In practice, serological detection with sensitive and specific anti-HIV-2 or HIV-2 antigen detection assays provide a frontline laboratory diagnosis, enriched and augmented by the application of molecular techniques for genome detection.

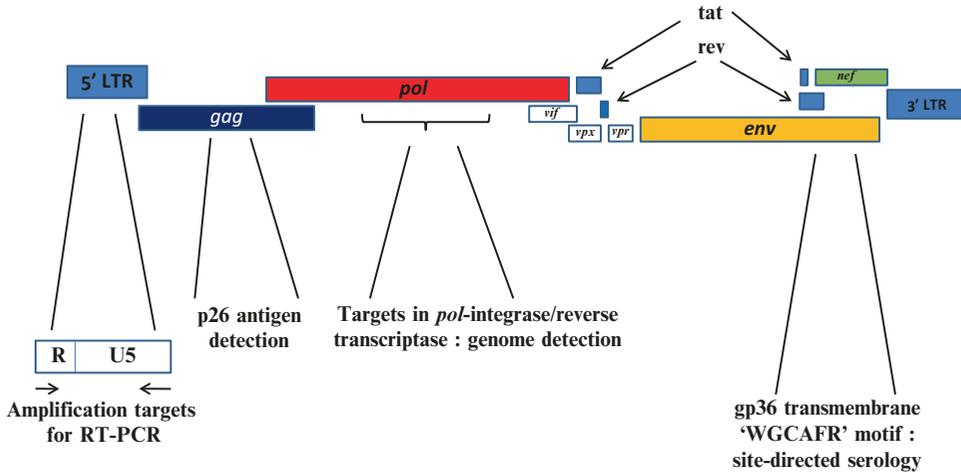
HIV-2 presents a number of particular challenges when effecting a reliable diagnosis, stemming from its evolutionary origins and entry into human populations. One important consideration when identifying diagnostic approaches to virus infection is knowledge of the range and spectrum of the infecting agent; HIV-2 viruses are no exception. Such considerations need to be taken into account when developing diagnostic strategies for HIV-2.

Origins and Diversity of HIV-2

HIV-2 is closely linked to countries of West Africa where it remains endemic, following initial

identification in patients with AIDS-defining symptoms and its recognition as a novel human pathogen (► [Natural history and clinical features of HIV-2 infection](#)). Phylogenetic analyses have confirmed HIV-2 was introduced into humans from Old World primates by multiple cross-species transfers from sooty mangabey monkeys (*Cercocebus torquatus atys*) of SIVsmm viruses (Sharp and Hahn 2011). By comparison, pandemic HIV-1 group M is the result of a single transfer of SIVcpz from the common chimpanzee (*Pan troglodytes troglodytes*). In this key respect, HIV-2 differs markedly from HIV-1 stemming from a different viral lineage which transcends many issues relating to HIV-2 biology, including successful implementation of diagnostic procedures. The phylogenetic relationship between HIV-2 and closely related viruses is depicted in Fig. 1.

Detailed molecular epidemiological and bioinformatics approaches have revealed a broad spectrum of HIV-2/SIVsmm viruses encompassing naturally infected sooty mangabey monkeys in



HIV-2 Diagnosis and Viral Load Measurements, Fig. 2 Genomic structure of the HIV-2 genome highlighting different regions targeted by diagnostic assays. Both coding and noncoding regions provide targets

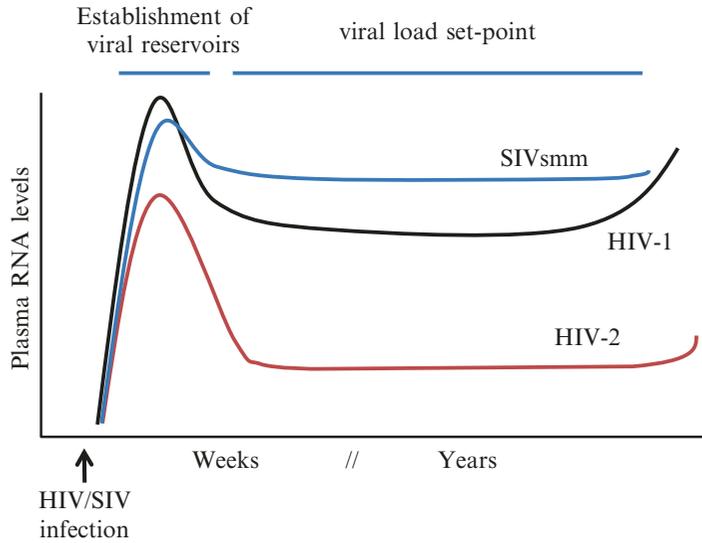
for antibody, antigen, and molecular detection systems where a common feature is the conservation of the genetic sequence or the response to conserved virally encoded epitopes or antigens

equatorial West Africa and at least nine putative HIV-2 subtypes. Each represents an independent transmission event from a reservoir of SIVsmm viruses in the wild, subsequently identified in humans, and highlights the diverse nature of lentivirus infections. However, detection of both clinically important viruses and what might be regarded as obscure, exotic infections is important, to provide a full picture of animal lentiviruses transitioning into human populations and their potential impact on human health.

Genomic Structure of HIV-2

HIV-2 is an exogenous retrovirus represented by two single strands of RNA in a diploid viral genome, encapsulated by retroviral capsid proteins and encompassed by an outer envelope glycoprotein (EGP). Like all retroviruses, HIV-2 undergoes reverse transcription as part of its life cycle. Viral or virion RNA (vRNA) is converted to proviral DNA able to integrate into the host genome. Cell-free vRNA in plasma (or serum) and cell-associated proviral DNA, representing different aspects of the viral life cycle, provide potential for direct viral genome detection in either RNA or DNA form. Virally encoded

proteins which act as targets for host immune responses to infection are also important components of diagnostic algorithms. Figure 2 shows the basic structure of the HIV-2 genome resembling a classical retrovirus *gag-pol-env* structure flanked by two long-terminal repeat (LTR) sequences targeted by antibody, antigen, and genome detection systems. *Gag* encodes group antigens, *pol* viral polymerases (reverse transcriptase, integrase), and *env* the envelope transmembrane (gp36) and external glycoprotein (gp105). Non-coding LTRs, transcriptionally important parts of the viral genome, which do not code for viral proteins, are less susceptible to immunological pressures. Regulatory/accessory genes (*tat*, *rev*, *nef*, *vpx*, *vif*, *vpr*) provide additional targets. Regions within the structural *gag*, *pol*, and/or *env* regions, however, have provided the main focus for serology, although molecular detection systems have employed both coding and noncoding regions to good effect. Highly conserved regions of the 5' R/U5 LTR of genomic RNA, involved in the initiation of reverse transcription in vivo, have provided secure targets for HIV-2/SIVsmm genome detection (Berry et al. 2011). Detailed analysis of HIV-2 molecular structure and properties is covered in the entry ► [Molecular biology of HIV-2](#).



HIV-2 Diagnosis and Viral Load Measurements, Fig. 3 Schematic comparing generalized viral load profiles for HIV-1, HIV-2, and SIVsmm infections during the infection time course. Plasma RNA levels reflecting systemic virus replication characterized by a peak of primary virus replication, as virus is seeded into

multiple lymphoid organs at centralized sites of the body during an exponential growth phase during acute infection. Steady-state plasma RNA levels differ for each virus group with HIV-2 infections having a generally low RNA loads correlating with prolonged survival

Acute Infection

Most HIV-2 infections, like HIV-1, are the result of sexual exposure. Following transmission, most likely at mucosal sites, a burst of virus replication occurs although is less well characterized than for HIV-1. Virus founder populations, limited to a few viral variants established during the first days of infection during an exponential viral growth phase, are preceded by an initial eclipse phase. Increased levels of detectable virus in blood during this acute period may be amenable to diagnosis of recent HIV-2 infection, prior to any form of immunological response. At this time, HIV-2 plasma vRNA is likely to be at its highest during a classical peak viremia although extensive studies of acute HIV-2 infection are lacking. Set-point virus loads, ~ 30 -fold lower than HIV-1, are most likely a direct consequence of a more controlled early viremic phase following primary HIV-2 infection and seroconversion (Andersson et al. 2000).

However, the long-lived nature of HIV-2 infections also stems from early virus dissemination

with establishment of viral reservoirs during this acute period (Fig. 3). HIV-2 infects CD4⁺ T lymphocytes and macrophages eliciting broad immunological responses following initial exposure. Both humoral and cellular responses to major virally encoded gene products occur, although serological responses to viral antigens remaining in the circulation for detection in serum/plasma are critical for viral diagnostic approaches. Hence, although the biology of HIV-2 and HIV-1 infections differs, similarities in general parameters of infection may be applied in a diagnostic setting.

Anti-HIV-2 Detection

Detection of HIV-2 antibody provides a frontline means of initially identifying infection in viral screens. Sensitivity and specificity are central aspects of viral diagnosis, although the nature of cross-reactive epitopes between HIV-1, HIV-2, and SIV makes the choice of viral antigen and assay format critical. Indeed, initial identification of a novel retrovirus in human populations, later

identified as HIV-2, was made with SIVmac viral antigens detecting cross-reactive antibodies to major structural proteins (► [The antibody response to HIV-2](#)), reflecting high levels of antigenic relatedness to HIV-2. It is important to bear this fundamental feature in mind when considering strategies and algorithms for diagnosis and clinical management of HIV-2 infections. Hence, detection of antibodies provides the most robust platform to screen for as many variants as possible within the HIV-2/SIVsmm/SIVmac lineage, irrespective of the originating species. Noninvasive sampling collection techniques may also be applied to identify related retroviruses (Sharp and Hahn 2011).

While the degree of cross-reactivity between HIV-2 and SIVsmm/mac is greater than between HIV-1 and HIV-2, interaction between anti-HIV-2 and anti-HIV-1 is sufficient to obscure accurate serodiagnosis in different circumstances. Responses to virally encoded antigens in *gag* and *pol*, generally more conserved than *env*, which is under greater mutational pressure from the immune system, have frequently resulted in misdiagnoses (Tedder et al. 1988). Specific identification of anti-HIV-2 remains a challenge to many assay systems. One problem of developing diagnostic assays is the need to target conserved epitopes which deliver a consistent antibody response, detectable in all cases. While the HIV-2 EGP (gp105) elicits both binding and neutralizing antibody responses, the transmembrane glycoprotein (gp36) has proved the most reliable platform upon which to base serodiagnoses. Commercially available and “in-house” anti-HIV-2 assays rely principally on the detection of antibody to immunodominant domains within gp36, primarily the WGCAFR motif. This linear epitope universally elicits antibodies of sufficient magnitude and consistency to provide an effective target for site-directed serological assays. Assay specificity has been further improved with synthetic peptides and recombinant antigens, compared to early assays which employed whole viral lysates often in western blot format. However, detecting a monotypic or type-specific response has proved challenging, even when using synthetic peptides to conserved regions. Optimized assays with high

specificity and sensitivity for anti-HIV-2 have often been difficult to achieve; hence, the choice of assay format is important with competition EIAs providing a high level of specificity (Tedder et al. 1988).

Combined HIV-1/HIV-2 assays for antibody and/or antigen are frequently employed in current testing algorithms. However, ability to detect low-level HIV-2 antibody/antigen may cause significant problems in HIV-2 diagnosis, particularly as the HIV-2 limb of any given assay may be suboptimal compared to its HIV-1 counterpart. Hence, ability to specifically diagnose HIV-2 infection may be compromised due to lack of sensitivity. Conversely, some HIV-2 sera remain capable of exhibiting strong cross-reactivity with HIV-1. Where HIV-2 sera are reacted with HIV-1 antigens and vice versa, cross-reactive antibodies to the heterologous virus have frequently been mistaken as evidence of dual HIV-1/HIV-2 infection (► [Dual HIV-1 and HIV-2 infection](#)). Where dual reactivity is present at low levels, distinguishing between the two infections, or identification of a genuine dual infection, may be achieved by serial dilution prior to assay. On the one hand, HIV-2 may be misdiagnosed due to cross-reactive HIV-1 antibodies on HIV-2 antigens. On the other, HIV-2 may be missed since validatory processes for a range of assay methodologies, including combination HIV-1/HIV-2 assays, may be deficient at HIV-2 detection. This raises concern with the introduction of more rapid testing and screening assays without an optimized HIV-2 limb, markedly compromising the ability to diagnose HIV-2 in different settings, either in endemic regions or traditionally low-prevalence countries such as the USA.

A balance also needs to be struck between the speed at which a diagnostic result is obtained with reliability and not poorly diagnose HIV-2 in such situations. Rigorous assay validation with well-characterized panels of reference sera, representative of the target population, is essential to highlight deficiencies in diagnostic tests and detection algorithms. Hence, the application of rapid HIV-2 diagnosis in endemic regions of West Africa raises a significant public health concern particularly where the ability to differentially diagnose HIV

infections in such settings should not be underestimated. As limitations in sensitivity and specificity for HIV-1 are well documented by rapid testing approaches, it raises particular concerns with respect to HIV-2 diagnosis by this approach. Notwithstanding these limitations, application of serological assays as important tools in clinical and epidemiological studies have extended our understanding of the distribution and relationship between primate lentiviruses and their relative impact on human health.

Molecular Diagnosis of HIV-2

While detection of anti-HIV-2 provides the most effective way to screen for HIV-2, detection and/or quantification of HIV-2 genome provides more detailed information to inform clinical decisions and investigate more in-depth epidemiological and virological aspects of HIV-2 infections. HIV-2 molecular diagnosis encompasses direct detection of genome, usually by *in vitro* amplification techniques, as well as investigations into sequence identity and viral phylogeny. HIV-2 exists as a viral quasispecies within infected individuals, where sequence populations of cell-associated provirus DNA or cell-free virion RNA in serum/plasma provide an opportunity to directly identify infection using molecular methods. Application of polymerase-chain reaction (PCR)-based techniques to clinically derived materials has greatly informed our understanding of HIV-2/SIV biology and epidemiology.

Early studies focused on detection and quantification of HIV-2 provirus (Berry et al. 1994; Ariyoshi et al. 1996) by sensitive PCR assays. Selection and application of diagnostically secure oligonucleotide primers is as important for HIV-2 as HIV-1. As full-length sequence became available from eight HIV-2 strains, so a database for prototypic HIV-2 subtype A (e.g., HIV-2_{ROD}, HIV-2_{NIHZ}, HIV-2_{BEN}) and subtype B (HIV-2_{EHO}, HIV-2_{D205}) viruses was established (see <http://www.hiv.lanl.gov>). Sequence comparison with SIVsmm/mac-related viruses enabled design of oligonucleotides to conserved regions, capturing the broad virus variability within the HIV-2/

SIVsm/SIVmac group. Conserved sequences located in *pol* or the LTR frequently provided consistent datasets, although 100% concordance with serological assays has always been difficult to achieve (Berry et al. 1994). This observation provided one of the first clues to the lower abundance of HIV-2 genome within infected individuals since 1–3% individuals confirmed anti-HIV-2 positive remained genome negative.

Amplification of low levels of viral genome, potentially confounded by sequence divergence, is a key consideration when embarking on molecular investigations into HIV-2. Sequence-independent approaches, such as the branched-chain reaction and direct measures of reverse transcriptase activity, may also be applied in such situations, although the ability to reliably detect very low levels of viral genome is a key consideration in HIV-2 genome detection. Sensitive real-time PCR-based assays provide a means of detecting low levels of HIV-2 genome although inherently low viral load is an overriding factor in HIV-2 diagnosis. This also impacts on defining dual infections, since PCR primers designed and optimized for maximal sensitivity to one virus group are likely to be poor at the detection of the other, given the wide genetic diversity of HIV/SIV sequences. The genetic distance between HIV-1 and HIV-2 essentially obfuscates pan HIV-1/HIV-2 detection by molecular assays, given that PCR primers frequently struggle to cope with the genetic variance within the spectrum of HIV-1 group M viruses alone. Hence, specific identification of dual HIV-1 and HIV-2 genomes relies on the ability to detect genomic RNA and/or DNA with assays which focus primarily on one virus family (i.e., HIV-1 or HIV-2) or the other. However, if achieved, molecular detection of monotypic HIV or dual HIV-1/HIV-2 infections provides unambiguous diagnosis, irrespective of serological detection.

Molecular detection of HIV-2 in certain scenarios may be the only means of diagnosing HIV-2, for example, in vertical transmission cases where perinatal or postnatal transmission events may be identified by PCR detection. This circumvents complications of serological diagnosis where passively acquired maternal antibody in

the first 18 months of life may obscure an accurate serodiagnosis. Vertical transmission of HIV-2 is relatively rare, however, frequently characterized by low virus load; hence, even genomic detection may be problematic in such circumstances.

Impact of HIV-2 Subtypes on Diagnostic Assays

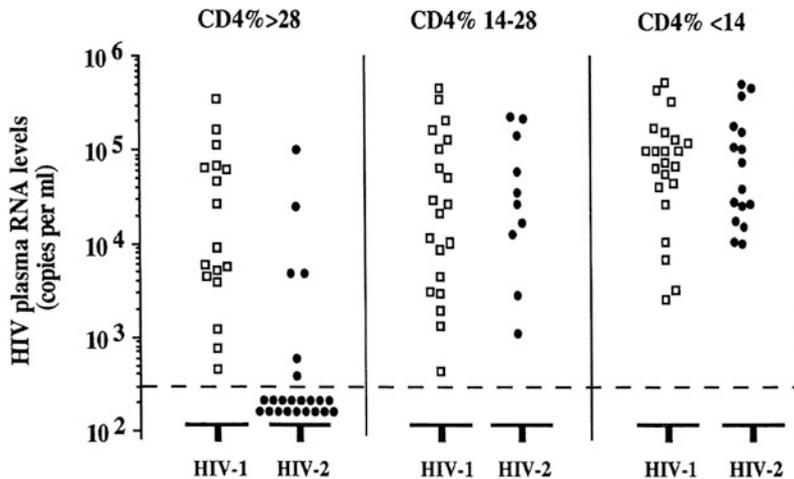
HIV-2 has a more restricted global distribution than HIV-1, both in prevalence and distribution of viral subtypes or genotypes. Only HIV-2 subtypes A and B are epidemically or clinically relevant accounting for the vast majority of HIV-2 cases identified to date. HIV-2 subtype A dominates, thought to have entered human populations in the first part of the twentieth century with estimates of a date for a most recent common ancestor to be 1940 ± 16 years (Lemey et al. 2003). Sequence variance within a single HIV-2 subtype from its estimated time of entry into humans is no greater than the sequence diversity within any single HIV-1 subtype. Hence, the spectrum of genetic variation likely to impact on diagnostic tests for HIV-2 genome is quantifiable and proportionate to the restricted course of the HIV-2 epidemic. As only HIV-2 subtypes A and B have been successful in onward human-human transmission, the focus of diagnostic testing is on these. HIV-2 subtypes A and B both have potential to cause severe lymphopenia and symptomatic disease albeit with differences in localized distribution. HIV-2 subtype B viruses are more closely linked with eastern parts of West Africa, mainly Cote d'Ivoire. The remainder (subtypes C–H) are minimally pathogenic or apathogenic with very low replication potential *in vivo*, considered biological and epidemiological “dead ends,” remaining largely restricted to the specific geographical locales where they were first identified with no onward transmission chain. The phylogeographical impact of HIV-2 has been defined (► [Epidemiology of HIV-2 infection in Europe](#)) relating onward spread to Europe and beyond to be largely attributed to HIV-2 subtype A which predominates in Guinea-Bissau, the country with the highest prevalence of HIV-2

(~8% of the general population). Portugal exhibits the highest prevalence of HIV-2 within Europe.

The development of subtype-specific molecular assays to identify HIV-2 subtypes A and B has delineated the distribution of HIV-2, although the identification of HIV-2 infections *per se* is usually sufficient. Subtype-specific regions in the U3 region of the LTR have been targeted to differentiate HIV-2 subtypes A and B to provide rapid subtyping of the principal HIV-2 subtypes (Berry et al. 2001). While the spectrum of HIV-2 sequence diversity is relatively well characterized, the first HIV-2 CRF (A/B) recombinant form has recently been described. Hence, vigilant monitoring and molecular epidemiological evaluation of circulating HIV-2 viruses will need to be maintained. Novel HIV-2 variants and subtype candidates inevitably impact on HIV-2 diagnosis, given the relatively higher propensity for sooty mangabey viruses to cross into humans leading to a high index of suspicion in regions where SIVsmm is endemic. Hence, the ability for diagnostic assays to detect such variants is always desirable. Ideally, equivalent amplification of all HIV-2 subtypes/clades, including SIVsmm variants, should be sought, achievable by targeting highly conserved regions of the HIV-2/SIVsmm genome (Fig. 2). A lower frequency of recombination events compared to HIV-1, most likely due to the lower replication of HIV-2/related viruses, suggests limited mixing of viral genomes between established viral genotypes. Moreover, rather than sequence diversity *per se*, perhaps the most significant problem facing HIV-2 genome detection is failure to identify low levels of viral genome.

HIV-2 Plasma Viral RNA Load

A major determinant of HIV-1 disease progression which informs clinical management is the level or magnitude of plasma viral load (PVL). With the advent of sensitive molecular assays suitable for measuring genetically diverse HIV, the significance of HIV-2 PVL was investigated in endemic groups in West Africa both in hospital-based studies in urban areas and community-



HIV-2 Diagnosis and Viral Load Measurements, Fig. 4 HIV-1 and HIV-2 RNA levels in a CD4-staged hospital-based cohort. HIV-2 plasma vRNA levels in asymptomatic individuals with a CD4%>28 were below the detection threshold (200 RNA copies/ml) in a high

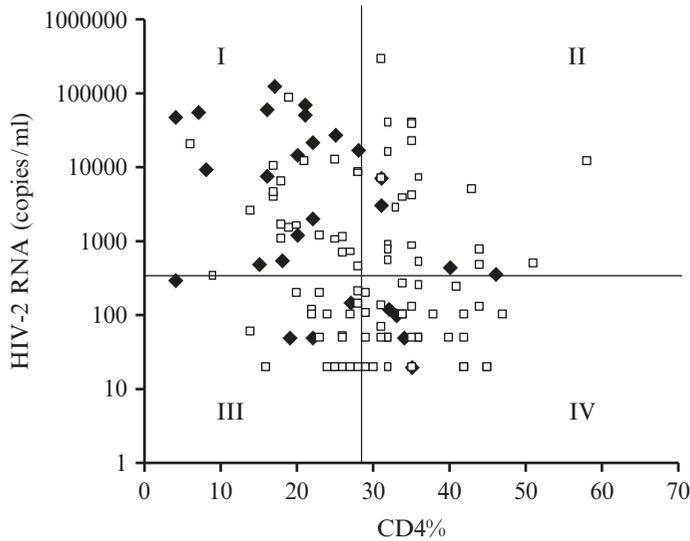
proportion of cases compared to similarly matched HIV-1-infected individuals. In the other CD4 bands, HIV-1 and HIV-2 RNA levels were similar (Reproduced from Berry et al. 1998)

based rural settings. These studies revealed insights into the dynamics of HIV-2 infections on a population basis. Perhaps the single most notable difference between HIV-1 and HIV-2 infections when considering broad distinctions made between the two viruses, their relative pathogenesis, and natural history is the quantity of cell-free virus detectable in the peripheral circulation. Definitively, the level of circulating HIV-2 RNA in peripheral blood during the asymptomatic phase of infection represents one of the largest single factors differentiating HIV-2 from HIV-1 infections in CD4-matched individuals in cross-sectional studies (Berry et al. 1998; Popper et al. 1999). Figure 4 depicts the first report of low HIV-2 RNA levels associated with asymptomatic infection, compared to HIV-1. Low set-point HIV-2 vRNA levels which frequently fall below the detection threshold of sensitive molecular analytical assays represent an underlying feature of HIV-2 infections.

Field-based, community studies of HIV-2 infection have furthered our insight into the long-term effects of HIV-2. A study of HIV-2-infected persons living in Caio, a remote village in northern Guinea-Bissau with an adult prevalence of ~8%, indicated low levels of plasma

viremia and high CD4% to be predictors of normal survival (Berry et al. 2002). Figure 5 compares HIV-2 RNA levels, CD4%, and mortality in this cohort where high levels of HIV-2 RNA broadly correlate with low CD4 and increased mortality. Extended studies of this unique cohort of long-term HIV-2-infection underscore the notion that HIV-2 RNA levels remain stable and largely unchanged over decades of infection (Schim van der Loeff et al. 2010). The net result is a high proportion of HIV-2-infected villagers who survive unaffected by HIV-2, essentially life-long, characterized by 2–3-fold excess mortality in HIV-2-infected subjects compared to age-matched uninfected villagers.

Low HIV-2 RNA load and by corollary high CD4 over many years of infection demonstrate humans can live for prolonged periods with a potentially lethal lentivirus and represent genuine cases of long-term survivors where natural life expectancy will be greater than the time taken to progress to life-threatening illness. Plasma viremia is frequently low, in the 100–1,000 RNA copies/ml range or undetectable (<50 HIV-2 RNA copies/ml). Such low levels of HIV-2 viremia present particular challenges to HIV-2 genome detection systems where <50 HIV-2



HIV-2 Diagnosis and Viral Load Measurements, Fig. 5 Plasma viral load in a community setting. HIV-2 plasma RNA levels and CD4% in 122 HIV-2-infected persons from the Caio village in northern Guinea-Bissau, taken at the 1991 baseline bleed. Stratified

according to median and mean values for HIV-2 RNA and CD4%, a high proportion of deaths (*solid symbols*) are represented in the high RNA, low CD4 grouping. In contrast, deaths were uncommon in the high CD4, low RNA group (Reproduced from Berry et al. 2002)

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RNA copies/ml or genome equivalents are not uncommon. While below any theoretical threshold required to lead to HIV disease, there would appear to be a balance between low virus replication and a more stable virus-host relationship enabling prolonged periods without extensive or overt pathology. Low vRNA levels and low viral replication represent improved chances of survival with baseline HIV-2 RNA levels providing an independent marker for disease progression during the asymptomatic period (Berry et al. 2002; Schim van der Loeff et al. 2010). However, the predictive power of HIV-2 RNA is reduced during the latter stages of infection when CD4 is the better indicator (Ariyoshi 2000).

In HIV-2 infections, as with HIV-1, the overall inverse correlation between plasma HIV-2 RNA levels and CD4 count indicates increased viral load reflects loss of immunological integrity in the host. In individuals where CD4 counts are low, levels of plasma viral RNA are often similar to HIV-1. Different studies indicate HIV infection levels, reflected in the PVL or vRNA concentration, irrespective of virus type (HIV-1, HIV-2, or dual infection) to be the most important factor

influencing disease progression (Gottlieb et al. 2002). High HIV-2 RNA levels identify those at most risk of disease and poorer outcome. However, the dynamics of HIV-2 infections in vivo remain understudied compared to HIV-1 where high turnover of virus and CD4+ lymphocyte subsets are compatible with an accelerated phenotype in vivo. Comparable studies of HIV-2 turnover are lacking though one preliminary study indicates HIV-2 to have a similar rate of production and clearance to HIV-1, although it is possible that turnover may differ at different disease stages.

The availability of reference materials for HIV-2 RNA testing has also advanced in recent years with collaborative studies improving efforts to accurately measure HIV-2 viral load (Damond et al. 2008), particularly discrepancies in relative detection efficiency of HIV-2 subtype B. Availability of an international standard for HIV-2 RNA to augment standardization and control testing for HIV-2 RNA (Holmes et al. 2011), particularly in light of lack of commercially available assays for HIV-2 RNA detection/quantification, should further improve HIV-2 diagnostic testing. However, it is not mandatory to test for

HIV-2 genome in many countries, including the USA. One additional caveat is the focus to date on HIV-2 subtype A with a need to characterize the range of HIV-2 subtype B sequence diversity and design appropriate assays to maximize detection of both HIV-2 subtypes A and B.

Intracellular Virus Detection and Viral Reservoirs

As HIV-2 plasma viral load is frequently very low, so characterization of cell-associated measures of virus infection assumes greater significance. Similar accumulations of proviral DNA, comparable at matched CDC stages of HIV-1 and HIV-2 infections, have been observed, yet HIV-1 and HIV-2 RNA levels differ during asymptomatic infection (Berry et al. 1998; Popper et al. 1999). The nature of cellular HIV-2 proviral populations and distribution of HIV-2 in different cell subsets *in vivo*, however, remains poorly studied, particularly relative proportions of integrated and unintegrated provirus. Despite a lower viral burst and lower systemic virus levels, HIV-2 seems capable of establishing disseminated virus reservoirs of HIV-2-infected cells, akin to HIV-1. Low transcriptional activity is compatible with low HIV-2 viremia and low burden of HIV-2-infected cells. Studies of quantification of unspliced and multiply spliced mRNA in HIV-2 infection are also scarce, although distinct mRNA species presented as unspliced (full length), partially spliced, or multiply spliced mRNA where unspliced mRNA also serves as genomic viral RNA have been described (MacNeil et al. 2007). The characterization of low-level mRNA transcripts in clinically derived materials suggests this may provide a more direct, sensitive means of detecting HIV-2 genomes in clinical samples. While low virus levels correlate with a low adverse impact on health, long-term effects of continuous, low virus turnover may not be completely benign. HIV-2 infects cells of the monocyte/macrophage lineage, which influence viral tropism for particular reservoirs of the body, including possible neurological complications. The long-term impact of HIV-2 on the brain and neurological

dysfunction is not fully understood, but studies suggest this might be the case (► [HIV-2 Neurological Manifestations](#)).

Moreover, the relatively low impact of HIV-2 infections with controlled viremia reflects lack of high intracellular virus replication in lymphoid organs and tissues. However, in individuals with undetectable plasma viremia and no demonstrable *in vivo* virus production, it is still possible to demonstrate replication-competent virus in peripheral blood using optimized HIV coculture protocols (Blaak et al. 2004). Low HIV-2 genome levels in these individuals may therefore not merely be composed of defective, non-replication-competent virus but cells containing very low levels of replication-competent virus. This low intrinsic replicative capacity of asymptomatic HIV-2 infections, with low peripheral viremia, underlies the distinct pathobiology of HIV-2 infections. This may be important when considering continuous stimulation of immunological responses at subthreshold levels and contribute to the better virus/host balance which seems to prevail in the majority of HIV-2 infections. While HIV-2 activity and the presence of different viral molecular species can be identified, it is the lack of high levels of productive infection which is the hallmark of the vast majority of HIV-2 infections.

Biological Significance of Low Viral Load

Comparative viral fitness studies have shown HIV-2 to be ~100-fold less fit than HIV-1 group M, though exhibit greater relative replicative capacity than HIV-1 group O. This broadly reflects the severity of the distinct human epidemics attributed to each virus group. Marked reductions in replication efficiency and transmission fitness have far reaching effects on the spread of HIV-2. This would seem to be a direct correlate of low viral burden and a key denominator of disease progression and transmission (► [HIV-2 Transmission](#)). HIV-2 is 5–10-fold less transmissible than HIV-1 between males and females and 20–30-fold lower from mother to child, explained by lower maternal HIV-2 PVL. Lower HIV-2

shedding than HIV-1 in female genital secretions and low seminal HIV-2 levels reflect overall low plasma vRNA loads. Taken together, these measures of low viral burden and shedding are a central feature of the reduced transmissibility of HIV-2 infections leading to a contraction in the HIV-2 epidemic.

Linked with prolonged long-term non-progression and survival, epidemiological data also suggest there are subsets of lifelong survivors with HIV-2. Although difficult to address definitively, survivors alive today in the 60–80 years age range may belong to the same birth cohort as those more susceptible to HIV-2 disease who have long since died. In this respect, can those remaining alive be viewed not merely as one part of a virological spectrum but as a unique subset, where measures of viral control is the central tenet of this survival status? Even this scenario would be quite distinct from naturally occurring, apathogenic SIV_{smm} infections in sooty mangabey monkeys, paradoxically typified by high plasma viral load frequently in the 10^6 – 10^8 SIV RNA copies/ml range (Fig. 3). Such levels for HIV-1 or HIV-2 represent uncontrolled virus replication compatible with a disease-progressing phenotype further characterized by systemic immune activation. In this key respect of viral measures, HIV-2 more closely resembles HIV-1, despite being the consequence of a different transmission lineage but presenting with an asymptomatic phenotype of low HIV-2 viral load. This alone marks HIV-2 as being distinct from either asymptomatic HIV-1 or natural SIV_{smm} infections where different scenarios of virus handling exist *in vivo*.

Impact of Virological and Host Factors on Viral Load

The inevitable interplay between virus and host will influence the ultimate outcome of HIV-2 infections where both viral and host genetic factors play a role in determining replication parameters and hence impact on viral load *in vivo*. Virological factors which influence virion output are crucial. HIV-2 LTR structure and differential

mRNA gene expression may be important determinants of cell-type specificity, HIV-2 transcriptional regulation, and viral integration. Differences in packaging and encapsidation of the viral genome are another feature of HIV-2 biology which may impact on viral output. Natural variation in *nef* gene sequence has been linked with low virus load in a cohort of Portuguese subjects (Padua et al. 2003). Minimal PxxP polyproline residues which correlate with low plasma viral load and asymptomatic infection suggest immunological receptor signaling differs according to disease stage. Recent advances in basic cell and molecular virology have identified novel classes of host restriction factors which act as broad inhibitors of retroviral replication, part of the innate response to infection. HIV-2 capsid interactions with TRIM5 α (tripartite motif), which acts as a postentry block to reverse transcription, relate to differences in HIV-2 viral load (► [Interactions between HIV-2 and host restriction factors](#)). Whether these changes represent cause or effect is unclear but highlights potential interactions between virus and host which may influence virus levels. APOBEC3G (A3G), a cytidine deaminase and RNA-editing enzyme which inhibits positive strand synthesis during reverse transcription, antagonized by the viral accessory gene *vif*, may also be active in limiting HIV-2 transmission. Interestingly, the identification of HIV-2_{F0784} in a Liberian rubber plantation worker (see Fig. 1) as the first definitive example of cross-species transmission of sooty mangabey SIV_{smm} to a human was noted for high levels of G>A hypermutation, a hallmark of A3G activity. Aspects of innate immunity which contribute to low set-point virus loads and a better virus/host balance may be key to understanding the prolonged outcome of HIV-2 infections.

Broader differences in host HLA genotypes and non-HLA genetic polymorphisms influencing HIV-2 disease progression and genetic predisposition of an individual to resistance or susceptibility to HIV disease progression represent further factors (► [Immunogenetics of HIV-2](#)). Identifying single measures or multiple factors which contribute to low plasma viral load *in vivo* is an important area which may reveal novel correlates of survival

or disease progression and inform novel areas of treatment or vaccination approaches. It is likely that a complex interplay between innate and adaptive immune responses and host genetics impacts on virus load.

Conclusion

Diagnosis of HIV-2 infections represents an important part of understanding the impact of this potentially AIDS-causing virus on human health, both at an individual level but also in broader comparative pathogenesis studies of human lentivirus infection. HIV-2 may be regarded as part of a spectrum of primate immunodeficiency viruses for which accurate identification of the infecting agent is the cornerstone of subsequent virological, clinical, and epidemiological studies. Sensitive and specific serological, molecular, and virological techniques are essential to properly diagnose HIV-2. The characterization of the differential viral dynamics of HIV-2 compared to HIV-1 and closely related simian viruses has illuminated our understanding of this enigmatic human pathogen.

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HIV-2 Envelope: Structure, Diversity, and Evolution

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Definition

The HIV-2 envelope is composed of trimers of gp125 and gp36, two heavily glycosylated proteins that are linked by noncovalent bonds and embedded in a lipid bilayer whose source is the host cell cytoplasmic membrane. The surface gp125 mediates the initial steps in the virus cycle binding to cellular CD4 and/or to chemokine receptors. The transmembrane gp36 mediates virus-to-cell fusion, participates in the assembly of new viruses, and counteracts the activity of

tetherin, enabling the release of new virus particles. HIV-2 Env of R5 isolates are intrinsically more sensitive to neutralizing antibodies than HIV-1. HIV-2 tropism and sensitivity to antibody neutralization are determined by the sequence, size, and conformation of the V3 loop. The evolutionary rate of HIV-2 gp125 (and the V3 region) is significantly higher than that of HIV-1 gp120.

Envelope Biogenesis

HIV-2 (► [HIV-1 Maturation](#); Tetherin Signaling) is a spherical enveloped virus (► [HIV-1 Maturation](#)) with a diameter of approximately 110 nm. The HIV-2 envelope is encoded by the *env* gene and consists of a lipid bilayer containing a transmembrane glycoprotein of 36 KDa (gp36) which anchors an outer surface glycoprotein with 125 KDa (gp125). In the mature HIV-2 virion, these heterodimers associate as trimers (gp125₃/gp36₃). In the infected cell, HIV-2 envelope glycoproteins are produced as a 97 KDa polyprotein by translation of a single-spliced mRNA in ribosomes bound to the endoplasmic reticulum. Glycosylation occurs in the Golgi apparatus on its way to the cell surface and results in a 140 KDa protein (gp140). Gp140 assembles in trimers and is cleaved by furin in the *trans* Golgi into gp125 and gp36 which remain linked by noncovalent bonds. The cleavage positions the fusion peptide at the N-terminus of gp36 and primes the spike for fusion activation. The trimeric envelope complex is transported to the cytoplasmic membrane where the assembly of the viral particles takes place. Release of the new virus particles occurs by budding from the cell membrane. Virus release can be inhibited by tetherin (► [HIV-1 Maturation](#)), a B-cell antigen that is an effector of the interferon-induced antiviral response. Tetherin presumably tethers the nascent virions to the cell surface. In HIV-1 tetherin is antagonized by Vpu. In HIV-2 tetherin is antagonized by the Env gp36. Tetherin binds specifically to the gp36 ectodomain and is subsequently downregulated from the cell surface and sequestered intracellularly in a perinuclear compartment that includes the trans-Golgi network (Hauser et al. 2010).

Envelope Structure and Function

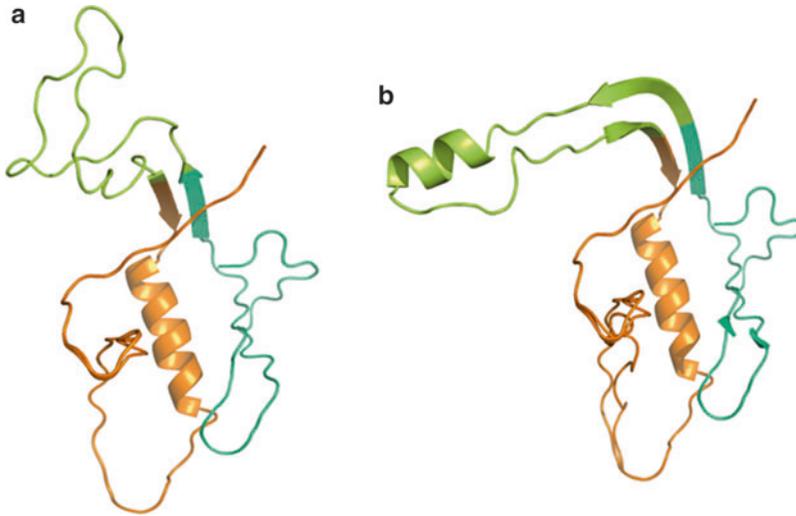
HIV-2 gp125 is a 501–515 amino acid long glycoprotein that is composed of five hypervariable regions named V1 to V5 separated by five more conserved regions named C1 to C5 (NH₂-C1-V1-V2-C2-V3-C3-V4-C4-V5-C5-COOH). In the envelope complex, gp125 binds to the CD4 receptor (► [HIV & SIV, CD8 T Cell Responses](#) to) (HIV main receptor) present in the surface of the host cells (T lymphocytes, monocytes, macrophages, and dendritic cells). The CD4-binding site in gp125 comprises selected amino acids in the relatively conserved C2, C3, and C4 regions as well as in V4. CD4 binding is thought to induce major conformational changes in gp125 and exposure of the coreceptor binding site (mostly comprising the V3 loop). In vivo, the major HIV-2 coreceptors are the chemokine receptors CCR5 and CXCR4 although some HIV-2 isolates from patients in late-stage disease can also use additional chemokine receptors (e.g., CCR1, CCR2b, CCR3, CCR4, Bob, and Bonzo) to enter cells. V3 loop charge and size influence coreceptor interaction such that increasing its size by 1–3 amino acids and charge by the presence of positively charged residues (R, K, or H) at positions 18, 19, and 27 is strongly correlated with CXCR4 usage (Shi et al. 2005; Visseaux et al. 2011). Based on these observations, new computational tools are currently being developed to infer coreceptor use and susceptibility of HIV-2 isolates to CCR5-antagonists such as maraviroc. Increasing the number of HIV-2 env sequences with known coreceptor usage will be crucial to assure that these prediction tools achieve similar performances for HIV-2 as they have achieved for HIV-1.

In primary isolates the size of the transmembrane glycoprotein gp36 varies between 343 and 354 amino acid residues. It has one extracellular domain, one transmembrane region, and one intracytoplasmic domain. The ectodomain starts with the N-terminal fusion peptide, which is highly conserved and hydrophobic in nature. The most conserved region in the fusion peptide is the hydrophobic core Phe₅₁₂-Val₅₁₃-Leu₅₁₄-Gly₅₁₅. Increasing the hydrophilicity of this

region by a single amino acid substitution in the hydrophobic core (Val₅₁₃ for Glu) has been shown to abolish HIV-2 fusion. The fusion peptide is followed by two α -helices containing leucine zipper-like motifs, the heptad repeats 1 (HR1, positions 558–586 in the ROD isolate) and 2 (HR2, positions 755–776). Between these two regions lies the immunodominant region (position 595–603) which contains two cysteine residues involved in a disulfide bond. All HIV-2-infected individuals produce IgG antibodies directed to this highly antigenic region (Marcelino et al. 2008). Within the immunodominant domain is the highly conserved Ala residue at position 598 that has been found to determine tetherin recognition by gp36. Despite the high conservation of the Ala₅₉₈ residue in HIV-2 isolates, not all envelope proteins downregulate tetherin, suggesting that other regions in the gp36 ectodomain might also contribute toward this recognition function.

The transmembrane region (positions 677–700) of gp36 is a highly hydrophobic region with a predominant α -helical content. Its main function is to insert and retain the envelope complex in the cell membrane. The intracytoplasmic domain has an average of 152 amino acids in primary HIV-2 isolates. It mediates the binding of the envelope to the matrix protein during the maturation of new viral particles. A membrane-proximal glycine-tyrosine-X-X-hydrophobic (G₇₀₆Tyr₇₀₇X₇₀₈X₇₀₉ θ ₇₁₀) motif acts to recruit adaptor protein complex 2 (AP-2) which is responsible for the endocytosis and intracellular sequestration of tetherin. In T-cell line-adapted isolates, this region is usually truncated to 138–146 amino acids (MW 32–34 KDa). This truncation has been associated with increased cytopathogenicity in some HIV-2 isolates.

Binding of gp125 to CD4 and coreceptor leads to major conformational changes in the gp36 ectodomain that result in the insertion of the fusion peptide into the host cell membrane. Then, the HR2 trimer folds back on an antiparallel fashion toward the HR1 trimer, forming a six-helix bundle structure stabilized by the hydrophobic interactions between the HR1 domains in the center (central coiled coil) and the HR2



HIV-2 Envelope: Structure, Diversity, and Evolution, Fig. 1 Secondary structures of C2-V3-C3 envelope region from R5 and X4 HIV-2 primary isolates. Secondary structures were determined by homology modeling using the 3-D structure of an unliganded SIV gp120 envelope glycoprotein as template. (a) Representative structure of the C2-V3-C3 envelope region from R5 isolates.

(b) Representative structure of the C2-V3-C3 envelope region from X4 isolates. The V3 loop of R5 isolates is characterized by absence or low amounts of regular secondary structural elements. In contrast, the V3 loop structure of X4 isolates fit into a β - α - β motif characterized by high β -sheet content

H

domains outside. This process brings together the viral envelope and the cell membrane which leads to the formation of a fusion pore through which the viral capsid enters the target cell. Formation of the six-helix bundle structure and subsequent fusion events can be inhibited in HIV-2 by HR2-like peptides that bind to HR1 (e.g., T-1249 and P3) (Borrego et al. 2012).

Binding to CD4 also leads to major conformational changes in gp125 and exposure of the coreceptor binding site (V3 loop). The rate at which the V3 loop becomes exposed after CD4 binding is faster in gp125 than in HIV-1 which promotes a faster Env-mediated fusion in HIV-2 (Gallo et al. 2006). However, it has been shown that, in contrast to HIV-1, CD4 binding may not be a prerequisite for HIV-2 cell entry as several primary isolates have the ability to infect cells via CCR5 and CXCR4 independently of CD4 (Reeves et al. 1999). This suggests that HIV-2 gp125 in its native trimeric state may be more prone to adopt spontaneously a CD4-bound conformation than HIV-1 gp120. As the CD4-bound state of gp125 is a functionally critical state, this

would predict HIV-2 to be highly vulnerable to neutralizing antibodies. In fact, in contrast to HIV-1, most HIV-2 R5 isolates are effectively neutralized by IgG antibodies from HIV-2-infected individuals (plasma or monoclonal antibodies) (de Silva et al. 2012). These antibodies are directed to the CD4 binding site, as well as to V2, V3 (likely to a conformational epitope comprising amino acids Phe-His-Ser-Gln in positions 315–318 and Trp-Cys-Arg in positions 329–331), and V4 (de Silva et al. 2012; Kong et al. 2012). However, X4 isolates such as the reference ROD strain and primary strains that emerge late in infection in some HIV-2 patients with low CD4 counts are highly resistant to antibody neutralization (Marcelino et al. 2012). The V3 loop conformation of X4 and R5 HIV-2 isolates are markedly different, supporting a direct role of this region in the different susceptibility of these viruses to antibody neutralization (Fig. 1). Differences in V3 loop conformation may also explain the higher maraviroc and TAK779 resistance of R5 isolates obtained from late-stage disease HIV-2 patients (Borrego et al. 2012). Interestingly, the fusion inhibitor

T-1249 is more active against X4 isolates than R5 isolates, suggesting that significant conformational changes might also occur in gp36 in advanced HIV-2 infection (Borrego et al. 2012). In summary, in the native envelope complex, the gp125 glycoprotein from R5 isolates seems to be in a CD4-bound conformation more often than HIV-1 gp120, and this may determine a higher susceptibility of HIV-2 to antibody neutralization. However, conformational changes in the V3 loop and gp36 occurring in late-stage disease might reverse this neutralization-sensitive phenotype and change the susceptibility of HIV-2 to entry inhibitors.

Diversity and Evolution

The HIV-2 envelope gene is characterized by high genetic diversity and high rate of molecular evolution. HIV-2 has diversified into 8 phylogenetic groups named A to H and one circulating recombinant form CRF01_AB that results from the recombination of the A and B isolates. Average envelope amino acid diversity between all HIV-2 groups is 23% and between isolates belonging to the most prevalent groups A and B is 14% and 17%, respectively. Diversity is higher in gp125 than in gp36 (20% vs. 14% in group A), which is likely to be related to the greater immunologic pressure that is exerted on gp125. Within gp125, the V3 region has been shown to be highly immunogenic and antigenic, to determine cell tropism, and to have immune modulatory properties (Visseaux et al. 2011; Marcelino et al. 2008, 2010). The multiple functional roles of HIV-2 V3 might explain its relatively low inter-patient amino acid diversity when compared to HIV-1.

The mean within-host *env* nucleotide diversity in patients with chronic infection is 2.1% (SD, 1.1%), increasing along the course of infection in most patients (Borrego et al. 2008). The genetic diversity of the HIV-2 *env* gene may be directly related to the period of infection even in the absence of plasma viremia or disease progression (Borrego et al. 2008; Barroso & Taveira 2005; MacNeil et al. 2007). However, some longitudinal studies have shown that higher variability in the V3 region is generally found in patients with

faster disease progression to AIDS and that in elite controllers (patients infected for ≈ 10 years with normal CD4+ T-cell counts without antiretroviral therapy and with low or undetectable viral load), the rate of *env* gene diversification is positively associated with the rate of CD4+ T-cell number decline (MacNeil et al. 2007).

Remarkably, it has been shown that the within-patient mean evolutionary rate of HIV-2 may be significantly higher than the rate of HIV-1, both in the gp125 (10.15×10^{-3} vs. 6.36×10^{-3} substitutions per site per year) and in the V3 region (29.37×10^{-3} vs. 12.26×10^{-3} substitutions per site per year) (Skar et al. 2010). Nonsynonymous sites evolve at a significantly lower rate than the synonymous sites in gp125, indicating that the higher evolutionary rate in the *env* gene in chronically HIV-2-infected patients is more likely to be related to a shorter generation time or higher mutation rate than to escape from immunological pressure. Indeed, other lines of evidence seem to rule out immunological pressure as the main driver of the high evolutionary rate of the HIV-2 *env* gene at least in chronically infected patients. Firstly, the HIV-2 envelope gene is globally under negative (purifying) selection, and the number and position of the few positively selected sites is usually highly variable. Secondly, the number and position of N-glycosylation sites in the *env* gene is usually highly conserved in HIV-2 patients along the course of infection. In HIV-1, variation in the number and position of N-linked glycosylation sites has been associated with more or less sensitivity to neutralizing antibodies. Thirdly, it has been found that variation of C2-V3-C3-specific IgG response over time in HIV-2-infected patients is inversely associated with the variation in nucleotide and amino acid diversity of the C2-V3-C3 envelope regions (Borrego et al. 2008). However, recent evidence indicates that some R5 isolates and most X4 isolates that emerge in patients with advanced disease and low CD4 counts can escape antibody neutralization. Higher sequence diversity and variation in putative glycosylation sites has been associated with higher resistance of HIV-2 R5 isolates to neutralizing antibodies (de Silva et al. 2012). Resistance to antibody neutralization in X4 isolates has been

associated with longer and higher-charged V3 loops compared to neutralization-sensitive R5 isolates as well as with major V3 loop conformational changes (Marcelino et al. 2012). Hence, neutralizing antibodies may play a central role in HIV-2 *env* evolution in a subset of patients with advanced disease and declining CD4 counts. In contrast to HIV-1, little is known about the neutralizing antibody response and the molecular evolution of the HIV-2 *env* gene in acute and early infection because the vast majority of HIV-2 patients are diagnosed many years after the original infection when the chronic phase is already established.

Conclusion

Biochemical, virological, and computer modeling data suggest that the HIV-2 envelope gp125 is in a CD4-bound conformation (open conformation) more often than HIV-1 and that the conformation of the V3 region differs significantly in R5 and X4 isolates. This is likely to explain the opposing susceptibilities of R5 and X4 HIV-2 isolates to neutralizing antibodies. Atomic or near-atomic-level structural models of the HIV-2 Env bound and unbound to CD4 and to neutralizing antibodies are urgently required to fully understand how gp125 interacts with these molecules and how this interaction affects the structure of the HIV-2 Env. This will be vital for the identification of neutralizing epitopes and subsequent development of an effective HIV-2 vaccine.

The determinants of the rapid evolutionary rate of HIV-2 gp125 are currently unknown. Escape from neutralizing antibodies plays a central role in the molecular and phenotypic evolution of the HIV-2 *env* in a subset of patients in late-stage disease, but it does not explain the rapid Env evolution in most chronic HIV-2 patients. Further research is needed on the molecular, structural, and functional evolution of the HIV-2 *env* gene in chronic as well as in acutely infected adult patients and/or children infected via vertical transmission as this will contribute crucially to a better understanding of the interplay between the virus, the host cell, and the immune response.

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HIV-2 Infection in Europe, Epidemiology of

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Definition

The incidence of HIV-2 infection shows signs of decline in the endemic regions of West Central Africa, while HIV-1 infection continues its pandemic global spread (Silva et al. 2008).

The HIV-2 epidemic remains largely confined to West Africa, where it peaked in prevalence in the 1970s and 1980s, with an epicenter in and around Guinea Bissau (Fig. 1).

The processes of globalization and migrant population mobility have contributed to and will continue to facilitate the spread of HIV-2 into European nations and other parts of the world.

In Europe, the countries with highest recorded prevalence of HIV-2 infection are those that share

historical, cultural, and economic relationships with the endemic West African countries, mainly represented by Portugal, France, and Spain.

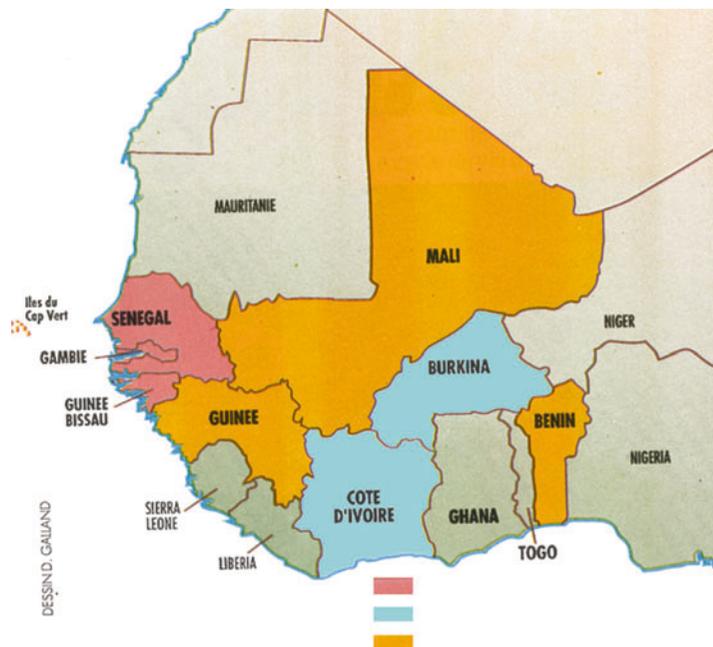
Previous estimates suggested that an estimated 1–2 million people were infected with HIV-2, but there are no data for the past two decades. Accurate data on HIV-2 prevalence would be very valuable in estimating the current burden of infection and identifying potential areas of increased transmission.

HIV Agents: Differences and Similarities

Acquired human deficiency syndrome (AIDS) results from the infection by two closely related viruses, members of the *Retroviridae* family and Lentivirus genus, HIV type 1 (HIV-1) responsible for the pandemic spread and HIV type 2 (HIV-2) mostly confined to some West African countries.

Both viruses share about 40% nucleotide sequence homology, the same life cycle, cellular targets, transmission routes (sexual, vertical, and blood-borne exposure), and pathogenic behavior that lead to chronic debilitating disease (Bock and Markovitz 2001).

HIV-2 Infection in Europe, Epidemiology of, Fig. 1 HIV-2 infection prevalence in West Africa: *Pink* areas: HIV-2 predominant; *Blue* areas: HIV-2 and HIV-1 in the same proportion; *Yellow* areas: HIV-2 less frequent (Caroline D et al. Science et vie 1992;179:38–41)



HIV infection is responsible for chronic inflammation and persistent immune activation which promotes progressive immunological dysfunction and exhaustion.

Natural History of Infection

The natural history of HIV infection is characterized by three stages: primary or acute infection, the asymptomatic stage, and AIDS, when opportunistic infections and tumors develop, reflecting a severely compromised immune system.

HIV-2 infection shows markedly lower transmission rates, slower immunologic depletion, and disease progression, with a prolonged asymptomatic stage and reduced mortality rates, compared with HIV-1 infection.

While the majority of untreated HIV-1 infected individuals progress to AIDS, only 20–30% of HIV-2 infected persons develop symptomatic disease in the same way as HIV-1 (Esbjornsson et al. 2012). An unusually high proportion of HIV-2 infected patients present as long-term non-progressors (LTNP), exhibiting a slower CD4⁺ T-lymphocyte decline and lower or undetectable plasma viral load and survival rates similar to the general noninfected population.

Since HIV-2 plasma RNA is frequently undetectable, disease monitoring is essentially based on clinical and immunological parameters, such as CD4⁺ and CD8⁺ T-cell counts, and levels of inflammatory markers, such as β_2 microglobulin.

Treatment options and the choice of antiretroviral regimens are limited by the fact that HIV-2 strains are intrinsically resistant to nonnucleoside reverse transcriptase inhibitors (NNRTIs) and to first generation fusion inhibitors (enfuvirtide) and are less susceptible in vitro to some protease inhibitors (atazanavir, amprenavir) (see chapter “► Antiretroviral Therapy and Drug Resistance in HIV-2 Infection”).

HIV Origin, Human Adaptation, and Dissemination

Phylogenetic studies have shown that both HIV-1 and HIV-2 evolved after multiple cross-species

transmissions of Simian Immunodeficiency Virus (SIV) that naturally infected African primates and which, subsequently, spread to humans (Hahn et al. 2000).

The two different types of HIV derive from distinct simian species: HIV-1 from SIV_{cpz} (chimpanzee) and HIV-2 from SIV_{sm} (sooty mangabey – *Cercocebus torquatus atys*) (Sharp and Hahn 2011). HIV-2 presents a high genetic sequence homology (~75%) with SIV_{sm} and only about 40% with HIV-1 (Bock and Markovitz 2001; Markovitz 1993).

SIV_{sm} was found to be highly prevalent in rural areas of West Africa, affecting sooty mangabeys both in captivity and the wild, but infection is not usually associated with the development of disease in its natural host.

The way humans became infected with these simian viral precursors of HIV is not known, but it is thought that transmission must have occurred after cutaneous or mucous membrane exposure during hunting practices, preparing bushmeat, or by maintaining wild animals in captivity as domestic pets. Nevertheless, the genetic similarities, phylogenetic evolution, geographic coincidence, prevalence of infection in natural hosts, and plausible routes of transmission together strongly support the evidence for zoonotic transmission of SIV into humans.

Extended viral replication and accumulation of mutations presumably permitted viral adaptation to the human host, and social circumstances such as the expansion of urban centers, population migration, increases in the prevalence of sexually transmitted infections, and large scale vaccination campaigns, all present in West Africa during the twentieth century, probably contributed to HIV-1 and HIV-2 adaptation and dissemination.

HIV-2 was first described in 1986 in isolates from West African patients, where it is currently endemic and seems to have been present before the 1960s (Clavel et al. 1987; Markovitz 1993; Peeters et al. 2013).

Since its first description, eight different lineages of HIV-2 have been identified and termed as group A to H, although only groups A and B are considered epidemic subtypes and have spread between humans to a significant degree (Lemey

et al. 2003). Group A is found mainly in West Africa and group B predominates in Ivory Coast and Mali (Sharp and Hahn 2011). The remaining six nonepidemic groups (C-H) were anecdotally found in single patients, assuming incidental infection with very limited or no secondary transmission.

HIV-2 Epidemiological Distribution

The African endemic areas of HIV-2 infection are Guinea Bissau, the country that recorded the highest HIV-2 prevalence in the 1980s, Senegal, Cape Verde, Ivory Coast, Gambia, Mali, Sierra Leone, Nigeria, Benin, Burkina Faso, Ghana, Liberia, Angola, Mozambique, and Sao Tome (Bock and Markovitz 2001).

In the late 1980s, some of these countries reported a prevalence above 1% of the national population, leading to the estimate of 1–2 million HIV-2 infected people living in West Africa.

In recent years, HIV-2 prevalence is reported to be declining in several African countries, especially among younger individuals. For instance, in a rural area of Guinea Bissau, the prevalence declined from 8.3% in 1990 to 4.7% in 2000; however, HIV-1 prevalence increased in the same population from 0.5% to 3.6% over the same period (Campbell-Yesufu and Gandhi 2011).

Additionally, in endemic West African regions 0.3–1% of patients with HIV infection are considered to be concomitantly infected (dual infection) with both HIV-1 and HIV-2 (Barin et al. 2007; Esbjornsson et al. 2012).

It is very difficult to estimate the exact prevalence of HIV-2 infection in Africa because of the high rates of population mobility, difficulties in diagnostic techniques, and deficient tracing and case notification.

While the overall prevalence of HIV-2 infection appears to be declining, even in endemic regions, there is a parallel increase in the spread of HIV-1, which may be explained by a lower efficiency in vertical and sexual transmission of HIV-2. Compared to HIV-1, HIV-2 vertical transmission by breast-feeding, in the absence of anti-retroviral therapy, is less than 4% (~25% in

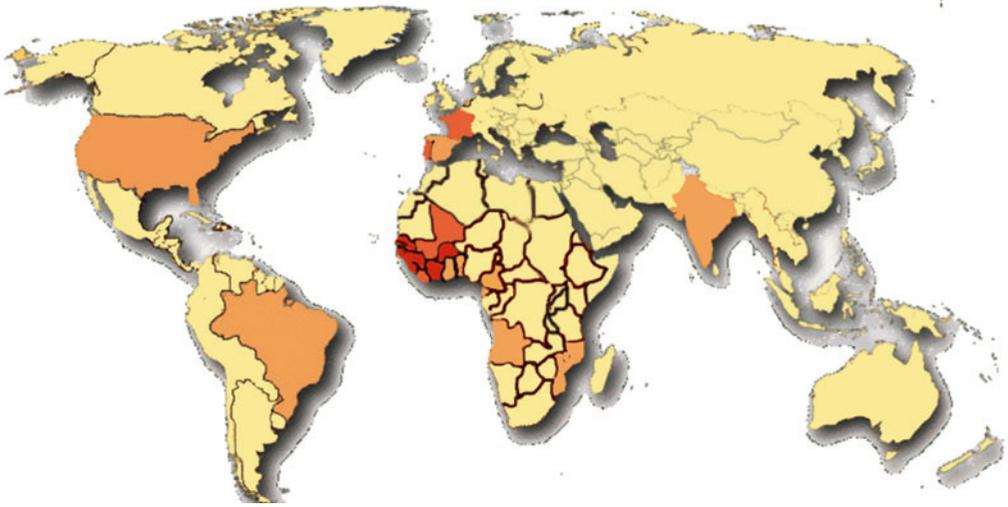
HIV-1). Similarly, shedding of HIV-2 in genital secretions is much lower and the virus levels in semen strongly correlate with plasma viral load levels (Sharp and Hahn 2011). The rates of sexual and vertical transmission of HIV-2 infection are around 5–9 times and 10–20 times lower, respectively, than for HIV-1 (Carvalho et al. 2011).

HIV-2 Infection: The European Scenario

In recent decades, HIV-2 infection has also expanded to some European countries, mainly Portugal and France, because of the historical and persistent social, cultural, and economic ties between these nations and countries in West Africa that promote a strong migrant population flow. Infection with HIV-2 has also been identified in North America, South America (Brazil), and in India (Fig. 2). The most frequent transmission route is usually heterosexual contact with individuals from endemic areas or who have traveled in those regions.

In Europe and other Western nations it is also difficult to assess the exact prevalence of HIV-2 infection, since asymptomatic patients do not usually seek medical attention: moreover barriers to accessing health services such as cultural beliefs and language constraints are likely to delay the diagnosis in immigrant populations. Furthermore, few clinicians outside West Africa are likely to consider HIV-2 infection in their differential diagnosis, further reducing the likelihood of diagnosing the infection.

In Europe, Portugal is the country with the highest reported prevalence of HIV-2 infection, estimated as being the cause of 4.5% of all AIDS reported cases at the end of twentieth century (Soriano et al. 2000). HIV-2 infection has been present in Portugal since the early 1980s. HIV-2 infection in Portugal is probably related to population flows, such as the mobilization of soldiers in the 1960s and 1970s to former Portuguese colonies that took place during the independence wars, and later repatriation and migration of people from African endemic territories. Nowadays, strong cultural and economical relations still remain and perpetuate the close relationships



HIV-2 Infection in Europe, Epidemiology of, Fig. 2 Global HIV-2 infection prevalence: ~1,000,000 people infected in West Africa. Highest prevalence: Guinea-Bissau (8–10% general population: >20% in

55–80 age group). Highest prevalence in Europe: Portugal, followed by France (Adapted: Rowland-Jones S, CROI 2007)

H

between Portuguese and African nations, particularly, Angola, Cape Verde, Mozambique, and Guinea Bissau.

HIV-2 infection in Portugal is almost entirely due to subtype A, with a cumulative number of cases of 1813 notified by the end of the year 2008 (Sharp and Hahn 2011). In the beginning of 1990s, HIV-2 infection accounted for about 10% of the annually notified AIDS cases, but a significant drop was reported in the following decade, showing a decrease to 2.3% in 2008. Dual infections (HIV-1 and HIV-2) accounted for a minority of reported cases (3.6%) (Carvalho et al. 2011).

A recent published study characterized a sample of 442 patients living in Portugal, representing a wide geographic distribution and 37% of all HIV-2 infection cases notified in this country by the end of 2007 (Carvalho et al. 2011). Cases were analyzed over a period of almost 20 years (1985–2007), revealing the presence of distinct characteristics according to the date of diagnosis. Until 2000, the majority of HIV-2 infected patients were of Portuguese origin and were mainly men living in the north of the country. However, from 2000 until 2007, patients diagnosed with HIV-2 infection were predominantly West African immigrant females, residing in the capital Lisbon, located in

the central region of Portugal. The most frequent transmission route identified was heterosexual contact (58.8%); blood transfusion was referred to in 15.4% of cases; injection drug use in 2.3%; and 1.1% of patients were men that have sex with men. Vertical transmission was a rare event (0.9%). As expected, the majority of patients were asymptomatic at diagnosis (64%) and 45% remained treatment naïve.

In France, of 10,184 new HIV diagnoses between 2003 and 2006, 1.8% were infected with HIV-2 (1.6% with HIV-2 monoinfection and 0.2% with HIV-1 and HIV-2 dual infection) (Barin et al. 2007). In this study, most patients infected with HIV-2 were citizens of a West African country (65%), mainly Ivory Coast, Mali, and Senegal, but 12% were European (20 from France and 2 from Portugal). Female gender accounted for more than half the cohort (63%) and 72% of patients acquired the infection by heterosexual transmission.

Data collected from the French National Agency for AIDS Research, published in 2001, characterized a cohort of 124 HIV-2 infected patients (Damond et al. 2001). Sequencing and phylogenetic analysis revealed significant diversity among HIV-2 strains isolated in France, to the

extent that one third of patients were infected with subtype B. There were no differences between the epidemiological or clinical aspects between patients infected with HIV-2 subtype A or with HIV-2 subtype B, suggesting that both subtypes show similar pathogenic behavior. However, as HIV-2 subtype B shows significant viral diversity with substantial differences from subtype A, this could have public health implications for HIV-2 screening, diagnosis, and appropriate treatment.

There is a lack of systematic and updated prevalence data or estimates for HIV-2 infection in other European countries.

A recent epidemiological study published in 2007 performed in a Northern Italian Center screened 2941 HIV-infected patients and reported a prevalence of 10.6% of HIV-2 infection (Costarelli et al. 2007). In that same evaluation, most HIV-2 patients had a West African origin, predominantly from Ghana, Senegal, Nigeria, and Guinea. No differences were noted in the patient gender distribution and, once again, the predominant transmission route was unprotected heterosexual intercourse.

Between 1985 and 2003, 1324 individuals were diagnosed with HIV infection in the United Kingdom and Northern Ireland who had probably acquired the disease in West Africa (Dougan et al. 2005). Of those, 69% were infected by HIV-1 and 6% by HIV-2 – the HIV type was not reported for the remainder. As expected, 96% reported heterosexual contact as the likely risk factor: of note, women with HIV-2 were older at diagnosis (median age 35 years) than those with HIV-1 (median 30 years).

In Spain, from 1988 to 2006, 146 new HIV-2 infections were identified. The Belgium-Luxembourg database included 65 patients with HIV-2 in data collected up to 2007 (Carvalho et al. 2011).

While the number of HIV-2 infection diagnoses remains relatively low and stable over time in European countries, clinicians should be aware that the HIV-2 prevalence can be high in particular African settings. The growing migrant African population should be considered as an important target to screen for infection with both HIV-1 and HIV-2, in order to identify and treat infected individuals promptly, thereby reducing both the risk

of onward transmission and the development of HIV disease.

The scarcity of available HIV-2 infection data emphasizes the need for a systematic data collection system in order to characterize the prevalence of HIV-2 infection in each community, trying to address the specific needs for diagnosis, clinical monitoring, and treatment management of this infection.

Monitoring the progression of HIV-2 infection is, essentially, based on the evolution of clinical and immunological markers. Plasma HIV-2 viral replication is usually lower compared to HIV-1 replication rates, and validated assays for clinical quantification are not yet available. Several laboratories provide different “in-house” real-time PCR assays with primers and probes located in various regions of the HIV-2 genome. A collaborative effort to generate an accurate and standardized plasma HIV-2 RNA assay has been made in recent years (ACHIEV₂E), which has significantly improved the determination of viral load for HIV-2 group A. Unfortunately, a similar improvement was not achieved for group B HIV-2 RNA quantification, probably a consequence of the high genetic diversity found in HIV-2 group B isolates (Damond et al. 2011).

Conclusion

Although less prevalent than HIV-1, HIV-2 infection remains an important public health issue in West Central Africa, as well as in some European countries.

Both viruses share several genetic, pathogenic, and epidemiological characteristics but, at the same time, they have distinct biological features regarding transmission efficiency, time of clinical latency, cellular receptor usage, viral replication level, immune activation response, and susceptibility to current antiretroviral drugs.

Understanding those differences may prove vital to the development of more efficient treatment and prevention strategies for HIV-1 and HIV-2 infections.

Currently, there are several questions that remain unanswered that illustrate the difficulties

in achieving optimal management of HIV-2 infection, such as identifying the optimal time to start antiretroviral therapy, the ideal sequence of antiretroviral regimens, the validation of a gold standard RNA quantification assay, and an algorithm to analyze the impact of ART resistance mutations interpretation. The design of randomized controlled clinical trials would constitute a fundamental step in response to some of these persistent concerns in HIV-2 management.

Cross-References

- ▶ [Antibody Response to HIV-2](#)
- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Cellular Immune Response to HIV-2 Infection](#)
- ▶ [Epidemiology of HIV-2 Infection in West Africa](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [HIV-2 Diagnosis and Viral Load Measurements](#)
- ▶ [HIV-2 Envelope: Structure, Diversity, and Evolution](#)
- ▶ [HIV-2 Infection: The Role of Immune Activation in Pathogenesis](#)
- ▶ [HIV-2 Transmission](#)
- ▶ [Immunogenetics of HIV-2 Infection](#)
- ▶ [Molecular Biology of HIV-2](#)
- ▶ [Role of Dendritic Cells in HIV-2 Pathogenesis](#)
- ▶ [Transmission HIV-2: Origin, Epidemiology, and Phenotype](#)

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HIV-2 Infection: The Role of Immune Activation in Pathogenesis

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Definition

Human immunodeficiency virus type 2 (HIV-2) is a retrovirus of the *Lentivirus* genus that may cause AIDS. However, the pace of disease development during an HIV-2 infection is much slower as compared with the course of an HIV-1 infection. In fact, many HIV-2-infected individuals may never develop AIDS. Several parameters influence the rate of HIV disease progression, and heightened level of chronic immune activation is one such factor. Chronic immune activation can be depicted by a wide range of dysregulated immunological responses, including increased frequencies of activated, but virus-unspecific, immune cells. Elevated levels of circulating cytokines, inflammation markers, and microbial products are also traits of chronic immune activation during an HIV infection.

HIV-2 Infection, a Long-Term Nonprogressive Lentiviral Disease

The human immunodeficiency virus type 2 (HIV-2) is a *Lentivirus* with distinct pathogenesis, despite its close relationship to HIV-1 (Table 1) (de Silva et al. 2008). HIV-2, which was introduced to humans by the zoonotic spread of simian immunodeficiency virus (SIV) from sooty mangabeys, is in contrast to the globally spread HIV-1

HIV-2 Infection: The Role of Immune Activation in Pathogenesis, Table 1 Comparison of HIV-1 and HIV-2 in relation to pathogenesis

	HIV-1	HIV-2
Distribution	Pandemic	Endemic in West Africa
Clinical outcome	Majority develop AIDS, LTNP only ca 2%	Majority LTNP
CD4+ T-cell decline	Steady Decline	Normal in LTNP, but declining in progressors
Plasma viral load	Set point 10 ⁴ –10 ⁵ RNA copies/ml	At least 1 log lower, and many times undetectable
Immune activation	Elevated	Less pronounced, but increasing in progressors

mainly confined to West Africa (► [The Epidemiology of HIV-2 Infection in West Africa](#) and ► [Phylogeographic Insights into the Origins and Epidemic History of HIV-2](#). Furthermore, the rates of transmission (► [HIV-2 Transmission](#) and ► [Transmission HIV-2: Origin, Epidemiology, and Phenotype](#)) and progression (► [Natural History and Clinical Features of HIV-2 Infection](#)) towards AIDS are slower in HIV-2 infection as compared to HIV-1 infection. In fact, the common clinical course of HIV-2 infection is in several aspects similar to that of the small group of HIV-1 long-term nonprogressors (LTNP) (► [Long Term Non-progressors & Elite Controllers](#)). Moreover, many HIV-2-infected individuals also display features of HIV-1 elite controllers (► [Long Term Non-progressors & Elite Controllers](#)), since plasma viral load is commonly not detected. This means that most HIV-2-infected individuals remain asymptomatic and display viral load control for an extended period of time. However, a subset of HIV-2-infected individuals still progresses at a rate similar to most HIV-1-infected individuals.

Chronic Immune Activation in Progressive HIV Infection

In HIV-1 infection it is well established that chronic and progressive disease is characterized

by heightened level of systemic immune activation (Douek et al. 2009). This condition, which is known as ► [chronic immune activation](#), can be depicted by a wide range of dysregulated immunological responses. This includes increased T-cell turnover higher frequencies of T cells expressing activation markers (► [Lymphocyte Activation Markers](#)), such as CD38 and HLA-DR; as well as polyclonal B-cell activation translating into hypergammaglobulinemia. In addition, elevated levels of proinflammatory cytokines (► [Inflammatory Cytokines](#)) and a broad range of soluble immune activation markers (► [Soluble Markers of Immune Activation](#)), including beta-2 microglobulin (β 2m), can be detected in peripheral blood of chronically infected HIV-1 individuals. Indeed, level of chronic immune activation has been shown to be a marker that independently predicts HIV-1 disease progression, even better so than plasma viral load. Multiple instigating factors behind chronic immune activation have been proposed, including excessive innate immune activation (► [Chronic Immune Activation](#) and ► [Immune Activation and HIV Transmission](#)) elevated T-cell turnover. Additionally, microbial translocation (► [Microbial Translocation](#)) from the gut, as the result of preferential replication of the virus in the gut-associated lymphoid tissue (GALT), has been implicated in immune activation. However, the relative contributions of the different factors to chronic immune activation in HIV-1 infection are not well established. Furthermore, the underlying mechanisms behind the long-term nonprogressive disease course of most HIV-2-infected individuals and the influence of chronic immune activation on this outcome are not fully understood. Yet, accumulating findings point to a complex interplay between the virus and the host that influences level of chronic immune activation also in HIV-2 infection.

HIV-2 Clinical Progression, Viral Load, and Immune Activation

It is well acknowledged that the clinical progression rate and decline in CD4+ T cells (► [Natural](#)

[History and Clinical Features of HIV-2 Infection](#)) in the common HIV-2 infection are significantly slower than that generally observed in HIV-1 infection (Table 1) (de Silva et al. 2008). One explanation for this can be attributed to the fact that the plasma viral load set point (► [TRIM5 Alpha and HIV-2 Infection](#)) is low or undetectable in HIV-2 infection. Still, elevated plasma viral load in HIV-2 infection can be linked to shorter survival. However, it is clear that plasma viral load is not the sole determinant of HIV-2 disease progression. Level of immune activation, for example, assessed by the analysis of the concentration of β 2m in plasma, has been shown to be independently predictive of survival also in HIV-2 infection (Jaffar et al. 2005). Elevated β 2m plasma levels have also been documented with decreasing CD4+ T-cell count and disease severity (Whittle et al. 1993). Furthermore, HIV-2-infected individuals display, in a manner similar to HIV-1 long-term nonprogressors and elite controllers, heightened level of immune activation markers as compared with HIV-seronegative subjects. Both increased concentrations of soluble immune activation markers and higher frequencies of activated T cells can be observed, also in HIV-2 infections where plasma viral load cannot be detected. Still, in line with the milder disease course, the common feature of HIV-2 infection is less apparent immune activation as compared to the general pattern of HIV-1 infection. The causal relationships between CD4+ T-cell depletion, plasma viral load, and immune activation in HIV-2 infection are nonetheless a matter of discussion. One report showed that numbers of activated T cells were elevated with lowered CD4+ T-cell count, despite the fact that a majority of the study subjects had undetectable viral loads, suggesting that immune activation is directly associated with the CD4+ T-cell count and only secondary to viral load (Sousa et al. 2002). Another study conducted on a different cohort observed no relationship between CD4+ T-cell count and markers of immune activation in HIV-2-infected individuals with undetectable viral load (Leligdowicz 2010a). The latter study, which also included viremic HIV-2-infected subjects, instead found a direct relationship between

levels of immune activation and plasma viral load (Leligdowicz 2010). The explanation for the divergent findings may be several, including more viremic subjects in one group, indicating individuals experiencing longer duration of infection. As of today, no study has yet addressed the impact of HIV-2 duration on immune activation. This is mainly because information on HIV-2 seroconversion dates is rare or nonexistent. Another explanation for the different observations could be the fact that the two study cohorts resided in two distinct locations, which may translate into divergent genetic background (► [Immunogenetics of HIV-2 Infection](#)) and also altered exposure to various copathogens (► [Immunopathogenesis of HIV Co-infections](#)). Thus, higher microbial burden, including parasites, helminths, and other pathogens, may elevate immune activation independent on HIV infection disease stage. Studies addressing the impact of duration of HIV-2 infection and copathogens on immune activation in HIV-2 infection may further clarify the causative relationships.

Innate Immune Activation During HIV-2 Infection

The detailed mechanisms that trigger chronic immune activation in pathogenic lentiviral infections are to be revealed; however, several lines of evidence suggest multiple causes. From studies of SIV infections in the sooty mangabey (► [Non-Pathogenic SIV Infection of Sooty Mangabeys](#)) and African green monkeys (► [SIV Infection of African Green Monkeys](#)), it is clear that in these natural SIV hosts, viremia may exist without causing chronic immune activation and pathogenesis (Bosinger et al. 2011). These studies indicate that the downregulation of the strong innate immune response, triggered during the acute infection (► [Immune Activation and HIV Transmission](#)) of both HIV-1 and SIV, is associated with non-pathogenic lentiviral infections. Instead, in HIV-1 infection the hyperactivation of innate responses (► [Chronic Immune Activation](#) and ► [Immune Activation and HIV Transmission](#)), including type 1 interferon signaling, appears to be

maintained and linked to the establishment of chronic immune activation. Suggested mechanisms behind the benign outcome in the natural SIV hosts include weaker signaling via the toll-like receptors (TLRs) expressed by plasmacytoid dendritic cells (pDCs). Since acute phase HIV-2 infections have not been described, studies on innate immune responses and the establishment of chronic immune activation during the transition from the acute to the chronic phase of the infection are not available. However, it has been reported that frequencies of circulating pDCs (► [Natural Killer Cells and Innate Immunity in HIV-2 Infection](#) and ► [Role of Dendritic Cells in HIV-2 Pathogenesis](#)) in chronically infected HIV-2 individuals, both viremic and aviremic, are reduced as compared to HIV-seronegative individuals, in a manner similar to observations reported for HIV-1-infected subjects (Cavaleiro et al. 2009). Moreover, the degree of pDC depletion in HIV-2-infected subjects appears to be negatively correlated with the expression of activation markers on both CD4+ and CD8+ T cells. In addition, mRNA expression levels of *MxA*, a gene mainly induced by interferon-alpha (IFN- α), were found to be increased in parallel with elevated immune activation, higher viral load, and decreasing frequency of pDC. Reduced in vitro responsiveness of pDC, assessed by IFN- α expression after TLR9 stimulation, also seems to correlate with elevated expression of immune activation markers, as well as declining CD4+ T-cell count and higher viral load (Cavaleiro et al. 2009; Nowroozalazadeh et al. 2009). Thus, these studies suggest that pDCs are hyperactivated and depleted/anergized in vivo and less responsive to in vitro restimulation during progressive HIV-2 infection. These observations have been made despite the fact that in vitro experiments indicate that pDCs, as well as myeloid DC (mDC), are less susceptible to HIV-2 infection as compared to HIV-1 infection (Duvall et al. 2007). Hence, mechanisms, independent of productive infection, may influence the frequency and function of these cells in vivo. On the other hand, studies have revealed that HIV-1 and HIV-2 are differently sensitive to an intracellular restriction factor (► [Interactions](#)

Between HIV-2 and Host Restriction Factors) present within myeloid cells, sterile alpha motif and HD domain 1 (SAMHD1) (Laguette and Benkirane 2012). The Vpx (► [Molecular Biology of HIV-2](#)) protein, which is produced by HIV-2 and related SIV strains but is lacking in HIV-1, counteracts SAMHD1. Accordingly, replication of HIV-1 is restricted by SAMHD1 in myeloid cells, whereas HIV-2 is not. These results may seem paradox, but it has been suggested that HIV-2 antigen presentation by myeloid cells favors a more efficient antiviral immune response. However, the impact that the SAMHD1-Vpx interaction has on chronic immune activation and disease progression in HIV-2 infected is to be revealed. Similarly, the frequency and function of myeloid cell, monocytes, and mDC, in HIV-2-infected subjects at different disease stages, are also to be disclosed. Nevertheless, it appears that the production of mDC-secreted cytokines after in vitro TLR7/8 stimulation of the whole blood from HIV-2-infected subjects is comparable to that of HIV-negative individuals, whereas corresponding in vitro responsiveness appears defective in HIV-1 infection (Nowroozalizadeh et al. 2009). Still, studies on the kinetics of innate immune activation during the transition phase, from acute to chronic disease, in HIV-2 infections may reveal regulatory properties that control systemic immune activation.

Regulation of T-Cell Activation in HIV-2 Infection

Another factor that has been proposed to influence chronic immune activation is the interaction between the negative regulatory factor (Nef) (► [Molecular Biology of HIV-2](#)) protein and the T-cell receptor (TCR)-CD3 complex. The interaction between Nef of HIV-2 mediates downregulation of the TCR-CD3 complex and inhibition of cell death, whereas Nef of HIV-1 does not (Table 1) (Schindler et al. 2006). In HIV-2-infected individuals with low viral load, being either nonprogressor or progressors, the ability of Nef to downregulate TCR on CD4+ T cells has been found to correlate with the level

of T-cell immune activation (Feldmann et al. 2009). However, the TCR down-modulating effect of Nef did not appear to protect against disease progression in these HIV-2-infected subjects. Instead, in viremic HIV-2 infections, it has been shown that increased potency of *nef* alleles to mediate down-modulation of TCR-CD3 and CD28 correlates with higher CD4+ T-cell counts (Khalid et al. 2012). It was also shown that down-modulation of TCR-CD3 and CD28 suppresses the expression of markers linked to T-cell exhaustion (► [HIV Associated Immune Exhaustion](#)) and death, such as Fas, Fas ligand, programmed death-1 (PD-1), and cytotoxic T-lymphocyte antigen 4 (CTLA-4). Thus, these findings suggest that the ability of HIV-2 Nef to act on T-cell activation may translate into better prognosis for the viremic individuals by suppressing T-cell exhaustion and death. In line with these observations, it has been reported that elevated expression levels of PD-1 and its main ligand, PD-L1, on CD4+ and CD8+ T cells of HIV-2-infected individuals, correlate with increased immune activation and CD4+ T-cell depletion (Tendeiro et al. 2012). Thus, similar to the immunopathogenic events in HIV-1 infection, it appears that immune activation in progressive HIV-2 infection triggers the upregulation of inhibitory receptors, such as PD-1, which in turn may downregulate virus-controlling immune responses. Indeed, strong Gag-specific T-cell responses (► [The Cellular Immune Response to HIV-2 Infection](#)) are elicited in long-term nonprogressive HIV-2 infections, and frequency of highly avid and early differentiated HIV-2 Gag-specific CD8+ T cells appears to be inversely correlated with the level of immune activation (Leligdowicz 2010b). Furthermore, in vitro findings suggest that the HIV-2 surface envelope glycoprotein (► [HIV-2 Envelope: Structure, Diversity and Evolution](#)), gp105, may suppress T-cell activation in a monocyte-dependent but T regulatory cell-independent manner (Cavaleiro et al. 2007). In contrast, gp120 envelope glycoprotein of HIV-1 did not suppress T-cell activation. Thus, the interaction between virally encoded proteins and host defense structures may regulate the level of T-cell immune activation in HIV-2 infection. Moreover, this regulation may

take place within infected target cells or on a bystander cell level.

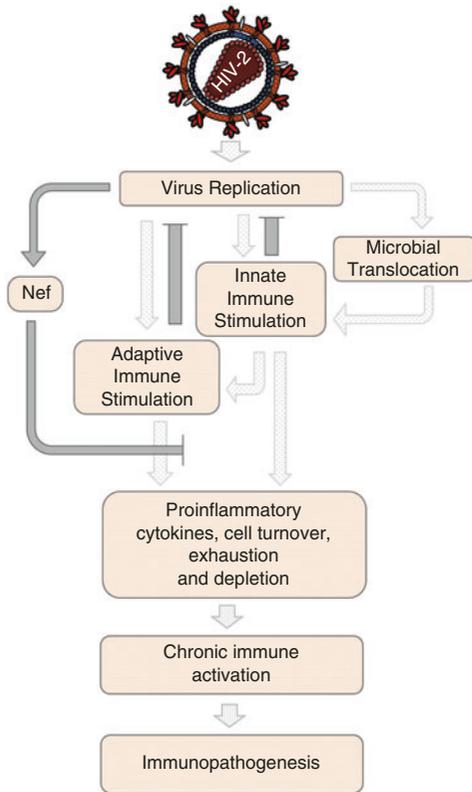
Microbial Translocation and HIV-2 Infection

With the identification of massive depletion of CD4⁺ T cells in the GALT (► [Microbial Translocation](#)) during the acute phase of HIV-1 infection in humans and SIV infection in nonhuman primates, the focus on pathogenic effects mediated by these viruses has been shifted to events taking place in the gastrointestinal tract. Therefore, in line with damage to the gut mucosa, the level of microbial translocation (► [Microbial Translocation](#)) has been studied in different pathogenic and nonpathogenic lentiviral infections. Microbial translocation, being translocation of microbes and/or microbial products from the gut to the peripheral blood without the cause of overt bacteremia, is usually analyzed by quantification of lipopolysaccharide (LPS), and LPS triggered host-related factors in plasma. In these studies it has been shown that elevated microbial translocation can be detected in chronically HIV-1-infected humans and SIV-infected rhesus macaques (Douek et al. 2009). Furthermore, it has been proposed that microbial translocation contributes to chronic immune activation in HIV-1 infection since degree of microbial translocation correlates with plasma concentrations of IFN- α and frequency of activated CD8⁺ T cells. In addition, HIV-1-infected elite controllers display lower levels of microbial translocation, in parallel with reduced immune activation, than detected in viremic individuals. Similarly, microbial translocation in HIV-2-infected subjects with undetectable plasma viral load has been found to be significantly lower than in those with viremic infections (Nowroozalizadeh et al. 2010). In addition, it has been noted that microbial translocation correlates with severity of disease independently of type of HIV infection, as studied in a cohort of HIV-1- or HIV-2-infected individuals residing within the same West African location. However, care has to be taken when drawing causal relationships between microbial translocation, chronic immune

activation, and disease progression in HIV infections. This since HIV-mediated microbial translocation can be masked in cohorts residing in locations where infections with other gastrointestinal pathogens are abundant (Redd et al. 2009). Thus, for the clarification of causal relationships between microbial translocation, chronic immune activation, and viremia in HIV-2 infections, longitudinal studies also taking into account copathogens would be helpful. Furthermore, studies on the distribution and degree of CD4⁺ T-cell depletion in GALT of HIV-2-infected subjects, which are lacking, could also shed light on the relationship between pathogenic events localized to the gut.

Conclusions

Findings from accumulating studies suggest that chronic immune activation is not only a contributing factor to HIV-1 disease progression but also part of the pathogenesis of HIV-2 infection. However, along with the long-term nonprogressive disease, chronic immune activation is less pronounced in HIV-2-infected individuals, as compared to that detected during the common course of HIV-1-infected subjects. The reason for this is likely multifactorial, including low or undetectable viremia in HIV-2 infection, which can be the consequence of viral replication features, as well as the efficacy of virus-controlling immune responses (Fig. 1). Still, immune activation elevated above the level found in HIV-seronegative individuals is detected in aviremic HIV-2-infected subjects, similar to HIV-1 elite controllers, during chronic asymptomatic infections, suggesting that early events in the infection may play an important role in the trigger of chronic immune activation. The knowledge on immunological and virological events taking place in acute HIV-2 infections is, however, lacking and if available could shed light on the causal relationships between immune activation, viremia, and disease progression. Additional mechanisms that influence level of immune activation and disease progression in HIV-2 infections have been shown to be related to the



HIV-2 Infection: The Role of Immune Activation in Pathogenesis, Fig. 1 Schematic view on factors that may contribute to (light grey arrows) or restrict (dark grey arrows) HIV-2 chronic immune activation

interactions between virally encoded proteins, such as Nef, and target cell structures linked to the regulation of immune activation. Thus, comparative longitudinal studies on the different HIV-2 progressor groups, meaning HIV-2-infected subjects which disease progression rate is similar to HIV-1-infected subjects and the long-term nonprogressive HIV-2-infected individuals, would be helpful in delineating full understanding of factors that drives disease progression in HIV-2 infection. The relative impact of chronic immune activation to disease progression in different HIV-2-infected progressor groups would optimally also be conducted in cohorts longitudinally followed from the time point of infection and resident within the same geographical area, with similar genetic background and comparable environmental exposure to other pathogens. However,

with the declining HIV-2 incidence, the window of opportunity to study the long-term non-progressive lentiviral disease caused by this virus is becoming smaller.

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HIV-2 Neurological Manifestations

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Definition

Neurological manifestations of HIV-2 include neurocognitive dysfunction and peripheral neuropathy. They may include primary complications caused by the HIV-2 virus or secondary complications due to opportunistic illnesses or toxicity of therapy that develops during chronic HIV-2 infection.

Neurological Disease from Human Immunodeficiency Virus (HIV) Infection

One of the most underappreciated aspects of retroviral infection has been the serious neurological complications that commonly occur. In the case of HIV-1, it is known that shortly after infection, the virus enters the nervous system and can be recovered from it at almost any time during the ongoing years of infection. As immune dysfunction develops, there is increasing prevalence of both neurocognitive decline and peripheral neuropathy. Furthermore, opportunistic neurological conditions such as cryptococcal meningitis, toxoplasmic encephalitis, cytomegalovirus encephalitis, progressive multifocal leukoencephalopathy, and primary CNS lymphoma develop as immunodeficiency emerges. The neurological complications, both central cognitive and peripheral nerve system related, are believed to be driven by the HIV virus itself but mediated through inflammatory responses and viral protein toxicities. While the severity of these problems has been attenuated by the development of effective antiretroviral therapy, the problems persist even in optimally treated patients. Thus, current HIV-1 patients are known to have ongoing neurocognitive impairment and peripheral neuropathy, which become clinically significant and especially concerning as patients age. Much less is known about the parallel situation with HIV-2 which is endemic in West Africa and seen in populations emerging from West Africa.

HIV-2 Virus Epidemiology and Biology

HIV-2 is a retrovirus relatively confined to West Africa, with the highest prevalence (8%) reported in Guinea-Bissau (Poulsen et al. 1993). Emigrants from West Africa with HIV-2 may also be seen in developed countries, most commonly in Portugal, France, and Spain, but are rare enough that coordinated studies have never been attempted outside of West Africa. The importance of HIV-2 as a model for evaluation of mechanisms of key biological properties of HIV-1 has been underutilized, including in reference to

neurological disease. Like HIV-1, HIV-2 uses co-receptors CCR5 and CXCR4 to infect CD4 cells and demonstrates similar proviral loads; the two viruses share 60% homology of key genes *gag* and *pol* (Leligdowicz and Rowland-Jones 2008). Despite the genetic similarity between HIV-1 and HIV-2, most individuals infected with HIV-2 are long-term nonprogressors (LTNPs) with a normal life span, undetectable viral loads, and normal CD4 counts (Leligdowicz and Rowland-Jones 2008). Often treatment is used late in HIV-2, in part because of the more indolent characteristics of the disease, in part because of the limitation of resources in endemic areas, and in part because of the more limited armamentarium of antivirals active against HIV-2. With the current HIV research goals including ways to cure those infected or at least achieve preservation of health without pharmacologic interventions, HIV-2 provides hope as a potential model. HIV-2 patients can progress to AIDS, but they die at a higher CD4 count compared to their HIV-1 counterparts (Martinez-Steele et al. 2007). Less immune activation, neutralizing antibodies, and broad cytokine production by CD4 and CD8 cells are among the proposed factors of disease attenuation in HIV-2 (Leligdowicz and Rowland-Jones 2008). Because more than 80% of individuals with HIV-2 are LTNPs (compared to less than 2% of individuals with HIV-1), HIV-2 has been studied in the hopes of discovering the immune responses responsible for better viral control and ultimately the increased survival seen in the majority of HIV-2-infected individuals (Leligdowicz and Rowland-Jones 2008).

HIV-2 and Neurological Disease

Since HIV-2 is largely found in West Africa where formal neurological evaluations are rarely performed, there is a paucity of observations relevant to neurological manifestations of HIV-2. However, limited observations are consistent with neurological disease being part of the pathophysiology of chronic HIV-2 infection. The characterization of HIV-2 neurological disease has mostly been limited to case reports and rather

simple studies with imprecisely defined criteria for neurologic diagnosis. Studies in the early 1990s from West Africa noted myelopathy, neuropathy, meningoencephalitis, and pathologically diagnosed HIV-encephalitis among HIV-2 individuals (Ramiandrisoa et al. 1991; Lucas et al. 1993). Coinfection with HTLV-1 and HIV-1 is common in West Africa, and careful exclusion of these coinfections is not always perfectly documented. However, the evidence published is consistent with HIV-2 sharing neuropathologic stigmata also described with HIV-1. More recently, Martinez-Steele et al. (2007) noted “neurological impairment sufficient to prevent independent daily activities” (taken as a presumptive diagnosis) in 10.3% of its HIV-2 cohort. Other case reports and studies have reported demyelinating encephalomyelitis, toxoplasmosis, cryptococcal meningitis, and spastic paraplegia (Klemm et al. 1988; Mabey et al. 1988; Moulignier et al. 2006; Martinez-Steele et al. 2007), all neurological complications seen in HIV-1.

Biological markers of neurological involvement are even less commonly reported. The evidence of HIV-2 CSF infiltration and CNS histopathology indistinguishable from HIV-1 indicates a basis for neurological disease in HIV-2 individuals; multinucleated giant cells in brain specimens and HIV-2 RNA in CSF have been demonstrated in small numbers of HIV-2 individuals (Lucas et al. 1993; Arvidson et al. 2004). However, CSF and neuropathological studies remain rare in areas where HIV-2 is endemic, and the benign clinical course seen in the majority of HIV-2 individuals has discouraged invasive evaluations of those living with this disease. Thus, the details of the neurobiology of HIV-2 chronic infection are poorly described currently.

Because HIV-2 has been limited predominantly to West Africa, neurologic studies on HIV-2 have encountered the challenges of conducting research in the developing world. In developed countries, cognitive involvement has been recognized by quantitative neuropsychological testing, with significant normative data collected in the same countries. In the developing

world, neither the norms nor many of the tests are considered reliable. Much groundwork is required for neuropsychological testing to be validated and local matched control participants to be evaluated for normal values. An alternative approach to gain reliable insights has been to make blinded comparisons of community matched volunteers with and without the retroviral infection. Recently, a blinded, randomized controlled study was undertaken in Guinea-Bissau that systematically evaluated HIV-2 individuals and community controls by clinical, neurological, and neuropsychological examination (Choi et al. 2011). This study included patients with advanced HIV-2 (CD4 < 200) and patients with HIV-2 for over 20 years as well as comparative participants in the same community.

HIV-2 and Cognitive Impairment

One area of interest in HIV-1 has been the prevalence of HIV-associated neurocognitive disorders (HAND), which varies from severe (HIV-associated dementia or HAD), to mild functional impairment (minor neurocognitive disorder or MND), to subtle disease associated with sub-optimal testing performance but without clinical impairment (ANI or asymptomatic neurocognitive impairment). One of the special tools that has been used to tackle the challenge of assessing cognitive performance in the developing world is the International HIV Dementia Scale (IHDS) (Sacktor et al. 2005). This test includes immediate and delayed recall of four common words in the local language, a motor learning task with alternating hand movements, and timed, rapid, alternating hand movement task. This task is probably most appropriate for detecting HIV-associated dementia. HAD, is seen primarily in advanced or untreated patients (McArthur et al. 2005). Based on the rate of >80% LTNP with HIV-2, a relatively low prevalence of dementia has been assumed in HIV-2 individuals. In Guinea-Bissau, there was no evidence of a difference in dementia rates among HIV-2 and seronegative controls, as assessed by the IHDS. However, both HIV-1 and

HIV-2 studies have suggested that lower education attainment may have a greater negative effect on this measure than the effect of HIV serostatus itself (Waldrop-Valverde et al. 2010; Choi et al. 2011), making the IHDS a less sensitive measure in populations without formal education. The relatively simple IHDS tasks are not well designed for the detection of more subtle neurocognitive impairment.

Exclusion of the milder forms of HAND has been very difficult even under optimal research conditions in developed countries, and the task is substantially more difficult across cultural and language barriers. Thus, it is unclear how prevalent MND or ANI are in the developing world. It is possible that similar to other clinical manifestations being milder compared to HIV-1, HIV-2 yields a smaller effect size for the measures of cognitive impairment. In HIV-1 studies, several investigations in geographically diverse areas of the world have demonstrated HAND in the developing world in a pattern similar to what has been seen in developed countries. It remains to be determined whether milder forms of neurocognitive disease are prevalent in HIV-2. There is a suggestion that those with more severe HIV-2 by CD4 count have more subjective complaints of concentration and more difficulty in verbal fluency, as assessed by an animal naming task (Choi et al. 2011). These findings may reflect early HAND that testing is unable to confidently measure. However, with the limited observations to date, this remains unconfirmed.

HIV-2 and Peripheral Neuropathy

Peripheral neuropathy is the most common neurologic disorder in HIV-1 (Pardo et al. 2001), and it appears that HIV-2 may demonstrate a similar pattern of peripheral neuropathy as HIV-1. Retroviral disease severity is a risk factor for peripheral neuropathy in HIV-2, with neuropathy more likely in those HIV-2 individuals with lower CD4 counts, as has been widely demonstrated in HIV-1 (Lichtenstein et al. 2005). However, the rate of peripheral neuropathy and neurological exam findings has been shown to be no different

from the general population. To investigate this area more reliably in HIV-2-infected individuals, there is a need for studies controlling for meaningful comorbidities such as alcohol intake, undiagnosed diabetes, and other nutritional deficiencies that are likely to contribute to peripheral nerve disease.

HIV-2 and Myelopathy

Myelopathy, presenting with increasing spastic paraparesis and often loss of bladder function, as well as a variety of sensory deficits, is well recognized as a complication of HIV-1. In HIV-1, vacuolar myelopathy (VM) is the most common neuropathological manifestation of myelopathy, but it is usually found alongside those with AIDS (McArthur et al. 2005). While myelopathy has been documented in HIV-2 patients (Ramiandrisoa et al. 1991), given that most individuals have higher CD4 counts in HIV-2, myelopathy has rarely been described in HIV-2 patients (zero cases in the recent study by Choi et al. (2011) where coinfection with HTLV-1 was also carefully excluded).

Conclusions

There is yet to be a study with a large battery of neuropsychological tests evaluating all the major domains of function in HIV-2-infected patients. Performing such testing has serious limitations in the populations mostly infected by HIV-2. In particular, low formal education level in some countries limits the type and complexity of testing traditionally performed. For example, the Color Trails has been used as a substitute for the widely used Trail Making Test, but this test requires knowledge of Western numerical conventions. It is thus impossible to use in the nonreading populations in rural African countries such as Guinea-Bissau where HIV-2 is most prevalent. Furthermore, norms from developing world populations (Sacktor et al. 2005) may be higher than the participants' in West Africa and limit some of the neurologic tools available at this

time. Attention to robust collection of norms is only now underway to better allow neurocognitive characterization in the developing world populations.

There is much room for further characterization of HIV-2 neurological complications. Given the frequency of neuropathy, quantitative measures of peripheral nerve integrity, such as nerve conduction studies and skin-punch biopsies, could be performed to better characterize peripheral neuropathy. Better metabolic and nutritional testing would help characterize comorbid factors as well as the type of peripheral nerve pathology seen in HIV-2. It appears that specific studies of the cognitive impact of HIV-2 will be difficult to conduct with the challenges of neurocognitive assessments across cultures, but increasing attention to norms and appropriate testing modalities should allow for more nuanced description of cognitive performance in the future. Biomarkers for neurocognitive dysfunction are being evaluated, and when they become available, HIV-2 may yet be finely characterized and become a fruitful neurological model of long-standing HIV-1.

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HIV-2 Transmission

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Definition

HIV-2 transmission encompasses the spread of the virus from one person to another. Human-to-human HIV-2 transmission can occur through

multiple routes including sexual, mother to child, and blood-borne.

Introduction

Not long after the discovery of HIV-1 in 1983 (Barre-Sinoussi et al. 1983) and HIV-2 in 1986 (Clavel et al. 1986) as the etiologic agents of AIDS, it became clear that the global HIV/AIDS pandemic was caused primarily by HIV-1 (De Cock et al. 1993). Of the ~34 million people living with HIV/AIDS and the >30 million AIDS-related deaths since the beginning of the epidemic, only ~1–2 million infections have been due to HIV-2 (although accurate global HIV-2 disease burden data is not available) (Gottlieb et al. 2008a). HIV-2 is endemic in West Africa but has had limited spread to other locales worldwide (see HIV Database: <http://www.hiv.lanl.gov>). Ultimately, the reason why there is no HIV-2 pandemic is due to different transmission dynamics between HIV-1 and HIV-2.

Genetic and epidemiologic evidence suggests that HIV-2 started as a zoonosis, originally transmitted from West African *sooty mangabeys* to humans, and that this probably occurred at least 8 times, represented by the HIV-2 groups A through H (see HIV Database: <http://www.hiv.lanl.gov>). Examination of stored sera suggests that HIV-2 has been circulating in humans in West Africa since at least 1966 (Kawamura et al. 1989), and phylogenetic data suggests it may have been present since the early 1900s (Lemey et al. 2003). HIV-2 appears to have reached a peak prevalence in West Africa in the 1980–1990s, with Guinea-Bissau having >10% adult prevalence (Poulsen et al. 1993). More recent data suggest that HIV-2 prevalence may be slowly declining in West Africa, although the reasons for such a decline remain unclear (Eholie and Anglaret 2006).

Routes of HIV-2 Transmission

Similar to HIV-1, routes of HIV-2 transmission have been reported to include heterosexual

(De Cock et al. 1993; Poulsen et al. 1993), men who have sex with men (MSM) (Brucker et al. 1987), mother to child (MTCT) (Matheron et al. 1990), and blood-borne (e.g., injection drug use (IDU) (Ferroni et al. 1987), blood transfusions (Dufort et al. 1988) and blood-derived products, and unsafe medical and traditional practices (Pepin 2011). The epidemiology of routes of transmission appears to have changed over the course of the epidemic, with unsafe medical practices possibly playing an important role in the 1960–1970s during the multiple wars of independence in West Africa during that time period (Lemey et al. 2003; Pepin 2011). The current predominant mode of HIV-2 transmission is thought to be heterosexual.

Risks of HIV-2 Transmission

Heterosexual Transmission of HIV-2

In West Africa, where the majority of HIV-2 transmission occurs, heterosexual contact has been the dominant mode of transmission (De Cock et al. 1993; Poulsen et al. 1993). Several studies suggest that HIV-2 is significantly less transmissible via heterosexual routes than HIV-1 (De Cock et al. 1993; Donnelly et al. 1993). A study by Gilbert and colleagues in Senegal suggests that HIV-2 infectivity is ~3.5 times less than that of HIV-1 (Gilbert et al. 2003). The most important correlate of HIV-1 sexual transmission is plasma viral load (Quinn et al. 2000), and while this is likely to be the case for HIV-2, it has yet to be demonstrated. The significantly lower viral loads in HIV-2 infection, compared HIV-1, are thought to be the reason for the substantially reduced transmission of HIV-2 (Simon et al. 1993; Gottlieb et al. 2002). Other HIV-1 transmission correlates include low CD4⁺ T-cell count, advanced clinical stage infection, and concurrent sexually transmitted infections (STIs); all of which are also likely to be important for HIV-2 sexual transmission.

HIV-2 MTCT

Similar to sexual transmission, mother-to-child transmission of HIV-2 infection has been shown

to be significantly less efficient than HIV-1, with several studies showing rates <5% without ART (Adjorlolo-Johnson et al. 1994).

Other Modes of HIV-2 Transmission

Although case reports of HIV-2 transmission in MSM and in IDU have been reported, the absolute and relative risks compared to HIV-1 are undefined; however, like heterosexual and mother-to-child transmission, the relative risk compared to HIV-1 is likely low. Given the high prevalence of HIV-2 in some regions of West Africa and its apparent inefficient transmission, some have suggested that unsafe medical and traditional practices may have amplified the epidemic early on (Pepin 2011).

Transmitted HIV-2 Drug Resistance

Assessing the extent and prevalence of HIV-2 transmitted drug resistance (TDR) has been hampered by the lack of validated and clinically available HIV-2 resistance genotyping assays. In addition, because of the relatively recent availability of ART for HIV-2 in West Africa and the small numbers of HIV-2 cases in developed countries, reports of transmitted drug resistance are rare. Transmitted drug resistance must also be differentiated from *de novo* mutations and surreptitious ARV use which may be difficult.

Jallow and colleagues reported the occurrence of the multi-nucleoside resistance mutation, Q151M, in a 66-year-old, ARV-naïve, HIV-2-infected female from rural Caio, Guinea-Bissau, from multiple samples dating back to 1997 suggesting transmitted drug resistance (Jallow et al. 2009). Ruelle and colleagues reported the occurrence of possible TDR in 2 of 21 samples ARV-naïve HIV-2 patients from Burkina Faso: one with the M184V NRTI mutation and Q151M + M184V in the other (Ruelle et al. 2007). In Senegal, one ARV-naïve female subject had the HIV-2 reverse transcriptase M184I mutation detected in genital tract secretions (but not PBMCs) that is known to confer 3TC/FTC resistance in HIV-2 (Gottlieb et al. 2008b). In a study in Belgium and Luxembourg, Q151M or

M184I was found in 2 ARV-naïve HIV-2 patients suggesting transmitted drug resistance (Ruelle et al. 2008). The Q151M mutation was from a recently diagnosed ARV-naïve MSM patient originating from Belgium, and phylogenetic analysis showed that this virus was linked to a 2nd HIV-2 strain from another MSM which also had the Q151M mutation suggesting a TDR of HIV-2 between MSM (Ruelle et al. 2008). There have also been reports of HIV-2 protease polymorphisms/mutations in ARV-naïve individuals in West Africa and Europe, but the role in resistance has not been established (Gottlieb et al. 2008b; Ruelle et al. 2008). To date there have not been any reports of TDR of HIV-2 integrase inhibitors (INIs), which is likely due to lack of availability of INIs in West Africa, and only limited and recent use of raltegravir in HIV-2 in developed countries (Gottlieb et al. 2011).

HIV-2 Genital Track Shedding

Similar to other sexually transmitted infectious agents, risk of HIV transmission is associated with the presence and quantity of HIV in genital tract secretions (Quinn et al. 2000). Factors previously shown to be associated with cervicovaginal HIV-1 RNA or DNA detection include systemic factors such as high HIV RNA plasma load and low CD4⁺ T-cell count and local (genital) factors such as elevated vaginal pH, bacterial vaginosis, genital ulceration, and infection with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, herpes simplex (HSV), *Candida albicans*, or human papillomavirus.

Thus, understanding the patterns of and risk factors for genital HIV-2 shedding is important for developing strategies to control its transmission. Unfortunately, to date, there are only three published studies of HIV-2 female genital shedding (Ghys et al. 1997; Seck et al. 2001; Hawes et al. 2008). Two of these studies were cross-sectional in design and compared detection of HIV RNA or DNA in the genital secretions of African women with HIV-1 and HIV-2 and found genital HIV RNA or DNA among 25–50% of HIV-1- and 5–15% HIV-2-infected

women (Ghys et al. 1997; Seck et al. 2001). The largest study to date by Hawes (Hawes et al. 2008) and colleagues in Senegal, West Africa, evaluated HIV DNA and RNA detection in cervicovaginal specimens among 168 HIV-1- and 50 HIV-2-infected women, and in a subset of 31 women (20 with HIV-1, 11 with HIV-2), they conducted a prospective study in which cervicovaginal specimens were taken at 3-day intervals over a 6-week period. They found significantly lower rates and levels of HIV-2 RNA (58% shedding; 13% with >1000 copies/ml) in the female genital tract than HIV-1 RNA (78% shedding; 40% with >1,000 copies/ml) and shedding correlated with plasma viral load irrespective of virus type (odds ratio = 1.9, 95% confidence Interval = 1.3–2.8 for each log₁₀ increase in HIV viral RNA). Plasma viral load, not HIV type, was the strongest predictor of genital viral load. Over 80% of closely monitored women, regardless of HIV type, had at least intermittent HIV RNA detection during every 3-day sampling over a 6-week time period. Concurrent STIs were infrequent in this population.

Correlates of HIV-1 shedding in semen have been less well studied, but plasma viral load appears to be a strong correlate as do concurrent STIs. To date, the sole study examining male genital tract shedding of HIV-2 which found a significantly lower level of semen HIV-2 RNA than that of HIV-1 (Gottlieb et al. 2006). In this small cross-sectional study, HIV RNA was detected in semen in 21 of 22 (95%) of HIV-1- and 7 of 10 (70%) of HIV-2-infected subjects ($P = 0.07$). However, the levels of HIV RNA present in semen were markedly different between those with HIV-1 and HIV-2, with a mean of 4.4 log₁₀ copies/ml among those with HIV-1 and a mean of 2.6 log₁₀ copies/ml among those with HIV-2 ($P < 0.001$). In multivariate analysis, plasma viral load and HIV type, but not CD4 cell count, were independently predictive of semen viral load ($P = 0.03, 0.05, 0.48$, respectively) (Gottlieb et al. 2006).

Although the four, small, published studies on HIV-2 genital shedding suggest lower transmission rates of HIV-2 are likely due to lower genital tract viral loads, further natural history data would be of interest given HIV-2's slower disease

progression, slower decline in CD4⁺ T-cell count, lower plasma viral loads, and lower rates of mother-to-child and sexual transmission. In addition, determining whether ART reduces HIV-2 genital tract shedding, comparable to HIV-1, is important for assessing treatment as prevention strategies where HIV-2 is endemic.

Prevention of HIV-2 Transmission

There have been no clinical trials specific to HIV-2 that have assessed potential interventions that might reduce the risk or prevent HIV-2 transmission. Whether any of the global interventions that have been implemented to reduce HIV transmission in general are at least partially responsible for the apparent declining HIV-2 prevalence in West Africa is unclear (Eholie and Anglaret 2006). Interventions such as the use of condoms, sterile injection equipment in IDUs, implementing safe medical practices, and screening the blood supply for HIV have likely contributed to reduce transmission on HIV-2, in addition to that documented for HIV-1.

Regarding the prevention of MTCT (pMTCT) in HIV-2, randomized trial data is lacking; however, data from an observational HIV-2 cohort of pregnant women (including 223 HIV-2-infected women and 367 births) in France from 1986 to 2007 suggest that reductions, in the already low level of transmission, may be improved with effective maternal ART (Burgard et al. 2010). In over 20 years in the French Cohort, there were only two cases of HIV-2 MTCT: one case in 1993 occurred following maternal primary HIV-2 infection, and the other case occurred postnatally in 2002 and involved a mother with severe immune deficiency. The overall mother-to-child transmission rate for HIV-2 was 0.6% (95% confidence interval, 0.07–2.2%) (Burgard et al. 2010).

Recent data from a randomized controlled trial of antiretroviral treatment as prevention as well as previous cohort studies have demonstrated a significant reduction in the risk of sexual transmission of HIV-1; these results are likely applicable to antiretroviral treatment as prevention for HIV-2, although no such data yet exists. Similarly, other

interventions that have been studied for HIV-1 transmission prevention such as sexual, IDU, and occupational postexposure prophylaxis (PEP), preexposure prophylaxis (PREP), and topical microbicides will likely have potential efficacy in HIV-2 transmission prevention.

Conclusion

Overall, HIV-2 is significantly less transmissible than HIV-1, and this is reflected in the relative prevalence of HIV types in the global HIV/AIDS epidemic. The likely proximal cause for the different epidemiology between HIV-1 and HIV-2 is the significantly lower viral load burden in HIV-2. The mechanisms for this difference remain to be elucidated but will hopefully eventually provide insight into strategies to end the HIV/AIDS pandemic.

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HIV-2, Phylogeographic Insights into the Origins and Epidemic History

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Definition

Multiple cross-species transmissions of SIV from sooty mangabeys gave rise to various HIV-2 groups in West Africa, but only HIV-2 groups A and B established an appreciable human transmission history since the 1940s. Phylogeographic analyses reveal considerable migration of HIV-2 group A in West Africa, with a relatively rapid growth in the number of infections in countries like Guinea-Bissau in specific past time periods, and dispersal patterns out of Africa that are in line with colonial history.

Viral Phylogeography

Phylogeography is a discipline that aims to explain the spatial processes that gave rise to the contemporary distribution of genetic lineages. Whereas historical biogeography has focused on macroevolutionary processes, phylogeography has traditionally dealt with microevolutionary patterns in populations of a single species or among closely related species. In viral epidemiology, phylogenetic inference has also been extensively used to examine the relatedness of viral lineages sampled at different geographic locations. Although this may represent timescales that are several orders of magnitude smaller than classical species-oriented phylogeography, many RNA viruses accumulate extensive genetic diversity during epidemic spread. As a consequence, processes such as viral population growth and ancestral migration can leave a measurable signature in RNA virus genomes (Holmes 2009). HIV is a primary example of this, and the changing epidemiology of the virus has frequently been scrutinized at the molecular level due to the availability of large amount of sequence data. In fact, many of the state-of-the-art developments in evolutionary and population genetic models and computational inference have been motivated by the desire to shed light on the epidemic history of HIV and other viruses (Holmes 2009).

Uncovering the genetic imprint of spatial processes in viral genomes is now considered to be only one aspect of the broader field of phylodynamics (Pybus and Rambaut 2009), which in general terms aims to explain infectious disease behavior that arises from a combination of evolutionary and ecological processes. Such studies can complement seroprevalence data from surveillance studies and may offer complementary detail to traditional epidemiology, which is limited in its ability to provide historical insights. This entry discusses the fundamental starting point offered by classical HIV-2 epidemiology (► [The Epidemiology of HIV-2 Infection in West Africa](#)) and molecular virology, the important insights gained from molecular epidemiology, and specifically how phylogeographic and phylodynamic

analyses can further contribute to our understanding of the epidemic history of HIV-2.

Discovery of the Zoonotic Origin of Human Immunodeficiency Virus Type 2 (HIV-2)

HIV-2 was identified in 1985 (► [The Epidemiology of HIV-2 Infection in West Africa](#)) in healthy Senegalese sex workers on the basis of different preferential serological cross-reactivity to SIV antigens compared to HIV-1. In 1986, the virus was isolated (► [The Epidemiology of HIV-2 Infection in West Africa](#)) from two immunodeficient heterosexual men who did belong to any particular risk group for HIV infection, one from Guinea-Bissau and one from Cape Verde (Clavel et al. 1986). Three years later, the primate species *Cercocebus atys*, which is indigenous in West Africa and is commonly named sooty mangabeys, was proposed as the reservoir species of simian immunodeficiency virus (SIV). The ability of SIVs to jump between nonhuman primate species has been established as an ancient feature intrinsic to SIV evolution (Sharp and Hahn 2011). Within a few years, several lines of evidence for a natural transfer of HIV-2 from sooty mangabeys into humans were presented: SIVsmm (► [SIV Infection of Sooty Mangabeys](#)) and HIV-2 show high similarity in genomic organization (► [HIV-2: Viral Structure, Sequence Variation and Pathogenesis](#)) and close phylogenetic relatedness; the prevalence of SIVsmm was relatively high in its natural host; there was a geographical range overlap for sooty mangabeys; and HIV-2 infection and plausible routes of transmission have been identified (Reeves and Doms 2002). Currently, HIV-2 accounts for around 1–2 million infections alone in West Africa (► [The Epidemiology of HIV-2 Infection in West Africa](#)).

Geographic Distribution of HIV-2 and its Donor Species

Several studies have suggested that HIV-2 infection is characterized both by an enhanced immune

response (► [The Antibody Response to HIV-2](#)) and lower viral replication (► [HIV-2 Envelope: Structure, Diversity and Evolution](#)) as compared to infection with its human counterpart HIV-1 (de Silva et al. 2008). As a consequence, HIV-2 infection results in a relatively low degree of viremia (► [HIV-2 Diagnosis and Viral Load Measurements](#)) and lesser genital shedding (► [HIV-2 Transmission](#)) that help explaining why the HIV-2 epidemic in and outside West Africa is of limited proportions compared to HIV-1. In fact, there is still a large overlap in the spatial distribution of HIV-2 infection and that of its primate donor.

The historical geographic range of sooty mangabey extends the coastal forest areas from the Casamance River in Senegal to the Nzo/Sassandra River systems in western Côte d'Ivoire (Fig. 1). However, due to habitat destruction and commercial hunting in the last decades, sooty mangabeys are now likely to be extinct in Senegal and Guinea-Bissau (Santiago et al. 2005). Epidemiological studies conducted in Liberia, Sierra Leone, and Côte d'Ivoire have shown that the prevalence of SIVsmm in sooty mangabeys can be as high as 60% in wild-ranging communities (Santiago et al. 2005). This primate has been frequently hunted for its meat, and high prevalence of SIVsmm has been found in bush-meat markets in Sierra Leone. In addition, sooty mangabeys are often kept as household pets, providing ample opportunity to cross-species transmission (Pepin 2011).

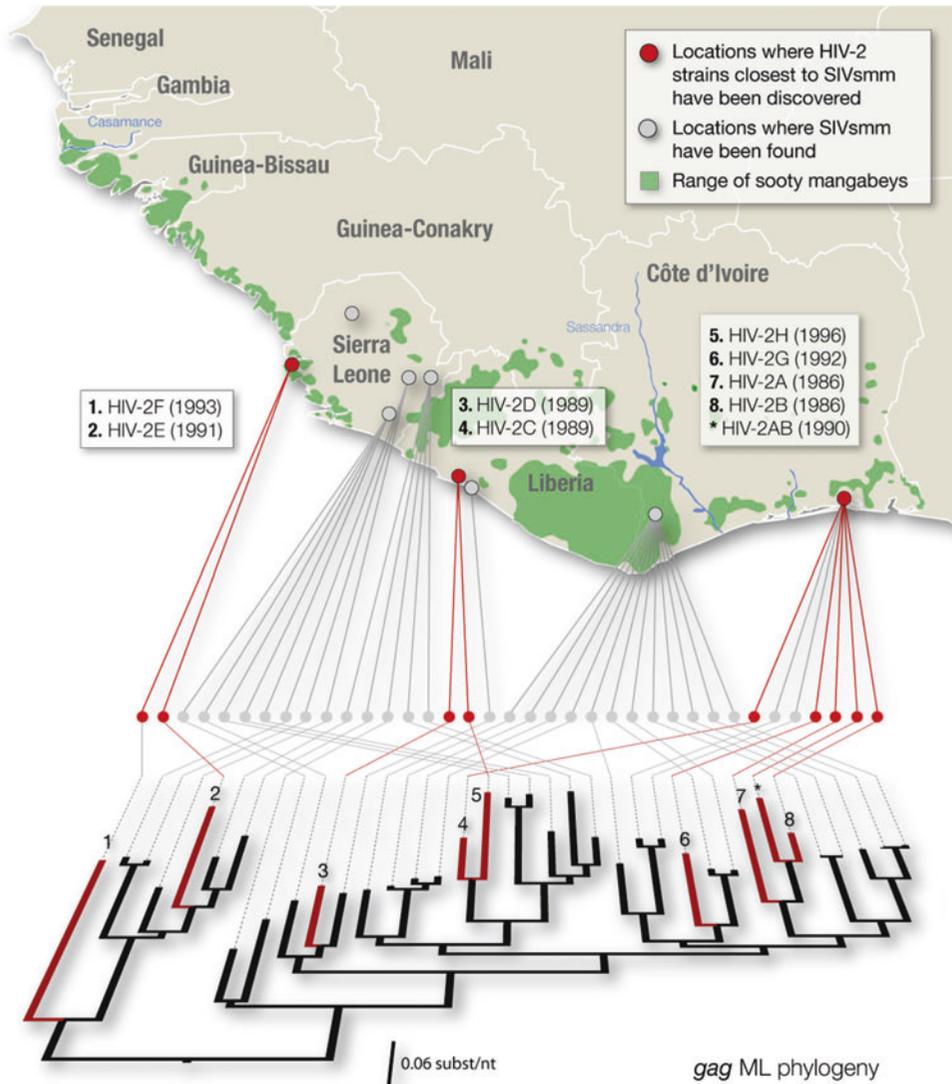
To date, eight different cross-species jumps of SIVsmm to humans have been identified in West Africa. While groups A and B represent virus lineages that evolved to establish sustainable epidemics within humans, groups C to H seem to represent dead-end infections since they were almost exclusively detected in one individual. Specifically, groups C and D have been identified in Liberia, while groups E and F in Sierra Leone. For these, only partial nucleotide sequences are available. Conversely, full-length genomic sequences from groups G and H strains have been collected in Côte d'Ivoire. Exceptionally, a second individual infected with a group F strain was recently identified in the United States. The immunodeficient patient originated from Freetown, Sierra Leone, and potentially acquired the

virus through human-to-human transmission in the patient's country of origin (Sharp and Hahn 2011). In addition, one circulating recombinant form of HIV-2 (HIV-2 CRF01_AB) (► [Recombinant Forms of HIV-2](#)) has been identified in 1990 in Côte d'Ivoire. This recombinant form was recently isolated from one Japanese and two Nigerian individuals living in Japan. Figure 1 illustrates the locations in Liberia, Sierra Leone, and Côte d'Ivoire where SIVsmm's have been identified to date and indicates where the phylogenetically closest HIV-2 groups (as well as the first isolate of CRF01_AB) (► [Recombinant Forms of HIV-2](#)) have been sampled. Traditionally, phylogeographic insights in viral epidemiology have been limited to interpreting the location distribution at the tips of phylogenetic trees as exemplified in this figure. Recent advances in the field, however, aim to uncover the process that gives rise to this distribution and attempt to estimate where virus resided at ancestral nodes in the tree. Below, the application of such techniques to HIV-2 group A is illustrated in more detail.

Epidemiological and Phylogenetic Origins of HIV-2 Groups

The great majority of SIVsmm strains (82%) have been identified in Sierra Leone (Santiago et al. 2005). Notably, this country has a large HIV-2 diversity (groups A, B, E, and F) but a very low overall prevalence of HIV-2 (0.02%). While the presence of groups E and F can be explained by local zoonoses from SIVsmm-infected sooty mangabeys that resulted in transmission dead ends (Pepin 2011), groups A and B in Sierra Leone have probably been imported from elsewhere. Moreover, epidemiological surveys of HIV-2 in West Africa (► [The Epidemiology of HIV-2 Infection in West Africa](#)) indicate a strikingly low prevalence in Liberia and Conakry, Guinea (De Cock and Brun-Vezinet 1989).

New insights into the geographic origins of the more prevalent groups A and B arose from a noninvasive molecular survey in 2002 of SIVsmm in a well-studied community of 120 wild-ranging sooty mangabeys in the Tai forest in southwestern



H

HIV-2, Phylogeographic Insights into the Origins and Epidemic History, Fig. 1 Geographical distribution of SIVsmm and the historical range of *Cercocebus atys atys* (sooty mangabeys). A maximum likelihood (ML) phylogenetic tree (genome numbering according to HIV-2 BEN: 1214–1993) including all available overlapping gag SIVsmm strains (black branches) and one

strain per HIV-2 group (red branches) was projected in geographical coordinates. The gray and red lines connect the terminal branches in the phylogeny representing SIVsmm strains and HIV-2 groups, respectively, to the locations in West Africa where these have been discovered. The dates represent the sampling year of the first available strains for each HIV-2 group

Côte d’Ivoire (Santiago et al. 2005). Phylogenetic analyses based on gp41 env fragments recovered from two SIVsmm strains isolated from sooty mangabeys clustered closely to HIV-2 group A with strong statistical support, providing the strongest evidence for a simian origin of this group in Côte d’Ivoire. Taken together, this

study supported an origin for groups A, B, C, G, and H in the southwestern region of Côte d’Ivoire. Accordingly, epidemiological data has shown that the geographic distribution of HIV-2 group B (► [The Epidemiology of HIV-2 Infection in West Africa](#)) seems to be mostly restricted to Côte d’Ivoire and the neighboring countries of

Ghana and Mali (de Silva et al. 2008; Reeves and Doms 2002), which lie outside the spatial range of sooty mangabeys.

Epidemiological and phylogenetic evidence seemingly disagree about the geographic origin of the predominant HIV-2 group A. The identification of regions with high seroprevalence in Guinea-Bissau (► [The Epidemiology of HIV-2 Infection in West Africa](#)) has led to the suggestion that this may represent the origin of HIV-2 group A, which is difficult to reconcile with the geographical origins of the closest SIVsmm strains found nearly 1,400 km southwards in the Tai forest in western Côte d'Ivoire (Pepin 2011; Santiago et al. 2005). While the closest SIVsmm from groups A and B have been identified in Côte d'Ivoire (Santiago et al. 2005), the prevalence of HIV-2 in Guinea-Bissau has been highest in West Africa, more than the double of the prevalence found in Côte d'Ivoire. However, high prevalences in particular areas may result from processes that do not necessarily require the virus to be originally introduced in these areas.

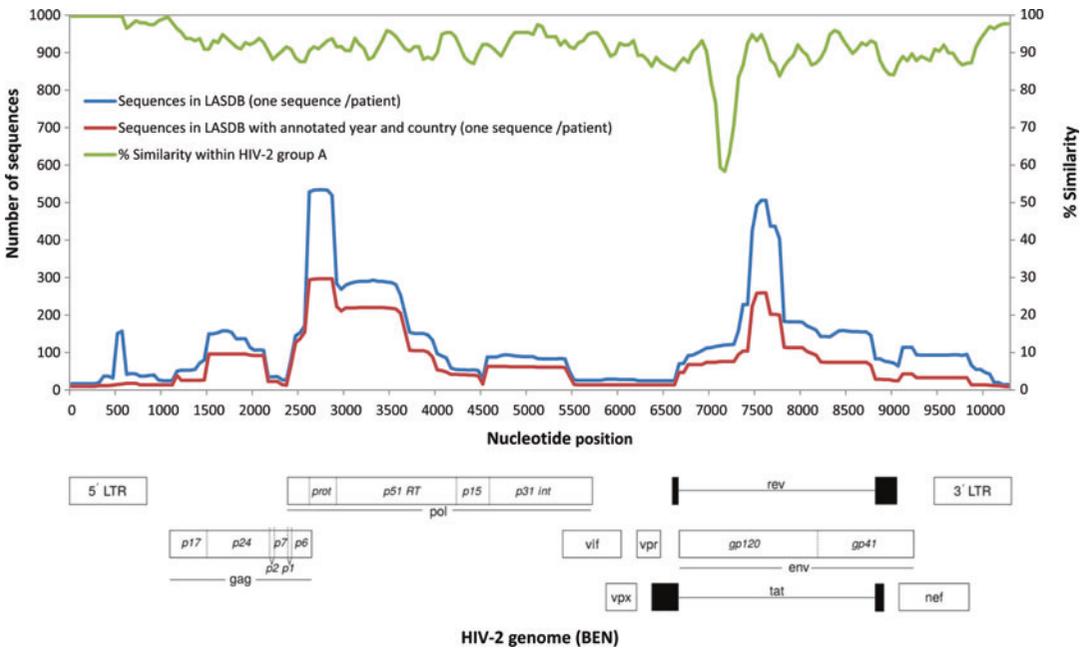
To shed light on the origins and dispersal patterns of group A, a comprehensive collection of 248 partial *env* and 320 partial *pol* nucleotide sequences sampled in 19 worldwide countries was recently investigated. Phylogeographic analyses geared toward time-calibrated genealogies that take into account uncertainty at the phylogenetic and dispersal level, placing Guinea-Bissau at the ancestral root location of group A (Faria et al. 2012). Moreover, molecular epidemiology insight revealed significant support for gene flow between localities within Guinea-Bissau, as well as from there to Senegal and between Gambia and Senegal. Interestingly, an epidemiological link between Côte d'Ivoire and Gambia was also recovered from the viral genetic data (Faria et al. 2012).

At face value, a spatial origin in Guinea-Bissau seems in line with the epidemiological evidence showing the HIV-2 prevalence among West African countries (► [The Epidemiology of HIV-2 Infection in West Africa](#)). However, accurate phylogeographic estimation of the ancestral root location of viruses is critically dependent on

dense, representative sampling, both in terms of year and location. In addition, genetic regions of sufficient length and variability are required to adequately inform such reconstructions. To illustrate the available sequence data for HIV-2, a search of the Los Alamos sequence database (in March 2012 <http://www.hiv.lanl.gov/>) was able to retrieve 761 geo-referenced sequences with known sampling date. The available sequences were distributed over the entire HIV-2 genome (► [Molecular Biology of HIV-2](#)) in a nonrandom fashion (Fig. 2), but even for the most covered regions in *pol* and *env*, the collective number of sequences available for analysis remained restricted. Particularly, the number of gene sequences available from Côte d'Ivoire appears to be insufficient compared to those available from Guinea-Bissau to build datasets robust to the effect of sampling bias. Taking this is in mind, the phylogeographic evidence for an origin in Guinea-Bissau can be considered as circumstantial, and it remains possible that the virus emerged in Côte d'Ivoire and was transported to Guinea-Bissau or surrounding countries early after entering the human population. Arguably, archival samples of group A from Côte d'Ivoire and Guinea-Bissau could provide the most compelling evidence for a geographic origin of group A.

The Chronological Origins of HIV-2 Endemic Groups

There are several indications that HIV-2 was present in West Africa during the 1960s and 1970s, and retrospective cases were confirmed mostly in Portuguese individuals who had lived in Guinea-Bissau during this period. In addition, retrospective testing of more than 3,000 stored sera suggested two positive samples from 1966 sampled in Côte d'Ivoire (Pepin 2011). These observations suggest that the predominant groups A and B emerged in the human population decades before its widespread molecular isolation. But when did these HIV-2 endemic lineages originate? Molecular clock analyses have often been used to offer insight in the age of the most



HIV-2, Phylogeographic Insights into the Origins and Epidemic History, Fig. 2 Number of available HIV-2 sequences (one per patient) across the genome (blue line) and the subset that is annotated with year and country (red line, potentially useful in phylogeographic analyses) in Los Alamos sequence database (LASDB;

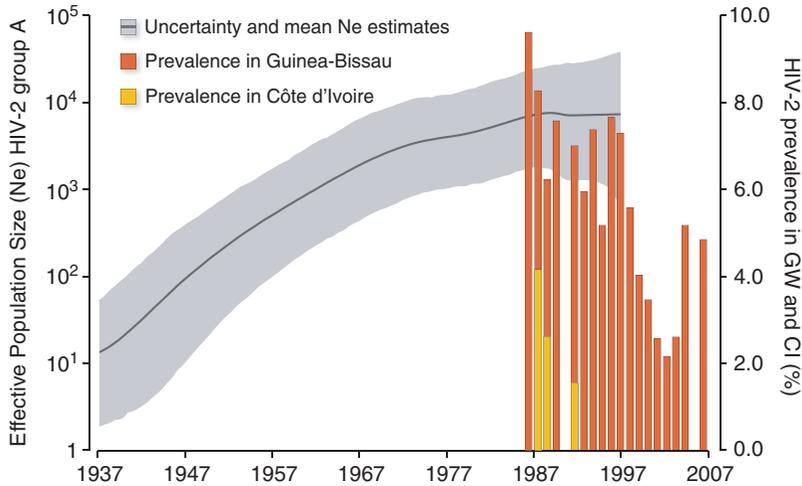
<http://www.hiv.lanl.gov/>). The green line displays the diversity over the genome within HIV-2 group A, as indicated by a similarity percentage. Data was produced using a stepwise sliding window approach. Bottom: map of the HIV-2 genome in relation to nucleotide positions of the top diagram

recent common ancestor of a viral lineage based on how fast they accumulate mutations in their genomes over an observable timescale. Molecular clocks, both strict and several relaxed versions, have recently been integrated with phylogenetic and phylogeographic inference models. Phylogenetic analyses aimed at investigating the time of origin of HIV-2 have found that group A arose during the 1930s (between 1906 and 1956) (Faria et al. 2012; Lemey et al. 2003; Wertheim and Worobey 2009). More recent estimates that included globally sampled data further suggested an introduction of this group in India in or around 1964 (between 1951 and 1974). Group B most likely arose within the same period of time (between 1907 and 1961). Finally, CRF01_AB (► **Recombinant Forms of HIV-2**) emergence has been dated between 1964 and 1973, although the small number of sequences available for this lineage resulted in estimates that are bounded by wide uncertainty intervals (1933–1986).

The Epidemic History of HIV-2 Group A

In addition to molecular clock and phylogeographic models, coalescent analyses have proven very useful in molecular epidemiology. These models are able to explain the phylogenetic tree shape as function of population size changes through time, which in turn may reflect important changes in epidemic patterns. After cross-species transmission from sooty mangabeys, a coalescent analysis of nucleotide sequences sampled in Caió, Guinea-Bissau (Lemey et al. 2003), suggested an initial period of stasis of group A, followed by an exponential increase in the number of infections between 1955 and 1970 (Fig. 3). By extending the sampling interval of the molecular epidemiological analysis, a more recent molecular epidemiological study corroborates a leveling off on the estimated number of HIV-2 infections since the early 1990s (Faria et al. 2012). Such decline in the number of





HIV-2, Phylogeographic Insights into the Origins and Epidemic History, Fig. 3 Demographic history of HIV-2 in Guinea-Bissau inferred using a molecular epidemiology approach. Bars indicate prevalence of HIV-2 in Guinea-Bissau and Côte d'Ivoire. Demographic history

was estimated using 80 partial *pol* HIV-2 group A strains sampled in Guinea-Bissau from 1986 to 1997 (location from start of HIV-2 BEN: 7553–7851) under a Bayesian skyride demographic model

new HIV-2 infections is in line with several epidemiological reports in West Africa (► [The Epidemiology of HIV-2 Infection in West Africa](#)) (Pepin 2011). Interestingly, in Guinea-Bissau, the highest prevalence of HIV-2 has been found in older groups and has been declining since the late 1980s (Pepin 2011). For example, a large study on 20,422 women in a maternity clinic in the capital of Guinea-Bissau showed a decline of HIV-2 prevalence from 8.3% in 1987 to 2.5% in 1999 (Mansson et al. 2009). Several explanations have been put forward to explain the exceptional rise of HIV-2 prevalence in Guinea-Bissau during this period and its subsequent decline.

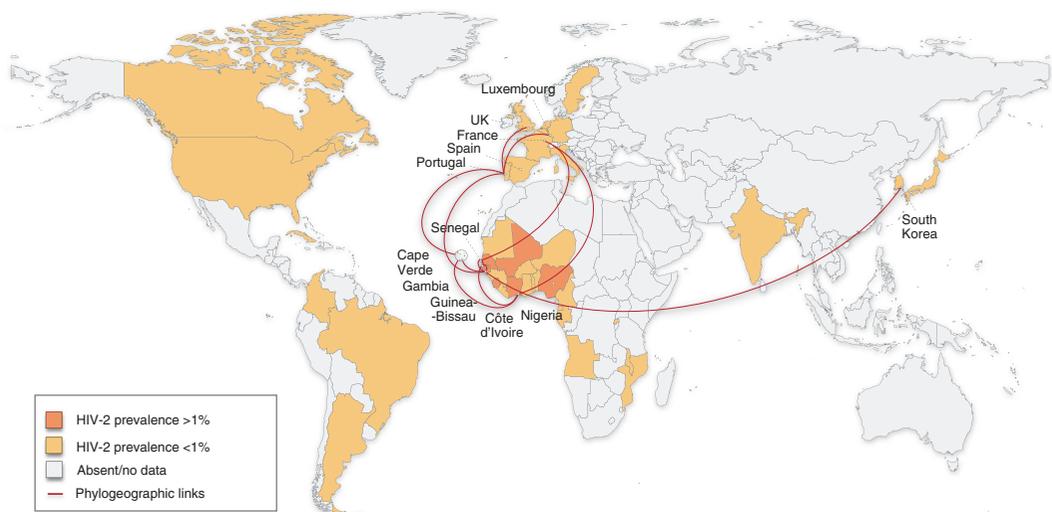
In Guinea-Bissau, the war against the Portuguese colonial power (1963–1974) has been suggested as a period offering increased opportunities for HIV-2 transmission. Prior and during this time, blood-borne transmission may have been facilitated by vaccination campaigns as well as other healthcare-related actions, such as blood transfusion and treatment of nosocomial infection. However, also increased commercial sex worker activity and higher migration of soldiers may have contributed to increased transmission. Pépin and colleagues inquired 1,608 individuals aged over

50 years and found significant associations between HIV-2 infection and treatment for sleeping sickness and tuberculosis (Pepin 2011). Outside West Africa, HIV-2 has also declined in the last decades. In Portugal, HIV-2 infections accounted for 10–12% of AIDS cases in the early 1990s but dropped to 3–8% around 2003 (Gomes et al. 2003). An investigation of the date of birth of HIV-2 infected patients attending the Egas Moniz Hospital in Lisbon between 1997 and 2002 (most with Guinea-Bissau origin) indicated that the majority of patients were born between 1940 and 1970, with median at 1957 (Gomes et al. 2003). This suggested that these patients were not sexually active during the period of colonial war, thus further arguing for a role of parenteral exposure as a key route for the spread of this virus during this period. In addition, a recent study suggests that the high incidence of genital ulcer diseases around the estimated ancestor of HIV-2 predominant groups might have created a unique opportunity for the initial spread of HIV in general (Sousa et al. 2010). Most likely, the explanation for the successful establishment of HIV-2 in West Africa stands on a combination of the above mentioned factors.

HIV-2 Out of Africa

In 1991, a pioneering study conducted on a geo-referenced database of the global incidence of HIV-2 infection has suggested a primary role of human migratory fluxes in the dispersal of this virus beyond West Africa (Smallman-Raynor and Cliff 1991). Since then, several countries have reported cases of HIV-2 infections. More recently, the study of the main corridors of viral diffusion out of West Africa was confirmed and further extended using molecular data (Faria et al. 2012). The map in Fig. 4 depicts the countries where HIV-2 has been detected and the dispersal links inferred through a phylogeographic analysis based on a comprehensive dataset of globally sampled nucleotide data. Interestingly, some of the strongest supported links were identified between West African countries and their former colonial powers, such as between Guinea-Bissau and Portugal, Cape Verde and Portugal, Côte d'Ivoire and France, and Senegal and France, demonstrating that molecular sequences contain information about viral spatiotemporal spread and phylogeographic analyses have the potential to uncover these.

Portugal has had the highest number of HIV-2 infections outside West Africa. Also, in France the virus accounted for 1.8% of 10,184 new HIV diagnoses between 2003 and 2006, with 3 HIV-2 cases among non-African men who have sex with men (Campbell-Yesufu and Gandhi 2011). Most of HIV-2 infections in Europe (► [Epidemiology of HIV-2 Infection in Europe](#)) can be traced to people from West Africa, or people who traveled there or had sexual contact with the West Africans. However, there have been reports of cases of HIV-2 transmission within Europe, particularly in Portugal and France. Moreover, phylogeographic analyses were able to uncover epidemiological links between Portugal and the United Kingdom and Luxembourg (Fig. 4). In the Americas, HIV-2 infection is rare, although present in the United States, Canada, Brazil, and Colombia (Campbell-Yesufu and Gandhi 2011; De Cock and Brun-Vezinet 1989). In Asia, HIV-2 infection is also uncommon and has only been reported in India, South Korea, and Japan. While in India the virus seems to be spreading, albeit at a limited pace, only few cases have been detected in South Korea and Japan, mostly linked to heterosexual contacts with people from West Africa.



HIV-2, Phylogeographic Insights into the Origins and Epidemic History, Fig. 4 Global map of HIV-2 epidemiology and phylogeographic links. Countries having a reported prevalence exceeding 1% in the national

population in the late 1980s are highlighted (Campbell-Yesufu and Gandhi 2011). Links represent statistically significant phylogeographic links of viral dispersal between countries (Adapted from (Faria et al. 2012))

Conclusion

Molecular analyses complement traditional epidemiology by providing a historical perspective on the contemporary distribution of viral pathogens. Such analyses have evolved from reconstructing evolutionary relatedness among strains to the application of probabilistic models that integrate sequence evolution, molecular clocks, processes of population size change through time, and spatial spread. Studies on HIV-2 evolution and molecular epidemiology have benefitted from these developments, but the lack of systematic and widespread sampling of HIV-2 sequence data represents a considerable limitation to the insights that can be obtained. This is the case for reconstructions of the origin of HIV-2 A in West Africa, which is sensitive to sampling heterogeneity in time and space. Nevertheless, recent phylogeographic estimates are in agreement with epidemiological links established by colonial history, providing evidence that such analysis captures important details of historical spread.

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HIV-2: Lessons from the Dakar Cohort

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Definitions

Acquired immunodeficiency syndrome (AIDS): a clinical syndrome caused by the human immunodeficiency virus (HIV). Its

pathogenesis is related to a qualitative and quantitative impairment of the immune system, particularly a reduction of the CD4⁺ helper T lymphocyte cell count (surrogate marker of the disease). After an average of 10 years if untreated, HIV⁺ individuals can develop opportunistic diseases (i.e., infections and cancers rarely detected in people with normal immune systems). The natural history of the disease can be dramatically modified with administration of combination therapy composed of antiretroviral (ARV) drugs. This is seen in both HIV-1 and HIV-2 diseases.

Beta chemokines: a family of proteins produced in response to acute or chronic inflammation to attract a variety of white blood cells. They are characterized by a double cysteine (C–C) in their primary sequence. They bind to cellular targets by specific beta-chemokine receptors that are one of the members of the 7 transmembrane chemokine receptors. They include: CCR5, MIP1 α , and MIP 1 β .

CCR5: is a cell membrane protein expressed on several cell types including peripheral blood-derived dendritic cells, CD34⁺ hematopoietic progenitor cells, and certain activated/memory Th1 lymphocytes. This receptor is well defined as a major coreceptor in conjunction with CD4⁺, implicated in susceptibility to HIV-1 and HIV-2 infection.

CD4⁺: is a large glycoprotein that is found on the surface of helper T lymphocyte cells, regulatory T cells, monocytes, and dendritic cells. Its natural function is as a coreceptor that assists the T cell receptor (TCR) to activate its T cell following an interaction with an antigen-presenting cell. CD4⁺ is a primary receptor used by HIV-1 and HIV-2 to gain entry into host T cells.

CD4⁺ T cell: an immune cell, lymphocyte (white blood cell) characterized by the CD4⁺ antigen (protein) on its surface. This is a T lymphocyte considered to have a “helper” function to enhance the cellular immune response. The CD4⁺ is the primary receptor for the HIV-1 and HIV-2 virus, and upon infection the virus can destroy the CD4⁺ cell. In HIV-infected people, the drop in CD4⁺ T lymphocyte cells is a major determinant of the progression of HIV infection to AIDS.

Coreceptor (CCR5 or CXCR4): protein molecules on the surface of lymphocytes or monocytes that bind to the gp120 protein of HIV and facilitate, with CD4, binding, fusion, and entry of the virus into the susceptible cell.

CXCR4: is an alpha-chemokine receptor specific for stromal-derived factor-1 (SDF-1, also called CXCL12), a molecule endowed with potent chemotactic activity for lymphocytes. This coreceptor is one of the several chemokine receptors that HIV isolates can use to specifically infect CD4⁺ T cells.

DNA (deoxyribonucleic acid): is a nucleic acid that contains the molecular basis of heredity for all known living organisms and some viruses and is found in the nuclei and mitochondria of eukaryotes. Chemically DNA consists of two polymer strands of units called nucleotides made up of one of four possible bases plus sugar and phosphate groups. The polymers are joined at the bases by hydrogen bonds to form a double helix structure.

Human immunodeficiency virus type 1 (HIV-1): the virus that causes acquired immunodeficiency syndrome (AIDS). It is a lentivirus belonging to *Retroviridae* family and was discovered in 1983 by Robert Gallo and Luc Montagnier. HIV infects and destroys helper T cells of the immune system causing a marked reduction in their numbers. Loss of CD4 cells leads to generalized failure of the immune system and susceptibility to life-threatening opportunistic infections. It is transmitted mainly through sexual intercourse, exchange of contaminated syringes among intravenous drug users, and contaminated blood transfusion. HIV-1 is the HIV type most frequently detected worldwide and responsible for the global pandemic.

HIV-1 subtypes or clades: genetically related HIV strains that are essentially phylogenetically equidistant, generating a star-like phylogeny. Subtypes A, B, C, D, F, G, H, J, and K are currently known; subtypes A, B, C, and D are highly prevalent; others have low prevalence and limited geographic distributions.

HIV-2: the second HIV virus discovered in registered sex workers in Dakar, Senegal in 1984, the virus is more closely related to the

simian immunodeficiency virus of primates. Although HIV-2 can cause AIDS, it has a distinct epidemiology, lower rates of transmission, and slower progression to disease.

Immunoblot: also termed “Western blot” is a technique to detect antibodies to proteins. The proteins of interest are typically electrophoresed and then transferred to nitrocellulose paper. Serum or plasma is incubated with the viral protein-impregnated paper, and using an antihuman Ig coupled with suitable colorimetric, the presence of the bound antibody can be detected by color.

Incidence: rate describing the number of new cases of disease occurring within a given time period, expressed as new cases per person-time.

Integrase: an enzyme found in retroviruses including HIV that permits the reverse transcribed viral DNA to be integrated into the infected cell’s DNA. Integrase is an enzyme encoded by the polymerase gene of HIV.

Prevalence: number of cases of disease in a defined population at a specific point in time; it is often expressed as a percentage.

Radioimmunoprecipitation: a technique where virus-infected cells were grown in media containing a radioactive-labeled amino acid, which results in radiolabeling of viral proteins which can be detected by antibodies directed to those viral proteins. The technique with SDS-polyacrylamide gel electrophoresis (PAGE) indicates that radiolabeled proteins are then lysed in SDS detergent and subsequently subjected to electrophoresis through a polyacrylamide gel.

RNA (ribonucleic acid): this a universal form of genetic material typically transcribed from DNA; it differs from DNA in that it contains ribose and uracil as structural components. In retroviruses like HIV, RNA is their primary genetic material and is found in a mature virus particle.

Human immunodeficiency: virus types 1 and 2 (HIV-1 and HIV-2) are members of the *Lentivirus* genus of the *Retroviridae* family of RNA viruses and are ~50% related at the genetic level. HIV-2, although more closely related to the simian immunodeficiency virus (SIV), is the second human immunodeficiency virus and

constitutes the closest known human virus related to the prototype AIDS virus, HIV-1. HIV-2 shares many virologic and biologic features with HIV-1. Although HIV-1 and HIV-2 are highly related lentiviruses, they maintain some distinctly different epidemiologic and biologic characteristics (Kanki and Meloni 2009; Barin et al. 1985). HIV-2 is largely confined to West Africa, while HIV-1 infection is prevalent worldwide and accounts for over 98% of all HIV infections globally. Importantly, disease progression to AIDS occurs much more slowly in HIV-2. In comparison to HIV-1, these biologically relevant characteristics of HIV-2 infection in vivo appear to model those of an attenuated HIV infection. To date, the precise mechanisms responsible for this attenuated phenotype of HIV-2 remain unclear. However, HIV-2, like HIV-1, causes AIDS, although more slowly, and it was based on these initial similarities that some believed that HIV-2 might cause a second worldwide AIDS epidemic. Now, 29 years after the discovery of HIV-2, no such epidemic has occurred. Rather, research studies conducted both in the laboratory and in HIV-2-infected people in West Africa have highlighted distinct biological differences between these related viruses (Kanki and Meloni 2009). The initial discovery of HIV-2 in registered sex workers in Dakar, Senegal, was conducted through a unique collaboration between Souleymane Mboup and his team, Cheikh Anta Diop University, Francis Barin, University of Tours (France), Francois Denis, University of Limoges, and the Harvard School of Public Health, USA (Barin et al. 1985). This interuniversity convention worked together for more than two decades to continue its studies on the biological and clinical significance of HIV-2. Much of the research was based on studies conducted on a cohort of registered female sex workers in Dakar, Senegal. A brief summary of the history and key studies are provided in this entry.

History of HIV-2’s Discovery in Sex Workers in Dakar, Senegal

In 1970, the Ministry of Health in Senegal established a public health program whereby

self-identified commercial sex workers were registered to attend a clinic, which provided regular evaluation and free sexually transmitted infection (STI) treatment. For many decades, the Institut d'Hygiene Sociale (IHS) clinic in Dakar, Senegal, has been the outpatient clinic administered by the Senegalese Ministry of Health responsible for overseeing all self-identified female commercial sex workers in the capital city, Dakar, as well as other urban centers throughout the country. Women attended the clinic where regular clinical and STI examinations were performed and free STI treatment was offered. The Laboratoire de Virologie at CHU Le Dantec hospital nearby provided the laboratory services for IHS and has been led by Professor Souleymane Mboup since the early 1980s.

In 1985, when 289 sera from registered female sex workers in Dakar, Senegal, were screened for antibodies to HIV-1 antigens by immunoblot, 20 (6.9%) of them showed extensive cross-reactivity for the virus gag antigens but minimal antibody binding reactivity for the HIV-1 envelope proteins (Barin et al. 1985). Yet, when the same sera were assayed on the then recently described simian immunodeficiency virus (SIV) antigens, they reacted strongly with the envelope proteins as well as the gag antigens, suggesting infection with a virus that was more closely related to SIV than to HIV-1. This serologic relationship was also demonstrated using S³⁵ cysteine-labeled SIV and HIV-1 antigens in a radioimmunoprecipitation and SDS-PAGE (RIP-SDS-PAGE) analysis (Kanki et al. 1985). It is now recognized that HIV and closely related viruses in primates, termed SIVs, exist. The close antigenic relatedness of both SIV and HIV-2 to the prototype HIV-1 virus prompted both the discovery and further classification of these related viruses.

In 1985, the number of AIDS cases reported in Senegal and most of West Africa was quite low. The original Senegalese sex workers from IHS that had evidence of HIV-2 were healthy, and testing of hospitalized patients in Dakar that might have AIDS or an AIDS-like disease revealed HIV-1 infection and often a connection to Central Africa. The isolation of LAV-2 from an

AIDS patient originating from Cape Verde (Clavel et al. 1987) and subsequent AIDS case reports prompted the AIDS research community to fear for a second global AIDS epidemic. It therefore became critical to assess the immunopathogenic potential of this newly described virus. With the recognition that progression to disease in HIV-1 infection might take years, it was reasoned that further serologic surveys of control, risk, and disease populations throughout West Africa might be informative. Serum samples from 4,248 individuals from Senegal, Guinea, Guinea-Bissau, Mauritania, Burkina Faso, and Ivory Coast were tested on HIV-2 and HIV-1 antigens using both immunoblot and RIP-SDS-PAGE analysis (Kanki et al. 1987a). HIV-2 prevalence varied by country with Guinea-Bissau showing the highest rates and Mauritania without positives in the 184 samples evaluated. Healthy sexually active individuals in the "risk" category had the highest prevalence compared with the hospitalized disease category of individuals. Reactive positive samples were tested on variously named HIV-2 isolates including: LAV-2, HTLV-IV (MS), SBL-6669, and even SIV with identical results.

With evidence that a virus more closely related to SIV than to HIV-1 was present in Senegalese sex workers, more extensive studies were undertaken to determine if the SIV-related virus was more widely distributed in Africa. AIDS cases were targeted to determine if HIV-2 could be associated with what was already recognized as a significant AIDS epidemic in Central Africa. The screening of 1,508 high-risk individuals from Zaire (DRC), Burundi, Tanzania, Kenya, Zambia, and Cameroon, including many individuals with AIDS and other STDs, revealed no evidence that HIV-2 was present in the same regions in which HIV-1 was so common (Kanki et al. 1987b). However, pockets of infection with HIV-2 were detected in Mozambique and Angola, which, though distant from West Africa, were often on the same Portuguese trade routes as Guinea-Bissau and Cape Verde, both West African countries with some of the highest rates of infection. Even within Senegal, prevalence rates for HIV-2 were substantially higher in the

southern region of Casamance, which borders Guinea-Bissau, compared to other regions of Senegal (Kanki et al. 1987a).

The close relatedness of HIV-2 to HIV-1 also meant that methods to diagnose the virus, through antibody, antigen, and even genetic material, required specific assays. Early on it was recognized that HIV-2 was less prevalent globally and therefore commercial companies failed to develop HIV-2 specific diagnostics. This became even more pertinent, when the description of HIV-2 and HIV-1 dually infected individuals was first noted, relying heavily on unequivocal type-specific diagnosis of both viruses. The World Health Organization assisted with the formation of the HIV-2 working group to develop recommendations for HIV-2 diagnosis, which served as the basis for research and public health surveillance on the virus.

Studies conducted in the late 1980s and 1990s with type-specific serologic methods documented the prevalence of HIV-2 in most West African countries, surprisingly a distinctly different worldwide distribution compared to that of HIV-1 (Kanki and Meloni 2009). In most other countries of West Africa, such as Burkina Faso, Ghana, Ivory Coast, Nigeria, and Mali, infection with HIV-1 was more prevalent than infection with HIV-2, ranging from a 3- to 24-fold rate ratio (HIV-1 versus HIV-2). In recent times, most national serologic surveys have not distinguished these virus types; however, it is widely considered that HIV-2 prevalence rates are diminishing. This supports the hypotheses raised by Anderson and May, who analyzed the available biological and epidemiological data on HIV-1 and HIV-2 and used simple mathematical models to study the competition between the two viral types and predict the eventual outcome at a population level (Anderson and May 1996).

Epidemiology of HIV-2

Risk Determinants for HIV-2 and HIV-1 in Dakar Sex Workers

The prospective study of all registered sex workers in Dakar began in 1985 and in 1987 expanded to two other smaller clinic facilities in

the urban areas of Kaolack in western Senegal and Ziguinchor in the South, near the Guinea-Bissau border (Kanki et al. 1992). All women were asked to participate and informed consent was obtained and annual testing for HIV-1 and HIV-2 serology was performed along with a questionnaire to assess risk. The clinic's dynamic nature resulted in an open cohort design in which sequentially followed women were included irrespective of their date of entry. All women visiting the clinic were counseled on HIV/STI prevention, provided with free condoms at each visit, and offered free medical care as needed. These self-identified sex workers ranged in age from 18 to 70 years old, and over 90% of the approximately 2000 HIV-infected and uninfected women regularly attended the clinic. In studying the risk determinants of HIV-2 versus HIV-1 infection between 1985 and 1990, the variable prevalence of HIV-2 by sex worker cohort was confirmed, with Dakar being the lowest at 10.0% (128/1275), Ziguinchor 38.1% (106 /278), and Kaolack 27.4% (43/157) (Kanki et al. 1992). Step-up logistic regression analysis revealed that increased years of sexual activity (OR = 1.64; 95% CI = 1.06–2.57) and history of traditional scarification (OR = 1.65; 95% CI = 1.25–2.14) were positively associated with HIV-2 infection. The approximate log-linear relation of HIV-2 infection with increasing years of sexual activity was consistent with the hypothesis that the virus had been in the population for at least several decades. This differed from risk determinants for HIV-1 where history of hospitalization (adjusted OR = 2.12, 95% CI = 1.11–4.03) and a shorter period of registered sex work (adjusted OR = 0.23; 95% CI = 0.09–0.59) were associated with infection. In the Dakar and Ziguinchor clinics, Ghanian and Guinea-Bissau nationalities were significantly associated with higher HIV-2 prevalence.

Incidence of HIV-2 and HIV-1 in Dakar Sex Workers

The most common modes of HIV transmission in HIV-2 endemic areas is perinatal and heterosexual transmission; since Senegal like most West African countries had evidence of both HIV-1 and HIV-2 infections, measurement and comparison of incidence rates for both viruses was possible.

The incidence of HIV-1 and HIV-2 in the Dakar sex worker cohort ($n = 1,452$) was studied between 1985 and 1993 (Kanki et al. 1994). The overall incidence of HIV-2 was 1.11 per 100 person-years of observation (pyo) and was 1.11 per 100 pyo for HIV-1. Over the 8-year period, the annual incidence of HIV-1 dramatically increased, with a 1.4-fold increased risk per year and thus a 12-fold increase in risk over the entire study period. The incidence of HIV-2 remained stable, despite a higher HIV-2 prevalence of 11.3% compared to 6.2% for HIV-1. In this high-risk group, the heterosexual transmission of HIV-2 was significantly slower than that of HIV-1, which strongly suggested differences in the heterosexual transmission potential of these two related immunodeficiency viruses.

In modeling studies by Donnelly et al. the male-to-female transmission rates of HIV-2 and HIV-1 were estimated using maximum likelihood estimation theory and goodness-of-fit methods. Data from 780 sex workers followed from 1985 to 1990 revealed a 5.8–8.9-fold increased transmission probability of HIV-1 compared to HIV-2 (Donnelly et al 1993). Using a larger set of data from the Dakar sex worker cohort ($n = 1,948$) between 1985 and 1999, Gilbert et al. performed a modeling study of HIV-2 and HIV-1 to compare transmission potentials, where new nonparametric competing-risks failure-time methods were used, which minimized modeling assumptions and controlled for risk factors for HIV infection (Gilbert et al. 2003). The HIV-1 versus HIV-2 infectivity ratio over time was estimated by nonparametric kernel smoothing of the HIV-1/HIV-2 infection hazard ratio adjusted by an estimate of the relative HIV-1 versus HIV-2 prevalence in the partner population. HIV-1 was found to be significantly more infectious than HIV-2 throughout the follow-up period ($P < .0001$). The HIV-1/HIV-2 infectivity ratio was inferred to be approximately constant over time, with an estimated ratio of 3.55. The mathematical model of the concomitant transmission of the two viruses within the same sexually active population suggested a positive association between pathogenicity and reproductive success, indicating that HIV-1 would competitively displace HIV-2 in the long term. In the

HIV-2: Lessons from the Dakar Cohort, Table 1 Key differences between HIV-1 and HIV-2

	HIV-1	HIV-2
Geographic distribution	Worldwide	West Africa
Heterosexual transmission		Three to sixfold lower
Perinatal transmission	15–45%	0–5%
Plasma viral load		30-fold lower
Time to AIDS	7–10 years	10–25 years
Treatment		NNRTIs ^a ineffective

^aNon-nucleoside reverse transcriptase inhibitors

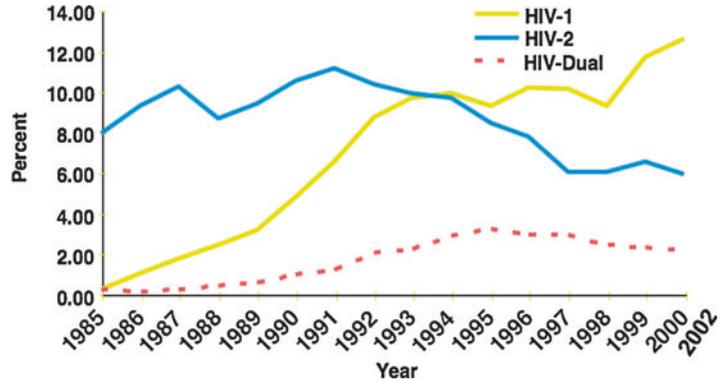
study of both viruses in Dakar, Senegal, over more than 25 years, the decrease of HIV-2 prevalence accompanied by the increase in HIV-1 prevalence was indeed observed (Hamel et al. 2007).

Maternal or perinatal transmission of HIV-2 also appears to be less efficient than for HIV-1. Perinatal transmission of HIV-2 and HIV-1 has been studied in Guinea-Bissau, Ivory Coast, France, and Senegal, with all demonstrating extremely low rates of HIV-2 perinatal transmission (0–3.7% transmission) in contrast to that of HIV-1 (15–45% transmission) (Kanki and Meloni 2009). In studies that measured perinatal transmission of both viruses, the rate of HIV-1 transmission was 10- to 20-fold higher than that of HIV-2 (Table 1).

A 20-year study of the prevalence and incidence of HIV-2 and HIV-1 subtypes in the Dakar sex worker cohort described the dynamic nature of these two related viruses (Hamel et al. 2007). Among the 3,910 women enrolled between 1985 and 2004, HIV-2 prevalence decreased from a range of 8–11% between 1985 and 1995 to 5.5% in 2003. HIV-1 prevalence has climbed steadily since its introduction in 1985 to just below 10% in 1993 stabilizing from 1993 to 1998, and then continuing to rise again to 13.8% in 2003 (Fig. 1). The annual incidence rate for HIV-1 increased from 0 in 1986 to 2.5/100 person-years of observation (PYO) in 1992, while HIV-2 and HIV-D have decreased to less than 0.3/100 PYO each, since 1999. In comparison to the previous assessment, this represented a fourfold decrease in HIV-2 incidence.

HIV-2: Lessons from the Dakar Cohort,

Fig. 1 Annual prevalence of HIV-1, HIV-2, and HIV dual infection in registered female sex workers, Dakar, Senegal (1985–2003)



Clinical Significance of HIV-2

HIV-2-Related Disease and Differences in Disease Progression

Early case reports described HIV-2-infected people with disease consistent with an AIDS diagnosis (Romieu et al. 1990). The disease characteristics, including tuberculosis, chronic diarrhea, and *Candida* infections, were similar to diseases seen in HIV-1-associated AIDS in the same settings. Central nervous system involvement has also occasionally been described in HIV-2 AIDS cases. However, classical African AIDS comorbidities, such as tuberculosis, often have had only a weak epidemiological association with HIV-2, even in HIV-2 endemic areas.

In 1988, a cross-sectional clinical study of compared 18 HIV-2-infected Dakar sex workers with 14 HIV-negative sex workers as well as HIV-negative surgery patients (Marlink et al. 1988). Significant elevations of T8 lymphocytes ($p = .03$), IgG ($p = .0001$), and beta₂-microglobulin ($p = .03$) were described. The mean T4 lymphocyte count in seropositive was not depressed and not different from seronegative sex workers (757 vs 1179, $p = .15$). There were no differences in lymphocyte stimulation studies. Skin-test energy to various antigens was also less pronounced in HIV-2 infection. Therefore, the clinical picture from this small study failed to demonstrate immunosuppression similar to HIV-1 but rather the immunologic alterations of a persistent viral infection. Similar cross-sectional studies of HIV-2 infection were conducted in the late 1980s; they were intrinsically limited in their

ability to describe the natural history of HIV-2 infection, which required a prospective study design (Romieu et al. 1990).

The prospective cohort study of Dakar sex workers initiated in 1985 provided the unique opportunity of measuring the infection and progression rates of both HIV-1 and HIV-2 infections (Marlink et al. 1994). Importantly, the study compared disease progression in women with known time of infection, with follow-up exceeding 22 years, representing one of the longest HIV natural-history studies in the literature to date. The Kaplan–Meier analysis of HIV-2-infected individuals indicates that 85% (95% CI, 50–96%) remain AIDS-free after 8 years of HIV-2 infection. These differences in survival probabilities between HIV-2 and HIV-1 were also seen for CDC stage IV disease and CD4 lymphocyte counts below 400 cells/mm³ and below 200 cells/mm³, as outcomes (Fig. 2).

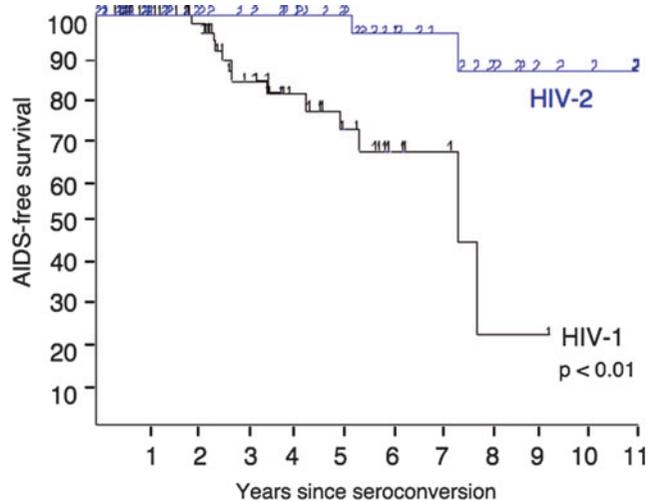
In the prospective study of HIV-2-infected individuals, individuals who fit a definition of long-term nonprogression were identified and further characterized (Kanki and Meloni 2009). Using a definition of long-term nonprogression of greater than or equal to 8 years of infection in the absence of AIDS or related symptoms and stable CD4 lymphocyte counts greater than 500 cells/mm³, 39 of 41 women (95%) could be classified as long-term nonprogressors.

Viral Characteristics of HIV-2

As studies continued to document HIV-2's distinct differences in epidemiology, transmission, and pathogenicity, other pertinent research

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Fig. 2 AIDS free survival HIV-1 compared to HIV-2 (Copyright © AIDS Reviews, 1999;1(2):101–8)



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questions emerged to identify and characterize the responsible viral and host immune mechanisms. Evidence for a lower viral burden in HIV-2-infected individuals was first reported from the decreased ability to isolate virus and later on quantitative PCR-based DNA and RNA studies. HIV-2 proviral DNA was measured with a quantitative competitive DNA PCR assay using nested gag primers and an internal competitor in the first round target sequence (Dieng-Sarr et al. 1998, 1999). DNA samples from 35 HIV-2 and 33 dually seroreactive Dakar sex workers were evaluated for levels of HIV-2 and HIV-1 proviral DNA, with assays demonstrating the same level of sensitivity. Despite similar CD4 levels, the median proviral loads differed significantly, with the HIV-2 group ranging from 63.2 to 669.8 copies/ 10^5 CD4+ cells and demonstrating an inverse correlation with CD4+ lymphocyte count. The HIV duals showed less variation in HIV-2 provirus levels, ranging from 9.9 to 43.3 copies/ 10^5 CD4+ cells; and in dually infected individuals low HIV-2 proviral load was correlated with low CD4+ lymphocyte counts. The HIV-2 proviral loads in HIV dually infected persons were significantly lower than those in HIV-2 monotypically infected individuals, despite comparable CD4+ lymphocyte counts. Immune-competent dually infected individuals regularly demonstrated both HIV proviruses, whereas those with low CD4

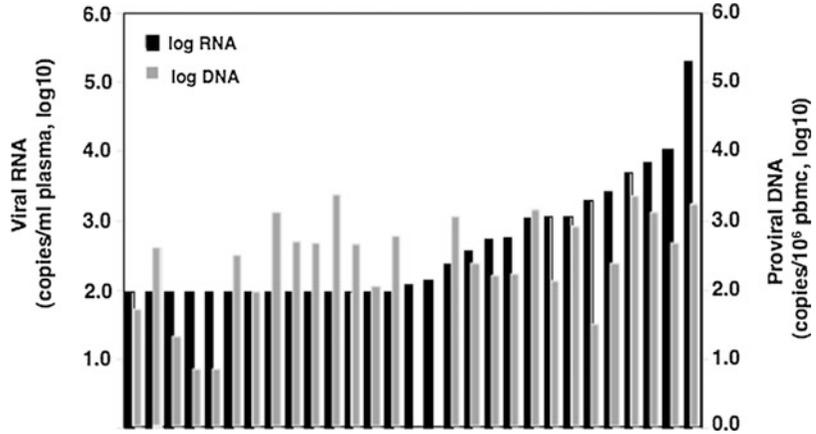
count were more likely to only have evidence of HIV-1 provirus (Dieng-Sarr et al. 1998). This suggests that during progression to disease, HIV-1 has a replicative advantage and outcompetes HIV-2 for available target cells, a phenomenon also observed in vitro.

The level of HIV virus in the plasma was also studied in the Dakar sex worker cohort with an internally controlled quantitative reverse transcriptase–polymerase chain reaction assay (Popper et al. 1999). HIV-2 viral RNA was detectable in 56% of all samples tested; the median plasma viral load was 141 copies/mL and inversely correlated to CD4 cell counts. HIV-2 and HIV-1 viral loads were compared among newly infected women in the cohort; the median viral load was 30-fold lower in the HIV-2-infected women ($p < .001$), irrespective of the length of time infected. This reinforced the concept that HIV plasma viremia was linked to the differences in the pathogenicity of the two viruses.

To further identify the source of this difference, viral RNA and proviral DNA were quantitated in matched samples from 34 HIV-2-infected individuals (Popper et al. 2000). Levels of HIV-2 proviral DNA were similar to those of HIV-1 but failed to correlate with levels of viral RNA. Thus, it appears that significant differences occur upon expression, release, and/or maintenance of HIV-2 virions in the bloodstream (Fig. 3).

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Fig. 3 HIV-2 DNA and RNA levels: No correlation between plasma virus and cellular provirus (Copyright © American Society for Microbiology, *J Virol.*, 2000;74(3):1554–7)



HIV-2 Integration and Replication

Based on an HIV-1 integration mapping study that demonstrated a major effect on viral transcription, 202 HIV-2 integration sites were mapped during *in vitro* infection of peripheral blood mononuclear cells with a primary HIV-2 isolate (p1629) (MacNeil et al. 2006). In addition, the *in vivo* proviral integration within heterochromatin in 21 HIV-1-infected and 23 HIV-2-infected PBMCs from the Dakar sex worker cohort was determined, using an alphoid repeat PCR assay. During *in vitro* infection, HIV-2 displayed integration site preferences similar to those previously reported for HIV-1. Notably, 82% of HIV-2 integrations mapped to RefSeq genes, and integration strongly favored regions of the genome with high gene density and high GC content. Though rare, the proportion of HIV-2 samples (5/23) with evidence of proviral integration within heterochromatin *in vivo* was significantly higher (p value = .05) than that of HIV-1-infected samples (0/21). Thus differences in integration sites did not appear to play a major role in differences between HIV-2 and HIV-1.

Subsequent studies demonstrated that HIV-2 is able to establish a stable, integrated proviral infection *in vivo*, but that accumulation of viral mRNA is attenuated in HIV-2 infection, relative to HIV-1 (MacNeil et al. 2007a). The differences in viral mRNA were consistent with the differences in plasma viral loads between HIV-1 and HIV-2 and suggested that lower plasma viral loads, and possibly the attenuated pathogenesis of HIV-2,

could be explained by lower rates of viral replication *in vivo*.

HIV-2 Viral Diversity

HIV diversity is considered a major determinant in the virus' pathobiology. In evaluation of the C2–C3 envelope sequences of blood and cervical samples from HIV-1- and HIV-2-infected Dakar sex workers, only rare tissue-specific subclustering was noted where most of the majority viral sequences from the two tissue compartments were intermingled (Sankalé et al. 1998).

The viral sequence evolution in the C2V3C3 region of the viral *env* gene was demonstrated in 8 HIV-2 infected individuals from Dakar, Senegal, over the course of approximately 10 years (MacNeil et al. 2007b). To compare results to HIV-1 infection, data was reanalyzed from a previous study that prospectively examined inpatient viral evolution in HIV-1-infected individuals from the same population. HIV-2 sequences from early and late time points were phylogenetically intermixed for all subjects, and no distinct trends were observed in terms of increases or decreases in fragment size or number of N-linked glycosylation sites. In overlapping *env* C2V3 sequence, rates of viral divergence and diversification were slower in individuals infected with HIV-2 compared to individuals with HIV-1. Viral evolution occurs slowly in HIV-2 infection, which is consistent with the slow disease progression observed in HIV-2 infection, and supports the notion that viral evolution

may be a relevant correlate for disease progression.

Antibody Responses to Virus-Specific Markers of HIV-2 Disease

Numerous HIV-1 studies examined the relationship between the antibody responses to viral proteins and disease. The responses to HIV-2 viral proteins were studied in 141 HIV-2-infected women from the Dakar cohort followed for up to 11 years (Popper et al. 1998). The absence of antibodies to p26, the virion-associated core protein of HIV-2, was a highly significant predictor of CDC IV category HIV disease ($p < .01$) in multivariate analysis. The antibody response to the virion-associated Vpx protein was also evaluated; while responses did not correlate to antibody responses to p26, they were similarly established early postinfection. In individuals lacking responses to p26 and with responses to Vpx, were six times more likely to be classified as CDC IV category disease ($p < .01$).

In HIV-1 infection some studies have suggested that anti-Tat antibodies may be associated with a better clinical outcome. The prevalence of anti-Tat antibodies was determined in 111 HIV-2 seroprevalent and 33 seroincident Dakar sex workers observed longitudinally between 1985 and 2003 (Rodriguez et al. 2006). Sixty-eight percent of the subjects tested positive for anti-Tat antibodies with reactivity notably established early after seroconversion and stably maintained over the course of infection. The absence of anti-Tat antibodies was significantly associated with progression to CDC IV-defined disease or CD4+ T cell count $< 200/\mu\text{L}$ during the study period, extending the importance of this prognostic marker for HIV-2 disease.

Genetic Determinants in HIV-2 Disease

Since slower disease course appeared to be common in HIV-2 infection, it seems reasonable to consider that certain subsets of the population would possess host characteristics that might predispose them to a more rapid disease course. Some human leukocyte antigen (HLA) class I alleles have been associated with slower or faster rates of HIV-1 disease progression, suggesting

that the presented epitopes or the restricting HLA class I molecule may play a central role in determining the ability to control viral replication.

A case-control study investigated possible associations between HLA and the risk of disease progression in HIV-2 (Diouf et al. 2002). The HLA class I status was molecularly typed in female sex workers from the Dakar cohort; HLA B35 was associated with lack of p26 antibodies ($P < .05$) and higher risk of disease progression. The same association was found for the class I haplotypes B35-Cw4 and A23-Cw7 ($P < .05$), similar to the association with HIV-1. The data showed that certain HLA molecules are associated with risk of disease progression in HIV-2; some of the alleles and haplotypes involved in susceptibility to disease are similar for both HIV-1 and HIV-2. Therefore, certain genetic factors may be shared by HIV-1 and HIV-2 with respect to susceptibility to enhanced disease progression. In addition, the role of the CCR5 delta 32 allele was also evaluated in 139 Dakar sex workers (Kokkotou et al. 1998). This is a known mutation in the CCR5 coreceptor that may reduce susceptibility to CCR5 HIV viruses. The presence of the CCR5delta 32 genotypes was found in 2 PBMC samples (1.44%) and was found to be less susceptible to in vitro infection by an M-tropic HIV-1 primary isolate.

HIV-2 Protection from HIV-1

Demonstrated differences in the infectivity and disease potential of HIV-2 compared to HIV-1 supported the notion that upon interaction, HIV-2 might protect from HIV-1 infection or disease, analogous to an attenuated virus vaccine model. In studies of the Dakar female sex worker cohort, the hypothesis that the attenuated phenotype of HIV-2 infection might protect from subsequent HIV-1 infection was addressed (Travers et al. 1995). HIV-1 infection in previous HIV negatives along with superinfection of HIV-2 infected was documented over the study period with both serology and PCR assays. A Poisson regression model was used to estimate the independent effect of demographic, behavioral, and biologic variables on the risk of HIV-1 infection. Despite higher incidence of other sexually

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Table 2 Natural protection from HIV-1 conferred by HIV-2 in Dakar, Senegal

HIV-1 incidence rate ratio (HIV-2, negative)	Fraction protected (%)	Years of observation	<i>p</i> value
0.23–0.32	68–77	9 years	<.05
0.26–0.36	64–74	11 years	<.05
0.33–0.42	58–67	12 years	<.05
0.34–0.44	56–66	13 years	<.03

transmitted diseases (STDs), HIV-2-infected women had lower incidence of HIV-1 than seronegatives, with an incidence rate ratio (IRR) of 0.32 ($p = 0.008$). When immunosuppression (reduced CD4+ cell count) was accounted for, the IRR associated with HIV-2 was reduced further to 0.23 ($p = 0.02$) (Table 2). This analysis led to the conclusion that HIV-2 infection conferred a significant reduction in the subsequent risk of HIV-1 infection.

The generalizability of these findings was questioned by studies from other West African sites. In Ivory Coast, Guinea-Bissau, and the Gambia, studies originally designed as cross-sectional surveys were analyzed for short periods of longitudinal observation; as a result of their design, they did not possess sufficient statistical power, capable only of detecting an extremely high protected fraction (>99%) of HIV-1 infection due to HIV-2 infection (Kanki and Meloni 2009). Continued analysis of the Dakar cohort extended the observation period from the first published report to over 13 years; HIV-2 protection ranged from 52% to 74% depending on the method of analysis. Notably, in 2012, Esbjornsson et al. reported slower progression to AIDS in individuals with dual infection where HIV-2 infection had preceded HIV-1 in Guinea-Bissau (Esbjornsson et al. 2012), thereby suggesting that HIV-1 disease progression is inhibited by concomitant HIV-2 infection and that dual infection is associated with slower disease progression.

Dieng-Sarr et al. sequenced the HIV viruses from 29 dually infected Dakar sex workers to determine if HIV-1 subtypes differed in dual infection. The majority (14/23) were infected with the prototype West African HIV-1 subtype,

CRF 02_A/G, and 9/23 formed a separate distinct cluster, later determined to be sub-subtype A3 (Meloni et al. 2004a). This was in contrast to what was found in HIV-1 singly infected women where the variant sub-subtype A3 was found in only 13/98 (13.3%). Thus, it seemed possible that the mechanisms of HIV-2 protection may be less efficacious with the sub-subtype A3 compared to the more common CRF 02A/G, resulting in the different proportion of the subtypes based on single or dual infection (Dieng-Sarr et al. 2000).

While numerous viral characteristics of HIV-2 were likely to play a role in its reduced transmission and slower disease protection, the host's immune response to the virus and potential cross-reactivity to HIV-1 was also considered. The neutralizing antibody response against heterologous isolates in HIV-2 infection and their relationship with established clinical markers of progression were examined (Rodriguez et al. 2007). Neutralizing responses against 7 heterologous primary isolates and 1 laboratory strain from 43 untreated HIV-2-infected subjects were analyzed using a pseudotyped reporter virus assay. Positive neutralization against at least 1 heterologous isolate was detected in 42 of 43 (98%) HIV-2-infected individuals tested, and significantly, 33% of chronically infected subjects neutralized all of the heterologous viruses in the panel. The magnitude of the heterologous responses was low overall, with a median IC₅₀ titer against heterologous isolates of 117, compared to 285 in HIV-1 infected subjects. The development of heterologous responses was analyzed in 10 Dakar sex workers assessed longitudinally for up to 14 years of HIV-2 infection. Longitudinal analysis demonstrated that these intratype heterologous responses develop fully after about 2.5 years of infection. Overall, there was a significant positive correlation between IC₅₀ titer and viral load, suggesting heterologous antibodies may be driven by viral replication. Plasma from a subgroup of 24 HIV-2-positive subjects was then tested for intertype neutralization against a panel of 6 HIV-1 reporter viruses. Here, only 10 of 144 (7%) plasma-virus combinations were positive for neutralization. When HIV-1 reporter viruses were pretreated with

sCD4 protein, however, the proportion of plasma–virus combinations positive for neutralization increased to 128 of 144 (88%). It was concluded that the extensive intratype cross-neutralization observed indicated the presence of conserved neutralization determinants among HIV-2 viruses circulating in Senegal. Further, it was demonstrated that HIV-2 infection elicits CD4-induced (CD4i) antibodies able to neutralize a virus as divergent as HIV-1.

T cell activation in HIV-2 infection was studied in the Dakar cohort where CD4⁺ and CD8⁺ T cell populations were independently assessed with the hypothesis that distinct factors mediate immune activation in specific T cell subsets (Hanson et al. 2005). CD8⁺ T cell immune activation has previously been strongly associated with HIV-1 disease progression. CD8⁺ T cell activation was significantly different between HIV-2 and HIV-1 volunteers ($p < 0.0015$), and both groups expressed higher activation levels compared to seronegative individuals ($p = 0.014$ and < 0.001 , respectively). A significant correlation between viral load and T cell activation was observed only in the HIV-1-infected group ($r = 0.57$, $p = 0.01$), but a similar association was not observed in the HIV-2-infected group ($r = 0.23$, $p = 0.47$). Surprisingly, similar levels of CD4⁺ T cell activation were observed in HIV-1- and HIV-2-infected subjects ($p = 0.52$), perhaps reflecting similar in vivo viral pathogenesis. CD4⁺ T cell activation in HIV-1-infected subjects correlated with CD4⁺ cell count ($r = -0.49$, $p = 0.03$) but not with viral load ($r = 0.16$, $p = 0.52$). In contrast, no correlation between CD4⁺ immune activation and CD4⁺ cell count or viral load was demonstrated in HIV-2-infected subjects ($p = 0.76$ and 0.90 , respectively). Thus, HIV-2 demonstrated distinct differences in both CD4⁺ and CD8⁺ activation compared to HIV-1.

The ELISPOT technique and a unique HIV-2 antigen delivery system to further prime and quantify the HIV-2-specific CTL response in HIV-2-infected individuals were described (Dieng Sarr et al. 2001). The frequency of HIV-2-specific gag CTL precursors in uncultured PBMCs from HIV-2-infected women was evaluated using a

modified nontoxic form of the anthrax toxin (*Bacillus anthracis*). The HIV-2 gag (P26) was fused to the terminal domain of the anthrax lethal factor (LFn; 255 aa). The LFn-P26 (HIV-2 gag) recombinant proteins were expressed and used as antigens to stimulate CTLs in an ELISPOT format and compared to the classic antigen delivery system using recombinant vaccinia virus (rVV) expressing HIV-2 gag. A majority of individuals (87.5%) in the study showed a specific gag CTL response using the LFn-P26 expression, far superior to the classic rVV expression. Interestingly, the individuals with strong cellular immune response had no detectable HIV-2 plasma load implicating the role of cellular immunity in the delayed pathogenesis of HIV-2.

In Vitro Evidence for HIV-2 Protection from HIV-1

Studies have described in vitro interactions of HIV-1 and HIV-2 that support the Dakar cohort in vivo observations; these range from virus–virus interactions to potential immune-mediated mechanisms for HIV-2 protection. Arya et al. have reported that HIV-2 inhibits the replication of HIV-1 at the molecular level. This inhibition was selective, dose dependent, and nonreciprocal. Though the exact mechanism remains to be defined, the inhibition appeared to be mainly due to an intracellular molecular event because it could not be explained solely on the basis of cell surface receptor-mediated interference. The results support the notion that the inhibition likely occurred at the level of viral RNA, possibly involving competition between viral RNAs for some transcriptional factor essential for virus replication (Kanki and Meloni 2009).

Using an in vitro HIV-1 challenge system, a significant proportion (60%) of PBMCs derived from HIV-2-infected women from the Dakar sex worker cohort could not support replication of a CCR5-dependent HIV-1 virus compared with CXCR4-dependent virus (Kokkotou et al. 2000). Resistance was transferable and CD8 dependent and strongly correlated with beta-chemokine production in the media. All resistant cultures were rendered susceptible by addition of a cocktail of antibodies to beta chemokines.

HIV-2 infection might dramatically influence beta-chemokine production by enhancing it in magnitude and/or duration, thus enabling HIV-2-infected individuals to cope favorably with subsequent exposure to HIV-1. This is supported by studies demonstrating that binding of the HIV-2 envelope to the alpha chain of CD8 stimulates dramatic levels of beta-chemokine production in comparison to HIV-1 gp120 activity. Further, unstimulated PBMCs from HIV-2-infected Dakar sex workers demonstrated diminished surface CCR5 receptor expression on CD4⁺ T cells (Shea et al. 2004). In vitro upregulation of the CCR5 receptor was readily demonstrated indicating that the receptor was functional. The in vivo downregulation of the receptor was not correlated with activation markers (HLA-DR), beta-chemokine levels, or plasma viral load. It was postulated that the downregulation of the CCR5 receptor in HIV-2 infection contributed to slower disease course, by decreasing susceptibility of target cells for infection in conjunction with HIV-2-specific cellular immune responses. It is also recognized that CTLs are able to lyse infected cells before progeny virions are produced and the production of granzyme particles will elicit beta chemokines therefore providing two mechanisms by which viral replication could be limited or prevented. Not only does this implicate a novel viral suppressive mechanism but one that may be adapted for immunoprophylaxis. Antiretroviral vaccine strategies that incorporate beta-chemokine induction or other receptor-blocking functions raise some encouraging possibilities for vaccine design and development.

Impact of HIV-1 Subtypes

Clinical and immunological differences have also been found between HIV-1 subtypes, and it was well recognized that multiple diverse HIV-1 subtypes are prevalent in West Africa with dynamic changes over time. The epidemiology of HIV infection in the Dakar cohort was further studied with diagnosis of the HIV-1 subtype in 328 HIV-1-infected women. A 385 bp C2-V3 fragment of the envelope gene was sequenced and classified into the following subtypes or recombinant forms: 239 (72%) were subtype

A [of which 180 (55%) were CRF02_AG, and 53 (16%) were A3], 10 (3%) were B, 12 (4%) were C, 11 (4%) were D, 18 (6%) were G, 24 (7%) were CRF06_cpx, and 7 (2%) were CRF09_cpx. An increasing proportion of CRF02_AG was observed over many years, but the more recently described sub-subtype A3 had overtaken CRF02_AG, with the largest proportion of new infections (Meloni et al. 2004a, b). The predominance of existing HIV-1 subtypes did not preclude the emergence and increase of other closely related subtypes or recombinant forms. This 20-year prospective serologic and sequence analysis of HIV viruses revealed a complex and changing HIV epidemic in Senegal and represents a unique long-term description of the epidemiology of these two HIV viruses in high-risk people (Hamel et al. 2007). In addition, women infected with CRF02_AG in the Dakar sex worker cohort had a significantly higher viral load during the early stage of infection than did women not infected with CRF02_AG (Kanki and Meloni 2009). Finally, women infected with CRF02_AG demonstrated slower progression to AIDS compared to women infected with other HIV-1 subtypes (Kanki et al. 1999).

Conclusions and Lessons Learned

HIV-2 was first described in the Dakar sex worker cohort in 1985 and was the dominant virus when HIV-1 was first entering this population. Between 1985 and 2004, HIV-2 prevalence dropped from 8% to 4%, a 50% decrease over 20 years. Concurrently, HIV-1 prevalence increased from 0% to 13% during the same time period. Anderson and May's prediction that HIV-2 would decrease in the face of an increasing HIV-1 prevalence is well supported by the Dakar sex worker cohort data. The attenuated rate of HIV-1 infection in a high-risk group of sex workers would also suggest that the interaction of the HIV viruses in this population may inhibit explosive increases in HIV-1 that have been described in similar HIV-1 studies of sex workers. In addition, the registration and health-care system for these women supported by a strong government HIV/AIDS control, prevention, and treatment program may have

contributed to the decline or stabilization of infection rates for both viruses (Kanki 2009).

HIV's propensity for genetic diversification has resulted in two closely related HIV types, HIV-1 and HIV-2. These viruses have a distinct geographic distribution, with HIV-2 predominating in West Africa. Current epidemiologic trends suggest that HIV-2 is unlikely to result in a global pandemic like HIV-1 and may in fact be decreasing in endemic regions. Major differences in the biologic properties between HIV-1 and HIV-2 suggest a more ancestral history to HIV-2 with adaptation to the human host and relative attenuation. This has resulted in lowered transmission potential and decreased pathogenicity. The diversification of HIV-1 appears to be more recent and less well understood. HIV-1 subtypes, sub-subtypes, and recombinant forms demonstrate a unique geographic distribution, even within the African continent. Current studies suggest that the epidemiology of these variants is dynamic, particularly within Africa. In the future, it will be important to molecularly characterize HIV subtypes in order to accurately map the molecular epidemiologic timeline. This may indicate important differences in the transmission and pathogenic properties of HIV types and subtypes which may inform our understanding of immune protection and disease pathogenesis.

Observational and *in vitro* studies suggest that the attenuated HIV-2 virus infection may provide cross-protective immunity for HIV-1, suggesting that one plausible explanation for the relatively low rates of HIV-1 infection may be due to the coexistence of HIV-2, a unique feature of West Africa. Unbiased, powerful studies, using sensitive and specific classification methods, will effectively address the generalizability of the observation of HIV-2's protective efficacy against subsequent HIV-1 infection described in the Dakar sex worker cohort for over a decade (Kanki and Rowland-Jones 2014). The long person-time of observation with few losses to follow-up and rigorous PCR testing supported these important findings. A potential mechanism could include the expression of beta chemokines from HIV-2-infected lymphocytes that inhibit HIV-1 infection. In addition to HIV-2's

demonstrated robust CTL responses, the accompanying granzyme production would elicit beta chemokines resulting in downregulation of the CCR5 receptor on CD4-bearing cells, rendering the target for HIV-1 and HIV-2 viruses less susceptible to infection. Therefore, HIV-2 infection resulting in active humoral, cellular immunity and beta-chemokine production could inhibit the replication of HIV-1 and ensuing disease progression.

Finally, the discovery and description of HIV-2 infection in Dakar, Senegal, also provided an important model for international and long-term collaboration and partnership. The initial discovery of HIV-2 in registered sex workers in Dakar, Senegal, was conducted through a unique collaboration between Souleymane Mboup and his team, Cheikh Anta Diop University, Francis Barin, University of Tours (France), Francois Denis, University of Limoges, and the Harvard School of Public Health, USA. This interuniversity convention worked together for more than two decades to advance our understanding of the biological and clinical significance of HIV-2 (Kanki 2009). Much of the research was based on studies conducted on registered female sex workers in Dakar Senegal. It is important to also recognize the contribution of these women who consented to participate in the research studies that advanced the HIV research community's understanding of HIV-2 and provided important lessons on HIV-2 infection and its implications.

Cross-References

- ▶ [Antibody Response to HIV-2](#)
- ▶ [Cellular Immune Response to HIV-2 Infection](#)
- ▶ [Epidemiology of HIV-2 Infection in West Africa](#)
- ▶ [Female, Male and Transgender Sex Workers, Epidemiology of HIV/AIDS](#)
- ▶ [HIV Transmission in Female Commercial Sex Workers and Host Protective Factors](#)
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HIV-Associated Cancers

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Definition

HIV-associated cancers are cancers whose incidence is increased in patients with HIV infection and especially those in which the increased incidence is a result of the HIV infection. These include both AIDS-defining cancers (cancers that confer a diagnosis of AIDS when they occur in HIV-infected persons) and non-AIDS-defining cancers (other cancers whose incidence is increased in HIV infection). The AIDS-defining cancers are Kaposi's sarcoma, certain high-grade B-cell lymphomas, and cervical cancer.

Introduction

On June 5, 1981, the Centers for Disease Control reported in *Morbidity and Mortality Weekly Report* a cluster of 5 cases of *Pneumocystis pneumonia* in young homosexual men. A month later, on July 3, they reported on both *Pneumocystis pneumonia* and Kaposi's sarcoma (KS) (see entry on “► [Epidemic Kaposi Sarcoma, Pathogenesis and Presentation](#)”) occurring among homosexual men in New York and California (Centers for Disease Control 1981). These were the first reports of the disease we now call acquired immunodeficiency syndrome (AIDS). KS had previously been a very rare skin cancer

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reported primarily in elderly men in Mediterranean regions, and its occurrence in young men who had sex with men (MSM) and the severity of many of these cases were a distinct departure from previous epidemiologic patterns.

AIDS-Defining and Non-AIDS-Defining Tumors in the AIDS Epidemic

As the AIDS epidemic unfolded over the next several years, it became apparent that AIDS was a complex disease characterized by profound immunodeficiency, especially of CD4+ T cells; immune dysregulation; and unusual opportunistic infections. Also, it became apparent that this syndrome was associated with certain cancers, especially KS (see entry on “► [Epidemic Kaposi Sarcoma, Pathogenesis and Presentation](#)”), central nervous system lymphoma (see entry on “► [AIDS-Related Primary Central Nervous System Lymphoma](#)”), and certain other high-grade B-cell lymphomas (see entry on “► [Presentation and Pathogenesis of B-Cell Lymphoid Cancers Associated with HIV Infection](#)”). As we now know, HIV/AIDS is associated with an increased risk of a number of tumors, some like KS that are rare outside the setting of profound immunodeficiency (Fig. 1) and others that develop in immunologically intact individuals but occur at a higher rate in patients with HIV/AIDS.

As the Centers for Disease Control (CDC) established criteria for this new and complex

syndrome, they considered Kaposi’s sarcoma and certain B-cell lymphomas [Burkitt or equivalent term (see entry on “► [Burkitt and Burkitt-like Lymphoma](#)”), immunoblastic or equivalent term, or primary brain lymphoma] as “AIDS defining” (Table 1) (see entry on “► [Epidemiology of AIDS-Defining Malignancies](#)”). This meant that the development of these tumors in an HIV-infected individual conferred a diagnosis of AIDS. These tumors tended to occur particularly in patients with low CD4 counts. Subsequently, in 1992, invasive cervical carcinoma was added to the list of “AIDS-defining tumors,” although it did not have as clear an association with profound immunodeficiency (see entry on “► [Cervical Cancer and HIV](#)”). It should be noted that the AIDS-defining lymphomas included in the 1985 CDC criteria are based on the classification of lymphomas in use at that time, which has since been superseded. There is some uncertainty as whether certain lymphomas in the current terminology should be considered AIDS defining. Primary effusion lymphoma, for example, was not described as a separate entity in 1992 but is generally considered an AIDS-defining lymphoma (see entry on “► [Primary Effusion Lymphoma](#)”).

These were not the only tumors that are increased in patients with AIDS (or infected with HIV). Epidemiological studies identified a number of other tumors with increased incidence, such as anal carcinoma, lung cancer, and non-Hodgkin’s lymphoma (see entries on “► [Anal Cancer](#),” “► [Lung Cancer](#),” and “► [Hodgkin](#)

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Fig. 1 Two patients with AIDS-associated KS. *Left:* patient with multiple lesions of cutaneous KS on the back. *Right:* lower limb, showing extensive involvement with confluent KS and lymphedema



Lymphoma in Patients with HIV Infection”). These additional HIV-associated cancers are now called “non-AIDS-defining tumors” (see entry on “► **Epidemiology of Non-AIDS-Defining Malignancies**”). This term is sometimes also used to refer to any cancer that arises in an HIV-infected patient (even if it is not HIV associated) and that is not one of the AIDS-defining tumors.

For a number of years, the fact that some tumors were markedly increased in incidence in HIV-infected patients, while others were not, was a puzzle to scientists. Some clues came from epidemiology. For example, KS was particularly common in HIV-infected patients who had had sex with men, while the incidence of lymphoma was relatively equal in all HIV risk groups. The epidemiology of KS suggested that another infectious agent besides HIV was responsible for its pathogenesis, but attempts to identify such an agent failed until 1994, when the husband and wife team of Patrick Moore and Yuan Chang reported sequences of a novel gammaherpesvirus in the KS lesions of AIDS patients using the technique of representational difference analysis (Chang et al. 1994). This virus, which is called Kaposi’s sarcoma-associated herpesvirus (KSHV) or human herpesvirus-8 (HHV-8), is now known to be the cause of KS (see entry on “► **Kaposi Sarcoma-Associated Herpesvirus (KSHV) or Human Herpesvirus 8 (HHV-8)**”). It has also been identified as the etiologic agent of a rare lymphoma called primary effusion lymphoma (PEL) (Cesarman et al. 1995) and of most cases of multicentric Castleman disease (MCD) that arise in HIV-infected individuals (see entry on “► **Multicentric Castleman Disease**”). With the discovery of KSHV, it became evident that most (but not all) HIV-associated tumors were caused by another oncogenic virus (Table 1).

The development of several effective anti-HIV drugs enabled the use of highly active combination regimens, often called highly active antiretroviral therapy (HAART). The use of HAART in the USA and other resource-rich countries started around 1996. In addition to markedly reducing the mortality of HIV, the widespread use of HAART led to a decrease in the incidence of some AIDS-defining cancers, especially those

(such as KS or central nervous system lymphoma) that occur in patients with very low CD4 counts and immunologic impairment (Shiels et al. 2011). There was initially optimism that these tumors would largely become a thing of the past. However, because of several factors, after an initial decrease, the number of cases of AIDS-defining malignancies has remained fairly constant in the USA since about 2002. One reason is that there has been more than a doubling of the number of individuals living with AIDS in the USA, because the rate of new HIV infection has not changed and HIV-infected patients are living longer. Another factor is that some HIV-infected patients do not realize they are infected, or do not engage with medical care, until they become substantially immunosuppressed and develop an AIDS-defining tumor. A third is that some AIDS-defining tumors, such as cervical cancer, develop in patients with relatively high CD4 counts. Finally, the population of HIV-infected patients is becoming older, and age is an additional major risk factor for many tumors.

As noted above, in addition to the AIDS-defining tumors, a number of other tumors develop more often in HIV-infected individuals than the general (age-matched) population (Engels et al. 2006). These include anal carcinoma, Hodgkin’s lymphoma, lung cancer, pharyngeal carcinoma (see entry on “► **Other HPV-Associated Cancers (Oropharyngeal and Penile)**”), and Merkel cell tumor (see entry on “► **Merkel Cell Carcinoma and Other HIV-Associated Skin Cancers**”). In addition, as HIV-infected patients live longer and this population is increasing in age, they are increasingly developing the wide range of tumors seen in the general population. The best evidence is that many of these tumors arise independent of HIV infection, although it is possible that HIV will be found to be a contributory factor as we learn more about the epidemiology and factors contributing to their pathogenesis.

Recent studies have shown that unlike the AIDS-defining tumors, the number of cases of non-AIDS-defining tumors has increased substantially since the introduction of HAART in 1996, and in fact, there are now more non-AIDS-defining tumors than AIDS-defining tumors in

HIV-Associated Cancers, Table 1 Selected malignancies that are more frequent in HIV patients and their associated viruses

Malignancy	AIDS defining	Principal-associated virus or viruses	Comment
Kaposi's sarcoma	Yes	KSHV	
Primary central nervous system lymphoma	Yes	EBV	
Diffuse large B-cell lymphomas (germinal center or activated B-cell subtypes) ^a	Yes	EBV	A substantial percentage of cases are EBV negative
Burkitt lymphoma	Yes	EBV	A substantial percentage of cases are EBV negative
Primary effusion lymphoma	Yes	KSHV	Often also EBV
Hodgkin's lymphoma	No	EBV	Sometimes EBV negative in HIV patients
Multicentric Castleman disease	No	KSHV	
Anal cancer	No	HPV	
Pharyngeal cancer	No	HPV	
Primary hepatocellular carcinoma	No	HBV or HCV	
Plasmablastic lymphoma (oral cavity associated) ^b	No	EBV	Occasionally EBV negative in HIV patients
Merkel cell tumor	No	Merkel cell polyomavirus	Some are negative for Merkel cell polyomavirus
Leiomyosarcoma in children ^c	No	EBV	
Conjunctival carcinoma	No	Unclear; possibly HPV	Reported almost only in Africa
Lung cancer	No	None yet identified	Much of increased risk due to high prevalence of smoking

Abbreviations: *KSHV* Kaposi's sarcoma-associated herpesvirus, *EBV* Epstein-Barr virus, *HPV* human papillomavirus, *HBV* hepatitis B virus, *HCV* hepatitis C virus

See entries on:

^a► [Diffuse Large B-Cell Lymphoma](#)”

^b► [Plasmablastic Lymphoma](#)”

^c► [Malignancies in Children with HIV Infection](#)”

HIV patients in the USA (Shiels et al. 2011). Also, with the widespread use of HAART, there are now fewer deaths from uncontrolled HIV infection, complications of profound AIDS immunosuppression, or opportunistic infections, and in some studies, cancer has now become the most common cause of death in patients with HIV/AIDS (Bonnet et al. 2009).

Pathogenesis of Tumors in HIV/AIDS

As noted above, the discovery of KSHV and its identification as the cause of KS (and several other tumors) was crucial, not just for its understanding of KS, but also because it led to an appreciation that most (but not all) tumors that are associated with HIV infection are caused by other oncogenic

viruses (Table 1). Our current understanding is that the immune system plays an important role in suppressing infection with these viruses and also suppressing malignant cells that express foreign epitopes from the viruses. Thus, in the setting of AIDS, the profound immunodeficiency permits an outgrowth of these viruses and cells transformed by them. In some cases, such as KSHV-associated multicentric Castleman's disease (MCD), the tumor is really a hyperproliferative state. In other cases, such as lymphomas caused by Epstein-Barr virus (EBV), our understanding is that a proliferation of virus-infected cells increases the likelihood of mutations that eventually lead to clonal tumors (see entry on “► [Epstein-Barr Virus \(EBV\)](#)”). Even HIV patients who are treated with HAART and have relatively normal CD4 counts have defects in their immune repertoire that over time can

enhance the risk of various tumors. An increased incidence of a tumor in the setting of HIV infection is a clue that this tumor may be caused by a virus. As an example, Merkel cell carcinoma, a rare skin cancer, is more likely to develop in HIV-infected patients, and an investigation of this relationship led to the discovery of Merkel cell polyomavirus as a new oncogenic virus (Feng et al. 2008).

Interestingly, not all tumors are more frequent in HIV-infected patients, and in particular, the incidence of some common tumors such as colon cancer, breast cancer, or cancer of the prostate is not increased. This observation suggests that either the immune system does not play an important role in keeping such tumors in check or time for such tumors to arise is longer than the survival of AIDS patients, at least until HAART was developed. It will be of interest to see if these tumors also become more common in the setting of HIV infection than in the general population as HIV-infected patients live for decades on HAART.

HIV can promote tumorigenesis in other ways as well. HIV infection is associated with immune dysregulation and B-cell hyperactivation, and this can promote the development of B-cell lymphoma and other tumors (such as KS) that respond to cytokine excess. Also, specific proteins of HIV may promote tumor development; for example, the Tat protein can penetrate cells and has been shown to enhance infection of cells with KSHV. Another possible factor is long-term exposure to antiretroviral drugs. Nucleoside reverse transcriptase inhibitors such as zidovudine act as chain terminators, and administration of high doses of certain of these drugs (including AZT) to pregnant mice has been reported to lead to an increased incidence of tumors in the offspring. However, it is important to note that epidemiologic studies of these drugs in humans have not yielded evidence that they contribute to tumorigenesis.

For some of the tumors whose incidence is increased in HIV-infected patients, the cause of the increased incidence may be in part or whole because of increased exposure to other cancer risk factors. For example, such patients have an increased risk of lung cancer that is due, at least in part, to increased cigarette use in the HIV-infected population. Studies trying to

determine if immunodeficiency or HIV infection independently contributes to the increased incidence of lung cancer have yielded conflicting results. Also, HIV-infected patients often have coinfection with hepatitis B virus (HBV) or hepatitis C viruses (HCV), as these viruses, like HIV, are spread through injection drug use, and hepatitis B can be spread sexually. Infection with these viruses can lead to hepatocellular carcinoma, which is also increased in incidence in HIV-infected patients. It appears that most of this increased incidence is due to the increased coinfection with HBV and/or HCV (see entry on “► [Hepatocellular Carcinoma in HIV-Positive Patients](#)”). However, there is reasonable evidence that immunosuppression also plays a role in the increased incidence of hepatocellular carcinoma. Similarly, HIV-infected patients have higher exposure to human papillomavirus than the general population, and this can work in conjunction with immunosuppression to lead to a higher incidence of cancer of the cervix, anus, penis, and oral pharynx (see entry on “► [Human Papillomavirus \(HPV\)](#)”). Thus, each HIV-associated cancer has its own complex story, and while there are some common themes, the pathogenesis of each has to be considered separately.

As the rate of HIV infection does not appear to be changing, at least in the USA, and as HIV-infected patients live for years with HAART, the HIV-infected population overall is increasing in age, and we are seeing an increasing population of HIV-infected patients over the age of 50 or 60 years. Cancer is in general a disease of aging, and some AIDS-defining tumors, such as KS, can occur in elderly patients outside the setting of HIV infection. It is not known exactly how the combination of aging, chronic mild immune defects of HIV and aging, coinfection with other oncogenic viruses, prolonged exposure to antiretroviral drugs, and enhanced exposure to other cancer risk factors such as cigarettes will interact to affect the development of tumors as we see a “graying” of the HIV-infected population. As HIV-infected patients age, they are increasingly susceptible to develop tumors on that basis alone. It is not known at this time how this process may be affected by long-term HIV infection. It will be

important to monitor the development of various tumors in the population living with long-term HIV infection to understand these issues and develop appropriate screening, preventive, and therapeutic strategies.

HIV-Associated Tumors in Resource-Limited Countries

Special attention must be given to HIV-associated cancers in resource-limited countries (see entry on “► [HIV Cancers in Resource-Limited Regions](#)”). In these regions, a higher proportion of cancers overall are due to infectious agents than in the more developed world, and many of these areas have a high prevalence of viruses that cause HIV-associated cancers. In particular, there is a high prevalence of KSHV infection in sub-Saharan Africa (Chokunonga et al. 1999), and in this region, KS is among the most common cancers; in some sub-Saharan countries, it is the most common tumor in males. Even before the spread of HIV, KS was a relatively common tumor in Africa, and KSHV-infected patients in Africa are more likely to develop KS than in other regions. The reasons for this are not clear. Possible explanations include exposure to other diseases, such as malaria, that cause inflammation; an earlier age of infection with KSHV; genetic factors that predispose to KS; or exposure to certain plants or other environmental factors that promote KS. Also, Burkitt lymphoma is relatively common in regions of sub-Saharan Africa even in HIV-uninfected patients. Most endemic cases of Burkitt lymphoma are caused by Epstein-Barr virus, and epidemiologic studies show that coinfection with malaria is an important contributor to tumorigenesis. There are certain HIV-associated tumors reported in Africa, such as carcinoma of the conjunctiva, that are not reported elsewhere, and there is much we need to learn about these unusual tumors (see entry on “► [Conjunctival Carcinoma](#)”).

Research on the epidemiology and pathogenesis of HIV-associated tumors in the developing world, and especially Africa, is hampered by the relatively undeveloped state of the medical and

research infrastructure in these regions. Patients with suspected tumors are often treated without biopsy or in setting with limited pathological expertise, and lymphoma, for example, can be confused with tuberculosis or other common diseases. As an example of this, while KSHV-associated multicentric Castleman disease has been diagnosed in a number of African immigrants to the USA, there are only few reports of this condition in Africa in spite of a very high prevalence of both KSHV and HIV. It is almost certain that many cases of this disease in Africa go unrecognized and are thought to be tuberculosis or a related illness.

The President's Emergency Plan for AIDS Relief (PEPFAR) is an ambitious program of the USA to provide effective therapy to HIV-infected patients in resource-limited settings. This program started in 2003 and has brought AIDS treatment to a substantial number of patients in Africa and other regions. As a result of this, we are starting to see a drop in AIDS-related tumors, especially KS. However, there is some evidence that, like in the resource-rich world, there is now the beginning of an increase in non-AIDS-defining tumors in these areas.

Treatment of Tumors in HIV-Infected Patients

The treatment of specific HIV-associated tumors is discussed in the specific sections on these tumors. However, it is worth presenting some broad principals. While KS has been, until recently, the most common tumor arising in AIDS patients in the USA, it was relatively rare in the USA prior to the AIDS epidemic, and relatively little was known about how to treat it. Thus, treatments for KS, including the use of interferon alpha, the identification of effective cancer chemotherapeutic drugs such as vincristine or paclitaxel, and the development of liposomal anthracyclines, were largely developed in the setting of AIDS (Krown et al. 1983; Welles et al. 1998). As we have learned more about the pathogenesis of this tumor, novel treatments aimed at blocking specific steps in its pathogenesis are now being developed.

With regard to systemic AIDS lymphomas, a challenge was that while these tumors were often curable outside the setting of AIDS with combination chemotherapy regimens, such regimens were initially viewed as too toxic to administer to AIDS patients. As a result, before the widespread use of HAART, low-dose chemotherapy regimens were developed specifically for HIV-associated lymphomas (Kaplan et al. 1997). An additional challenge at that time was that patients often died of their AIDS even if the lymphoma could be effectively treated. This was a problem with essentially all tumors developing in AIDS patients, and in fact, AIDS patients were almost universally excluded from experimental protocols to treat cancers except for studies of specific AIDS-related tumors.

With the development of HAART, this whole picture changed. The immune system of many AIDS patients could be restored to a large degree, they were not as frail, they had a better life expectancy, and they could tolerate toxic regimens better than before HAART. As a result, in a series of incremental clinical studies, it was shown that such patients could generally tolerate full-dose lymphoma regimens. There is recent information that HIV-infected patients can even tolerate allogeneic stem cell transplantation, and this approach is also being explored as a means of eradicating HIV in infected patients (see entry on “► [Stem Cell Transplantation](#)”). At the same time, a number of advances (such as rituximab and infusional regimens) were made in the therapy of various lymphomas (Dunleavy et al. 2010), and the expected survival of patients with certain types of AIDS lymphoma now approaches that of lymphoma patients without HIV.

While AIDS patients on HAART could often tolerate cancer chemotherapy, physicians treating such patients have often been reluctant to use full-dose therapy because of their experience with AIDS patients before the widespread use of HAART, and such patients have often been undertreated. Contributing to this problem has been the relative paucity of published information on the treatment of various tumors in HIV-infected patients as a result of their exclusion from cancer trials. Another concern for physicians

is that many HIV drugs, and especially the protease inhibitors that interact with cytochrome P450, can have substantial pharmacokinetic interactions with other drugs including cancer drugs (see entry on “► [cART and Supportive Care for HIV-Associated Malignancies](#)”). In many cases, there was little published information on these interactions, and this was another cause for caution. In 2008, the US National Cancer Institute determined that in most instances, there was no good reason to exclude HIV-infected patients from cancer trials, and they have made a concerted effort to open trials to such patients when feasible (Persad et al. 2008). Other cancer research agencies have also made an effort to study the treatment of cancer in HIV-infected patients, and as a result, there is an emerging body of information on the optimal treatment of a variety of cancers in the setting of HIV infection. This is an important development, as we see a shift from a few AIDS-defining tumors to a wide variety of AIDS-non-defining tumors arising in patients with HIV/AIDS.

Prevention of Tumors in HIV-Infected Patients

As noted above, a number of HIV-associated tumors are caused by oncogenic infectious agents, and they may thus be amendable to preventive strategies aimed at the underlying virus. Moreover, HIV itself plays an important role in the pathogenesis of the tumors, and strategies to prevent or treat HIV infection can therefore affect the development of these tumors. In fact, the development and widespread use of HAART caused a dramatic decrease in the incidence of AIDS-defining tumors, especially those that are associated with very low CD4 counts. It goes without saying that successful prevention of HIV infection, for example, by public health measures or, if developed, an HIV vaccine, will prevent HIV-associated cancers.

A number of approaches can also be used to prevent or reduce the extent of infection with the viruses responsible for causing many of these tumors. A vaccine against hepatitis B virus

(HBV) has been in use for several decades, and vaccines have recently been developed against strains of HPV responsible for the majority of cases of cervical, anal, and other HPV-associated cancers (Lowy and Schiller 2006). These vaccines must be given prior to infection with HPV, usually before the time of sexual activity. As a result, the population of adults today will generally not be protected by this vaccine, and its effects will be largely seen over the next several decades. Another concern is that, at least in the USA, the uptake of the vaccine in boys is very low, and MSM may remain an unprotected population for some time.

There are currently no vaccines developed against hepatitis C virus (HCV) or KSHV. Antiviral drugs have been developed or shown to be active against both of those viruses, and the use of ganciclovir (for cytomegalovirus retinitis) has been shown to reduce the development of KS (Martin et al. 1999). The spread of HCV, as well as HBV, has been reduced by the development of tests to screen the blood supply and can also be impacted by public health measures. KSHV is known to be secreted in saliva, although there is still much we do not know about the main causes of transmission. Unlike Epstein-Barr virus, KSHV appears to only be effectively transmitted in certain populations, and as we learn more about this, it may be possible to institute public health recommendations to reduce its spread. Strategies to prevent tumors in HIV-infected patients will be an important area of research and development as we move forward.

Conclusion

While it is still unclear whether HIV can be directly oncogenic, HIV infection and its associated immunodeficiency enhance the development of a variety of tumors. Some of these tumors, called "AIDS defining," confer a diagnosis of AIDS when they arise in an HIV-infected individual. The widespread use of HAART has dramatically reduced the incidence of several of these AIDS-defining tumors, especially those associated with very low CD4 counts. A number of

other "non-AIDS-defining" tumors are also more frequent in the setting of HIV infection. In some cases, HIV or HIV-associated immunodeficiency has directly contributed to their development. HIV-infected patients often have increased exposure to a variety of oncogenic agents, such as HCV or cigarette smoke, and this may contribute or even be the cause of the increased frequency of certain tumors. As HIV-infected patients live longer on HAART and as this population becomes older, we are seeing a decrease in AIDS-associated tumors and an increase in others. It is possible that new trends may emerge as patients live for decades with suppressed HIV infection, chronic subtle immune defects, and chronic exposure to antiretroviral drugs.

Most of the AIDS-defining or HIV-associated tumors are caused by oncogenic viruses, and it may be possible to effectively screen, prevent, or treat these tumors by strategies directed at the underlying virus. Substantial progress has already been made in this area, and it promises to be a fruitful area for future progress. At the same time, the trend toward development of a wider range of tumors in HIV patients is increasing the complexity of this field and will require teasing out and understanding the trends as they emerge.

Whether or not it is HIV associated, the treatment of cancer in an HIV-infected individual is often quite complex, as patients have two life-threatening conditions. Immunodeficiency and other manifestations of HIV may complicate the treatment of the tumor, and there is the potential for substantial drug interactions. It will be important to continue to develop improved therapy for those tumors that principally develop in HIV patients. At the same time, it will be important to learn how to optimally manage other cancers, whether or not they are HIV associated, that develop in the setting in HIV infection.

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HIV-Associated Immune Exhaustion

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Definition

The immune systems of HIV-infected patients show evidence of exhaustion. Immune exhaustion occurs at three different levels: (i) functional, (ii) clonal, and (iii) systemic. Firstly, HIV-specific cells present functional alterations, largely related to the overexpression of co-inhibitory receptors, upregulated as a result of chronic antigenic stimulation. Secondly, persistently activated HIV-1-specific CD8⁺ T-cell clones are driven towards a state of replicative senescence, resulting in the loss of cytotoxic T-cell populations important for the control of viral replication. Lastly, HIV-1-infected individuals are characterized by declining lymphocyte renewal capacities or lymphopoiesis, observed at the level of hematopoietic progenitors and reminiscent of old age. Thus, immune exhaustion in chronic HIV infection is multifaceted and affects the immune response in a number of ways, all of which are likely to contribute towards disease progression.

Introduction: Chronic Immune Activation in HIV Infection

The progressive depletion of and eventual deficit in CD4⁺ T cells represent hallmarks of HIV disease progression. However, the phenomenon of immune exhaustion may also play a key role in the decline of immune competence seen during the course of HIV infection. The plasticity of the immune system and its efficacy against pathogens are prodigious. Upon primary viral infection,

virus-specific naïve lymphocytes, that have not previously encountered the virus, become activated and develop into short-lived “effector” cells, capable of mounting a potent antiviral response. Upon viral clearance, a small proportion of these cells remains and acquires a memory state. These relatively long-lived memory lymphocytes will be responsible for orchestrating a rapid and robust recall response against the same pathogen. However, HIV is a persistent virus, which establishes chronic infection in its host, associated with the development of chronic immune activation. Elevated immune activation, which is a clear correlate of HIV disease progression, is believed to be an important determinant of immune exhaustion in HIV-infected patients (Appay and Sauce 2008).

A major contributor to immune activation in HIV-infected individuals is the persistent stimulation of immune cells that directly recognize HIV components, arising from incessant viral replication and elevated antigen loads (see sections “[Functional Exhaustion of HIV-1-Specific Humoral and Cellular Responses](#)” and “[Senescence of HIV-Specific T Cells and Clonal Exhaustion](#)”). Continued exposure of T cells to their cognate antigen can lead to their functional and clonal exhaustion. Systemic inflammation due to the activation of the innate immune response is another major issue in HIV pathogenesis. HIV-mediated depletion of CD4⁺ T cells (and to a lesser extent macrophages and dendritic cells) can disrupt the normal process of immune surveillance in mucosal lymphoid tissues, unleashing an avalanche of microbial products originating from commensal bacteria as well as microbial pathogens residing in the intestinal tract (Brenchley et al. 2006). Microbial products like lipopolysaccharide (LPS), flagellin, and CpG DNA are sensed by innate receptors such as TLRs present on dendritic cells and macrophages, leading to the release of proinflammatory mediators (e.g., tumor necrosis factor (TNF), interleukin 6 (IL-6), IL-1 β , macrophage inflammatory protein (MIP)-1 α , MIP-1 β , and RANTES) within both plasma and tissues. Moreover, the HIV-mediated depletion of CD4⁺ T cells and the creation of an inflammatory environment result in the

reactivation of latently integrated forms of persistent viruses (hepatitis B virus, HBV; hepatitis C virus, HCV; cytomegalovirus, CMV; or Epstein–Barr virus, EBV), which HIV-positive individuals are commonly coinfecting with. In this highly proinflammatory environment, a broad range of immune cell subsets, comprising CD8⁺ and CD4⁺ T cells, NK cells, monocytes, polymorphonuclear (PMN) neutrophils, and B cells, are activated. The release of large amounts of inflammatory mediators and reactive oxygen species (ROS) by these cell subsets propagates systemic immune activation. Heightened immune activation drives viral replication and, with it, the process of global immune exhaustion, affecting primary lymphoid organs and immune resources (see section “[Systemic Exhaustion of Immune Resources in HIV-1 Infection](#)”).

Functional Exhaustion of HIV-1-Specific Humoral and Cellular Responses

Given their important role in controlling HIV replication, many studies have focused on the immune exhaustion of CD8⁺ T cells. Exhausted CD8⁺ T cells cease the production of cytokines like IL-2, TNF, and interferon gamma (IFN γ) and show reduced cytotoxicity (Wherry 2011). Similarly, exhausted CD4⁺ T cells lose certain effector functions during chronic HIV-1 infection. Amongst the functions most affected is their ability to produce the homeostatic cytokines IL-2 and IL-21, which are important for maintaining CD8⁺ T cells alive and stimulating B cells to produce neutralizing antibodies. Chronic HIV-1 infection also interferes with normal B-cell function and differentiation pathways, giving rise to exhausted B cells. Exhausted memory B cells exhibit a CD21^{Low} CD10⁻ CD27⁻ phenotype, reduced ability to migrate to the T-cell-rich areas (sites of T-cell-dependent B-cell maturation) of lymphoid tissues, poor proliferative capacity, and decreased immunoglobulin diversity (Moir and Fauci 2009).

Functional exhaustion is to a great extent related to the expression of co-inhibitory receptors. Upon HIV infection, CD8⁺ T cells are subjected to considerable levels of antigenic

stimulation through the T-cell receptor (TCR). This excessive or chronic triggering of the TCR results in increased expression of immunoregulatory molecules such as programmed death receptor 1 (PD-1). PD-1 belongs to a class of co-inhibitory receptors with important roles in the negative regulation of the immune response. Signaling through PD-1 acts to preserve the delicate balance between immune activation in response to infection and immune contraction essential for maintaining tolerance. In chronically HIV-viremic individuals, high PD-1 levels on CD8⁺ T cells correlate positively with viral load and inversely with CD4⁺ T-cell counts (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006). Collectively, such observations and many others have labeled PD-1 as an important marker of immune exhaustion. It is however important to mention that PD-1 is also expressed on activated, yet fully functional, T cells during the acute phase of infection. Thus, the expression of PD-1 and other co-inhibitory signals on recently activated T cells should be considered as part of a normal mechanism of immune regulation, while sustained expression of these receptors may be an indicator of functional alteration or exhaustion. PD-1 expression is not limited to activated and exhausted CD8⁺ T cells and is found on the surface of many other immune cells, including CD4⁺ T cells, B cells, NK cells, and monocytes. Similar to CD8⁺ T cells, PD-1 expression on CD4⁺ T cells, NK cells, and B cells is related to functional exhaustion and more rapid HIV-1 disease progression.

In addition to the best-characterized immunoregulatory receptor PD-1, a multitude of other co-inhibitory cell-surface molecules, expressed on CD8⁺ and CD4⁺ T cells, have been identified in recent years (Crawford and Wherry 2009). These include cytotoxic T-cell antigen 4 (CTLA-4), T-cell immunoglobulin and mucin protein 3 (Tim-3), B- and T-cell attenuator (BTLA), 2B4 (CD244), lymphocyte-activation gene 3 (LAG-3), and CD160. Similarly, memory B cells can also upregulate co-inhibitory receptors besides PD-1. These tend to be different from T-cell co-inhibitory receptors and include Fc receptor-like 4 (FCRL4), sialic acid-binding Ig-like

6 (SIGLEC6), CD22, CD72, and CD85. Besides the various co-inhibitory cell-surface molecules, immunoregulatory soluble mediators can also contribute to immune exhaustion. During chronic HIV-1 infection, dendritic cells, monocytes, and certain subsets of CD4⁺ T cell produce the immunosuppressive cytokine IL-10. Another immunosuppressive modulator, the transforming growth factor beta (TGF- β), which is expressed by a wide variety of cell types, may also promote immune alteration during chronic HIV-1 infection. In many instances, a close connection exists between the initiation of regulatory signaling pathways through ligation of co-inhibitory receptors and the induction of immunosuppressive mediators. For example, PD-1 upregulation on monocytes following ligation of innate sensors such as TLRs may actually contribute to CD4⁺ T-cell dysfunction through the production of IL-10. Master regulators of these pathways are likely to be type I interferons (Teijaro et al. 2013; Wilson et al. 2013), whose adverse role in chronic HIV infection appears increasingly evident.

Senescence of HIV-Specific T Cells and Clonal Exhaustion

In addition to functional exhaustion, the replicative capacity and survival potential of HIV-specific cells can be affected. Accumulating evidence suggests that the Hayflick limit (which defines the irreversible state of growth arrest indicative of replicative senescence, initially observed in cultured human fibroblasts) applies to all cells of the immune system, meaning that their replicative lifespan *in vivo* is limited. The onset of replicative senescence is primarily related to the number of divisions undergone by a given cell and is associated with the shortening of telomeres (repeated hexameric DNA sequences found at the ends of the chromosomes). A cell experiencing substantial telomere shortening can succumb to chromosome instability, leading to growth arrest and/or apoptosis. During primary viral infection, upregulation of telomerase (the enzyme responsible for the maintenance of telomere length) enables newly activated virus-specific

T cells to retain long telomeres during this period of considerable clonal expansion. However, such capacity to upregulate telomerase decreases on repeated stimulation. As a consequence, continuously replicating memory T cells specific for persistent viruses present increasingly shorter telomere lengths and may rapidly reach a stage of replicative senescence. Such senescent CD8⁺ T-cell populations have been identified in HIV-viremic individuals and display a number of other functional defects such as the reduced capacity to produce IL-2 and lowered proliferative potential. Moreover, these memory T cells are characterized by a lack of CD28 and an increased expression of CD57, a cell-surface marker associated with cellular senescence (Papagno et al. 2004). Of note, reduced telomerase activity and telomere shortening have been directly reported in HIV-specific CD8⁺ T cells derived from infected patients (Lichterfeld et al. 2008). Persistent viral replication and repeated stimulation may therefore gradually drive HIV-specific CD8⁺ T cells towards an irreversible state of exhaustion, resulting in the eventual loss of important anti-HIV T-cell populations.

HIV-specific T-cell populations are heterogeneous and consist of cells displaying varying functional features and levels of antiviral efficacy. While all T cells are endowed with TCRs that determine their specificities for antigen, the term TCR “avidity” describes the combined affinities of these TCRs and provides a measure of the strength of a given T cell–target cell interaction. TCR avidity is thought to be a major determinant of the qualitative attributes of HIV-specific T cells, including their immunodominance, poly-functionality, HIV-suppressive capacity, and ultimately their control of HIV replication (Appay et al. 2008). Due to their superior sensitivity to antigen, high-avidity T cells may be particularly prone to viral antigen-mediated stimulation, resulting in a faster progression towards senescence and eventually the depletion of these clones from the repertoire of HIV-infected patients altogether (Almeida et al. 2007; Lichterfeld et al. 2007). The exhaustion and loss of these important T cells can play a significant role in the onset of HIV disease progression, despite

other functionally active, albeit lower avidity, HIV-specific CD8⁺ T cells remaining in circulation. Clonal exhaustion can also be blamed for the loss of specificity towards certain HIV epitopes. Ultimately, the maintenance of optimal immune pressure and control of HIV replication depends on the capacity of the immune system to continuously recruit new high-avidity T cells targeting a wide range of antigenic specificities.

Systemic Exhaustion of Immune Resources in HIV-1 Infection

In the face of their continuous depletion during HIV infection, the maintenance of adequate levels of CD4⁺ T cells depends not only on the ability to stop viral replication but also on the capacity to renew depleted lymphocyte stocks. However, continuous replacement and recruitment of new lymphocytes in HIV-infected patients is thwarted by the global decline of primary immune resources. It is indeed important to appreciate that activation-driven immune exhaustion in HIV infection may go far beyond the simple loss of virus-specific lymphocytes or cells actively depleted by the virus. HIV disease progression is actually associated with a general lymphopenia. As CD4⁺ and CD8⁺ T-cell counts drop during the chronic phase of HIV infection, the naive T-cell compartment is most strongly affected. NK- and B-cell numbers are also reduced during HIV infection. Similar to the observations made for the T-cell compartment, B-cell populations originating from HIV-infected patients are characterized by an underrepresentation of naive B cells. Collectively, these findings indicate that the integrity of all lymphocyte subsets is affected during the course of HIV disease progression. Given that all the cells that make up our immune system originate from hematopoietic stem cells (HSCs), the capacity to generate new lymphocytes ultimately depends on the availability of functional lymphoid precursors. However, accumulating evidence suggests that like other immune cells, HSCs have a limited replicative lifespan *in vivo*. Thus, it appears that the renewal capacity of the entire immune system has a limit.

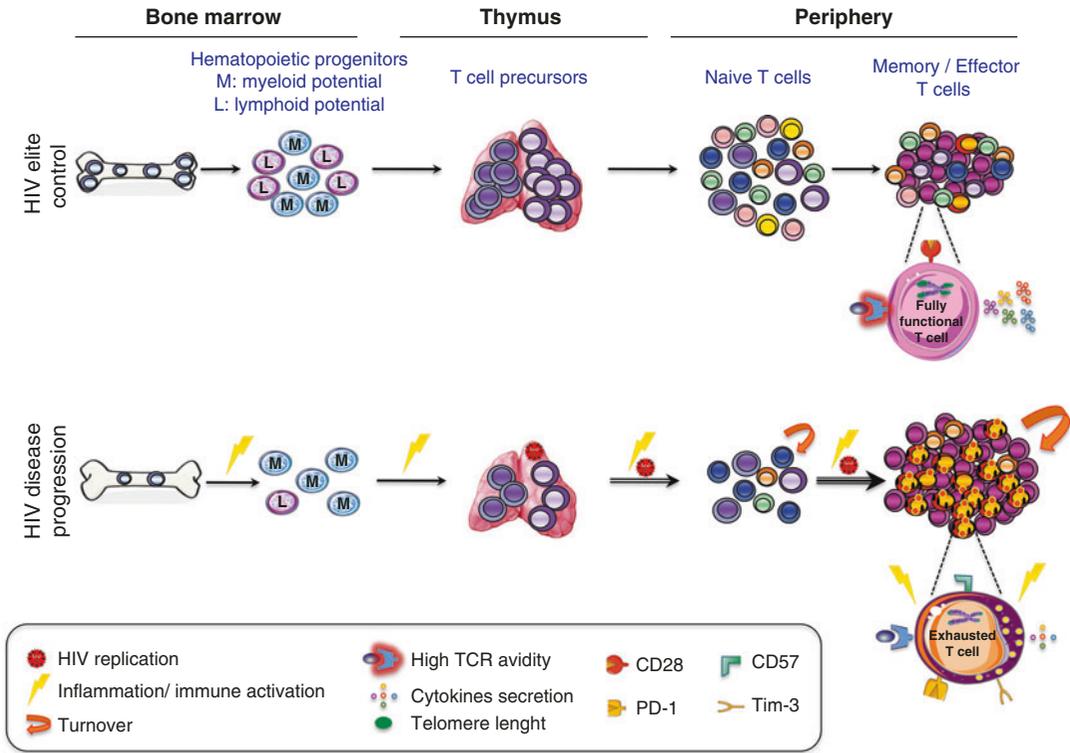
The reduced output of new T cells in HIV-infected patients can be partly caused by impaired thymopoiesis, relating to viral infection of the thymus or thymic involution. However, the general state of lymphopenia observed in chronic HIV infection appears to have a more profound origin, seeing as upstream elements of lymphocyte development are also affected. CD34⁺ hematopoietic progenitor cells (HPCs) represent the source of all blood cells. It was observed that HPCs derived from the bone marrow of HIV-viremic patients were functionally different from those found in healthy individuals, pointing to a case of impaired hematopoiesis in HIV infection (Moses et al. 1998). Recently, the detailed assessment of HPCs extracted directly from blood, allowing for the study of a large number of donors, revealed that patients progressing towards AIDS have decreased numbers of circulating HPCs. Moreover, CD34⁺ cells from these individuals displayed both functional defects and poor lymphoid precursor capacity, compared to healthy donors or HIV-infected nonprogressors (Sauce et al. 2011). These findings are in line with the impairment of T-cell progenitor function observed in chronic HIV infection. Results from fetal thymic organ culture experiments performed on CD34⁺ HPCs from HIV-infected progressors showed that these cells displayed a reduced capacity to generate both CD4⁺ and CD8⁺ T cells. Disrupted lymphopoiesis may thus significantly limit the production of new lymphocytes, including CD4⁺ T cells, and consequently disrupt the maintenance of adequate CD4⁺ T-cell numbers during HIV-1 infection. The onset of HIV disease progression appears therefore to be closely related to the condition of the primary lymphoid resources.

While lymphocytes are produced continuously throughout normal life, exacerbated consumption of cellular resources due to chronic or recurrent immune activation may eventually lead to the progressive decay of immune renewal capacities in HIV-infected subjects. This is reminiscent of the situation observed in the elderly, independently of HIV. Indeed, with age, the renewal capacity of the immune system gradually declines. Specifically, advanced age is

characterized by reduced lymphopoiesis due to dwindling HSC production and reduced egress from the bone marrow, accompanied by thymic involution. The end result is the shrinkage of the naïve lymphocyte pool and a loss of repertoire diversity. This deterioration forms part of the process of immunosenescence and is thought to be the result of successive rounds of immune activation throughout a lifetime. The parallels drawn between the state of primary immune resources in HIV-infected patients and HIV-negative elderly individuals suggest that premature immune aging occurs in chronic HIV infection.

Limiting Immune Exhaustion in HIV Infection

The different factors contributing to immune activation in HIV-infected patients eventually results in the perturbation of normal lymphocyte function, differentiation pathways, regenerative capacity, and ultimately a suboptimal immune control of viral replication. Better understanding of immune regulation has opened up new therapeutic avenues aimed at limiting the development of immune exhaustion during HIV infection. For instance, the functional exhaustion of lymphocytes mediated by co-inhibitory receptor overexpression appears to be a reversible phenomenon. Blockade of the interaction between PD-1 and its principal ligand PD-L1 resulted in improved CD8⁺ T-cell function in experiments performed *in vitro* using HIV⁺ human samples (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006). In the context of simian immunodeficiency virus (SIV) infection of macaques, animals receiving PD-1 antibody blockade showed improved T-cell and B-cell responses, in addition to a better control of viral replication and prolonged survival (Velu et al. 2009). Most recently, the therapeutic benefits of blocking PD-1 have been demonstrated in HIV-infected humanized mice, with a substantial decrease in viral load and a steady rise in CD4⁺ T-cell counts. Although promising, PD-1 blockade alone is not sufficient to fully rescue T cells



HIV-Associated Immune Exhaustion, Fig. 1 Exhaustion of T-cell immunity with HIV disease progression. Persistent HIV replication and chronic immune activation are associated with exhaustion of the T-cell compartment,

which occurs at three different levels: (1) functional, (2) clonal, and (3) systemic, as highlighted by the differences observed between HIV elite controllers and HIV-infected progressors

from exhaustion. One explanation is that the severity of functional exhaustion is influenced by the simultaneous expression of different co-inhibitory receptors on a single cell. Exhausted PD-1⁺ T cells express additional co-inhibitory receptors. The combined blockade of several co-inhibitory molecules can enhance functional restoration more effectively than abrogation of a single signaling pathway alone. Collectively, these observations expose the interesting possibility of different T-cell subsets being regulated by distinct combinations of co-inhibitory molecules, opening up opportunities for therapeutic reversion of immune exhaustion in a population-specific manner. It remains to be seen if these encouraging results can be replicated in humans, taking into account the potential development of immunopathological side effects when interfering with these immunoregulatory pathways.

While blockade of co-inhibitory signaling pathways is being exploited in the reversal of immune exhaustion during chronic HIV-1 infection, another approach involves tackling the problem of immune activation directly. Antiretroviral therapy (ART) represents the most effective antidote to HIV-driven immune activation developed to date. HIV-infected individuals receiving ART display reduced T-cell activation, diminished apoptosis, and lowered proinflammatory cytokine levels and show some reversion of immune exhaustion, with a partial recovery of cellular functions and primary immune resources. Of note, patients who fail to reconstitute their CD4⁺ T-cell compartment upon ART, referred to as immunological failure, present signs of profound and persistent damage to their hematopoietic system, with a fully exhausted capacity to produce lymphocytes (Sauce et al. 2011). Other therapies

aiming at reducing systemic immune activation are also being investigated. Amongst the candidates are inhibitors of T-cell activation (e.g., cyclosporine-A) and microbial product sensors (e.g., TLR antagonists), as well as blocking antibodies designed to target the proinflammatory cytokines themselves (e.g., IL-10 antagonists) (Rajasuriar et al. 2013). In cases of severe immune exhaustion (e.g., immunological failure), where restoration of immune function through decreased immune activation is simply not an option, alternative approaches aimed at endowing the immune system with younger, healthier cells may represent a solution. Examples of this strategy include the use of (i) homeostatic cytokines (IL-2, IL-7) to promote thymic regrowth and T-cell expansion, (ii) hematopoietic growth factors (e.g., growth hormone) to boost lymphopoiesis, and (iii) total reconstitution of the immune system through hematopoietic stem cell (HSC) transplantation.

Conclusions

Immune exhaustion caused by persistent HIV replication and chronic immune activation is likely to play an important role in the control of HIV infection and progression towards AIDS. The term “immune exhaustion” historically refers to the progressive decline of effector functions and proliferative potential observed in CD8⁺ T cells. However, exhaustion of other immune cell populations, including CD4⁺ helper T cells, memory B cells, NK cells, as well as hematopoietic progenitors, has also been reported in chronic HIV infection. Immune exhaustion can happen at three levels: (i) functional exhaustion of antiviral attributes of HIV-specific lymphocytes, (ii) clonal exhaustion of T cells important for the control of HIV replication, and (iii) systemic exhaustion of primary resources resulting in the decline of lymphopoiesis, accompanied by a general aging of the lymphocyte population (as exemplified in the Fig. 1). The development of strategies to limit or even reverse the onset of immune exhaustion is essential for securing the long-term survival of HIV-infected patients.

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Hodgkin Lymphoma in Patients with HIV Infection

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Definition

Hodgkin lymphoma (HL) is a type of lymphoma characterized by Reed-Sternberg cells and often associated with systemic symptoms. In is one of the more common non-AIDS-defining cancers, and it tends to be relatively more aggressive and to more frequently exhibit extranodal involvement. In the era of highly active antiretroviral therapy (HAART) the same regimens employed in HIV-negative patients with HL can be used in HIV setting with similar results.

Introduction

The availability of highly active antiretroviral therapy (HAART) has led to improvements in immune status among HIV-infected persons, reducing AIDS-related morbidity and prolonging survival. However, despite the impact of HAART on HIV-related mortality, malignancies remain an important cause of death (Bonnet et al. 2009). The use of HAART was also associated with reduced incidence of the two major AIDS-associated malignancies – ► [Kaposi's sarcoma \(KS\)](#) and high-grade non-Hodgkin lymphoma (NHL). However, among non-AIDS-defining cancers, an increased risk of Hodgkin lymphoma (HL), ► [anal cancer](#), ► [lung cancer](#), and hepatocarcinoma has been observed since the widespread use of HAART (Biggar et al. 2006).

Although HL is included in the World Health Organization's categorization of HIV-associated lymphomas, a number of questions remain regarding the relationship between HIV infection and HL. HIV-associated HL (HIV-HL) displays several peculiarities when compared with HL of the general population. First, HIV-HL exhibits an unusually aggressive clinical behavior, which mandates the use of specific therapeutic strategies and is associated with a poor prognosis. Second, the pathologic spectrum of HIV-HL differs markedly from that of HL in the general population (Tirelli et al. 1995). In particular, the aggressive histological subtypes of classic HL (cHL), namely mixed cellularity (MC) and lymphocyte depletion (LD), predominate among HIV-HL and pathologically, the tumor is characterized by an unusually large number of neoplastic cells, termed Reed-Sternberg (RS) cells. Finally, despite the great improvement in chemotherapy and supportive care, optimal staging and treatment is still a matter of controversy.

Epidemiology

In the HIV-negative population of western countries, HL is one of the commonest malignancies diagnosed in young adults, with 6 cases of HL observed per 100,000 inhabitants under 45 years

of. The epidemiology of HL is characterized by a peculiar age distribution pattern – a bimodal incidence curve with a first peak around the age of 30 and the second peak around the age of 50 years – that has been taken as suggestive of an infectious etiology. In immunosuppressed patients, HL occurs more frequently than in the general population of the same age and gender. In part because of the relative high frequency of HL in the population groups at high risk for HIV infection, a marked change in risk of HL was not initially appreciated in HIV-infected individuals, and HL was not included in the spectrum of HIV-defining cancers. However, with the spread of the epidemic and longer survival of infected people, the impact of HL was better recognized. A number of studies (Biggar et al. 2006; Serraino et al. 1997; Dal Maso et al. 2003; Clifford et al. 2005) strongly support the evidence that HIV-infected persons have, overall, a 10-fold higher risk of developing HL than HIV-negative persons. Such an excess risk is more pronounced in HIV-infected individuals with moderate immunosuppression; this is a different pattern than that observed for KS or certain other types of NHL such as ► [primary central nervous system lymphoma \(PCNSL\)](#) (Serraino et al. 1997). Thus, the epidemiological pattern of HL in HAART era substantially differs from those observed for KS or NHL – two neoplasms which drastically decreased after the introduction of HAART – and this pattern raises several new questions with regard of the relationship between degree of immunodeficiency, persistent viral infections, and cancer.

Of some interest is the recent observation of Powles et al. that have investigated the occurrence of cancers in a prospective cohort of 11,112 HIV-positive individuals, with 71,687 patient-years of follow-up (Powles et al. 2009). Standardized incidence ratios (SIRs) were calculated using general population incidence data. The incidence of HL in the HIV cohort was higher than in the general population (SIR 13.85; 95% CI, 9.64 to 19.26). There was a significant increase in the SIRs across the three study periods (1983 to 1995: 4.5; 1996 to 2001: 11.1 and 2002 to 2007: 32.4). Multivariate analysis demonstrated that

HAART was associated with an increased risk of disease (SIR 2.67; 95% CI, 1.19 to 6.02). Further multivariate modeling by class of antiretroviral agent showed that of the three classes of antiretroviral therapy, only the nonnucleoside reverse transcriptase inhibitors were associated with a significant increase in the incidence of HIV-HL (HR 2.20; 95% CI, 1.03 to 4.69); the reason for this association is not understood. The increased risk of HL with HAART might be explained because the risk of HL peaks when CD4 counts range from 150 to 199 CD4 cells/ μ L (Serraino et al. 1997). As the overall effect of HAART is to increase the CD4 count level, it paradoxically may increase HL incidence, leading to speculate that, with severe immune suppression, the cellular background surrounding the RS cells may be altered. A potential mechanism emphasizes the role of the RS cells producing several growth factors that increased the influx of CD4 cell and inflammatory cells, which, in turn, provide proliferation signals for the RS neoplastic cells. One can imagine that in the case of severe immune suppression, there are insufficient CD4 cells to maintain this feedback loop and there is less growth of the RS neoplastic cells (Gloghini and Carbone 2007). In addition, HIV-HL is associated with ► [Epstein-Barr virus \(EBV\)](#) in almost all cases, in contrast to the general population, in which this association is only observed in 20–50% according to histological type and age at diagnosis (Dolcetti et al. 2001). In summary, the use of HAART has improved the immunity of HIV-infected persons, diminishing the risks of developing other cancers or other opportunistic infections and paradoxically increasing the risk of HL.

Pathological Features

HIV-HL displays a number of different pathological features in comparison HL in HIV-negative patients. In fact, HIV-HL is characterized by the high incidence of unfavorable histological subtypes (i.e., MC and LD) (Tirelli et al. 1995). In the pre-HAART era, among HIV-infected persons, MC was the most frequent HL subtype and nodular sclerosis (NS) was less frequent than in

HIV-uninfected persons. For each HL subtype, incidence decreased with declining CD4 counts, but NS subtype decreased more precipitously than MC subtype, thereby increasing the proportion of MC subtype of HL seen in persons with AIDS. Thus, the greater proportion of MC and LD subtypes appears specifically related to severe immunocompromise in AIDS. By contrast, in the HAART era, HIV-infected patients with modest immunocompromise are more at risk for the development of the NS subtype (Biggar et al. 2006).

HIV-HL exhibits special features related to the cellular background (presence of fibrohistiocytoid stromal cell proliferation) and the high number of neoplastic cells, and both these features may pose relevant difficulties in diagnosing and classifying the disease. This finding contrasts with the rather low population of neoplastic cells usually found in HIV-unrelated HL. Moreover, a high frequency of EBV association has been shown in HL (80–100%) tissues from HIV-HL (Carbone et al. 1999). The EBV genomes in such cases have been reported to be episomal and clonal, even when detected in multiple independent lesions. The elevated frequency of EBV association with HIV-HL indicates that EBV probably does represent a relevant factor involved in the pathogenesis of HIV-HL. An etiologic role of EBV in the pathogenesis of HIV-HL is further supported by data showing that LMP-1 is expressed in virtually all HIV-HL cases (Carbone et al. 1999). On this basis, HL in HIV-infected persons appears to be an EBV-related lymphoma expressing LMP1.

It should be noted that RS cells of classical HL in HIV-negative patients represent transformed B-cells that originate from pre-apoptotic germinal center (GC) B-cells (Klein and Dalla-Favera 2008). By contrast, most HIV-related HL cases express LMP1 and display the BCL6-/CD138+/MUM1 IRF4+ (for multiple myeloma-1 interferon regulatory factor-4), phenotype, thus reflecting post-GC B cells (Carbone et al. 1999; Klein and Dalla-Favera 2008). The possible contribution of LMP1 to the loss of BCL6 expression seems plausible given that LMP1 can down-regulate many B-cell specific genes. Loss of

B-cell identity occurs during the normal differentiation of a GC B-cell into a plasma cell or memory B-cell.

Clinical Aspects and Treatment

As in HIV-NHL, one of the most characteristic features of HIV-HL is the widespread extent of the disease at presentation and the frequency of systemic “B” symptoms, including fever, night sweats and/or weight loss >10% of the normal body weight. At the time of diagnosis 70–96% of the patients with HIV-HL have “B” symptoms and 74–92% have advanced stages of disease with frequent involvement of extranodal sites, the most common being bone marrow (40–50%), liver (15–40%) and spleen (around 20%) (Tirelli et al. 1995). HIV-HL tends to develop relatively early in the course of HIV infection with a median CD4+ cell count ranging from 275 to 306/ μ L (Tirelli et al. 1995). The widespread use of HAART has resulted in substantial improvement in the survival of patients with HIV infection and lymphomas, due to the reduction of the incidence of opportunistic infections, to the opportunity to allow more aggressive chemotherapy and to the less aggressive presentation of lymphoma in patients in HAART in comparison with those lymphomas diagnosed in patients who never received HAART (Tirelli et al. 1995; Vaccher et al. 2003).

The Italian Cooperative Group on AIDS and Tumors (GICAT) has collected data on 290 patients with HIV-HL (Chimienti et al. 2008). Two hundred and eighty-one patients (87%) were males and the median age was 34 years (range 19–72 years). 69% of patients were intravenous drug users. The median CD4 cell count was 240/ μ L (range 4–1, 100/ μ L) and 57% of patients had a detectable HIV viral load. MC was diagnosed in 53% of cases, followed by NS in 24% and LD in 14%. Advanced stages of disease were observed in 79% of patients and 76% had B symptoms. The overall extranodal involvement was 59% with bone marrow, spleen and liver involved in 38, 30 and 17%, respectively. The authors split the series into two subgroups: the

first group was comprised of patients who received HAART throughout the 6 months before the onset of HL (84 patients); the second group was comprised of those patients who never received HAART before the diagnosis of HL or for less than 6 months (206 patients). Briefly, in comparison to patients who never received HAART, patients who received HAART before the onset of HL were older, had less B symptoms, and had a higher leukocyte count, neutrophils count and hemoglobin level. The following parameters were associated with a better overall survival (OS): MC subtype, the absence of extranodal involvement, the absence of B symptoms and a prior use of HAART. Interestingly, three parameters were associated with a longer time to treatment failure: a normal value of alkaline phosphatase, a prior exposure to HAART and an international prognostic score less than 3 (Chimienti et al. 2008).

A similar study was carried out within the Spanish group GESIDA where the authors compared the clinical characteristics and outcome of 104 patients with HIV-HL, treated (83 patients) or not (21 patients) with HAART (Berenguer et al. 2008). No differences were found between the groups at baseline, but the complete remission (CR) rate was significantly higher in HAART group (91% vs 70%, $p = 0.023$). The median overall survival was not reached in HAART group and was 39 months in no-HAART group ($p = 0.0089$); the median disease free survival (DFS) was not reached in HAART group and was 85 months in no-HAART group ($p = 0.129$). Factors independently associated with CR were a CD4 cell count >100 cells/ μL and the use of HAART; CR was the only factor independently associated with OS (Berenguer et al. 2008).

The optimal therapy for HIV-HL has not yet been defined. Because most patients have advanced stages of disease, they have generally been treated with combination chemotherapy regimens. Even so, the CR rate remains lower than that attained in HL of the general population with the OS being approximately 1.5 years (Tirelli et al. 1995). Due to the low incidence of HIV-HL, no randomized controlled trials have

been conducted. However, several phase II studies have evaluated the feasibility and activity of different regimens. In a prospective trial, conducted within the GICAT between March 1989 and March 1992, 17 previously untreated patients with HIV-HL were treated with epirubicin, vinblastine, and bleomycin (EVB). Overall, CR was achieved in 53% of the group, lasting a median of 20 months. The median OS for the group as a whole was 11 months and the 2-year DFS was 55% (Errante et al. 1994). In an attempt to improve upon these results, from 1993 to 1997, a second prospective trial consisting of full dose EVB plus prednisone (EVB/P) and concomitant antiretroviral therapy (zidovudine or didanosine) was conducted. The results of this trial in which 35 patients were enrolled, showed a CR rate of 74% and a 3-year OS and DFS of 32 and 53% respectively (Errante et al. 1999). The AIDS Clinical Trials Group (ACTG) reported the results of a phase II study in 21 patients treated with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) for 4–6 cycles and primary use of G-CSF. Antiretroviral therapy was not used. The CR rate, on an intent-to-treat analysis, was 43% with an overall objective response rate of 62%. Median survival for all patients was 18 months (Levine et al. 2000). The widespread use of HAART allows the use of more aggressive chemotherapeutic regimens. Within the European Intergroup Study 59 consecutive HL-HIV patients were treated with Stanford V regimen, consisting of short term chemotherapy (12 weeks) with adjuvant radiotherapy. This regimen was well tolerated and 69% of the patients completed treatment with no dose reduction or delayed chemotherapy administration. The most important dose-limiting side effects were bone marrow toxicity and neurotoxicity. Eighty-one percent of the patients achieved CR, and after a median follow-up of 17 months 33/59 (56%) patients are alive and disease-free. The estimated 5-year OS, DFS and freedom from progression (FFP) were 59, 68 and 60%, respectively. Probability of FFP was significantly higher ($p = 0.002$) among patients with an international prognostic score (IPS) of <2 than in those with IPS >2 , and the percentage of FFP at 2 years were 83 and 41% respectively. Similarly,

Hodgkin Lymphoma in Patients with HIV Infection, Table 1 Results of prospective studies in HIV-HL

Regimen / reference	# of patients	Stage III-IV (%)	Response rate (%)	Complete remission rate (%)	Overall survival
EBV (Errante et al. 1994)	17	88	82	53	11 months
EBVP (Errante et al. 1999)	35	83	91	74	16 months
ABVD (Levine et al. 2000)	21	81	62	43	18 months
Stanford V (Spina et al. 2002)	59	71	89	81	59% at 5 years
BEACOPP (Hartmann et al. 2003)	12	92	100	100	75% at 3 years
ABVD (Xicoy et al. 2007)	62	100	87	87	76% at 5 years
VEBEP (Spina et al. 2008)	71	70	78	67	69% at 2 years

probability of OS was significantly different ($p = 0.0004$), and the percent survival at 3 years was 76 and 33% respectively for IPS <2 and IPS >2 (Spina et al. 2002).

Within the German group, the very intensive bleomycin, etoposide, adriamycin, cyclophosphamide, oncovin, procarbazine, prednisone (BEACOPP) regimen has been tested in 12 untreated patients with a 100% of CR rate but a high incidence of opportunistic infections (Hartmann et al. 2003). Recently, the results of a large prospective phase II study with ABVD have been published. Within a cooperative network in Spain, 62 patients with HIV-HL received the standard ABVD plus HAART. The scheduled six to eight ABVD cycles were completed in 82% of cases. Six patients died during induction, 54 (87%) achieved a CR, and two were resistant. The 5-year OS and event-free survival (EFS) probabilities were 76 and 71%, respectively. The immunological response to HAART appeared to have a positive impact on OS ($p = 0.002$) and EFS ($p = 0.001$) (Xicoy et al. 2007). Finally, the GICAT has concluded the accrual of 71 patients in a prospective phase II study aiming to evaluate feasibility and activity of a novel regimen including epirubicin, bleomycin, vinorelbine, cyclophosphamide, and prednisone (VEBEP regimen). Seventy percent of patients had advanced stages of disease and 45% had an IPS >2 . The CR was 67% and 2-year OS, DFS,

TTF and EFS were 69, 86, 59 and 52%, respectively (Spina et al. 2008). The results of the largest prospective studies are shown in Table 1.

Recently, a stage-adapted approach has been reported on 112 patients with an excellent outcome similar to that of HIV negative patients (Hentrich et al. 2012). Moreover, a comparison between patients with HIV-HL and patients with HL without HIV infection showed that HIV patients have more advanced stages of disease with more adverse prognostic factors; however, when these patients were treated with ABVD, HIV infection does appear to not adversely affect OS or EFS (Montoto et al. 2012). Because a large proportion of patients with HIV-HL progress and relapse, the use of high dose chemotherapy and autologous stem cell transplantation (ASCT), which is considered the gold standard for salvage therapy, has been tested in this setting. Several data from different groups, including the GICAT, have demonstrated the feasibility of this (Re et al. 2009). Different conditioning regimens, varying in their inclusion of total body irradiation, have been tested. Recently, the AIDS Malignancy Consortium demonstrated in a multinstitutional trial that a regimen of dose-reduced high-dose chemotherapy, including cyclophosphamide and busulfan and ASCT, is well tolerated and is associated with favorable DFS and OS probabilities for selected patients with HIV associated NHL and HL.

PET Scanning

Positron emission tomography using [^{18}F]-Fluoro-2-Deoxy-D-Glucose (FDG-PET) was first introduced in the management of lymphomas in the early 1990s. It is now recognized as an important tool for staging and treatment response assessment in HL and NHL. In HIV-negative patients, residual FDG PET avidity after two cycles of ABVD has been shown to predict poor prognosis and therefore, has been proposed to guide future therapy (Gallamini et al. 2006). A negative PET scan after two cycles of ABVD predicted a 96% 2-year progression free survival (PFS). Nearly 80% of the HL patients show a complete normalization of PET scan after two courses of ABVD. This phenomenon, called “metabolic CR,” can be explained by the peculiar architecture and organization of the neoplastic tissue, where only few, scattered neoplastic cells (accounting for less than 1% of the total cellular population) are surrounded by a population of nonneoplastic mononuclear bystander cells. The latter cells are probably responsible for the immortalization of RS cells in HD by stimulating cytokine production by other CD4+ lymphoid cells (paracrine loop) or by inducing cytokine production by the RS cells (autocrine loop). In cases presenting with bulky lesions at diagnosis, a negative early PET is often associated with a persisting bulky lesion of more or less unchanged size. The explanation might be that chemotherapy switches off the production of chemokines by the activated lymphoid cells, as described for TARC (thymus and activation-related chemokine). The latter can be measured in the serum of HL patients and its level is correlated to the quality of treatment response: for patients in CR TARC levels are much lower than in patients with stable or progressing disease.

PET scanning within the HIV framework can be problematic. Some preliminary reports suggested that FDG activity may correlate with detectable lymphoma. Although initial staging may not alter the treatment plan, it can provide additional information, assess areas of possible, and help foresee and possibly avoid further complications. However, PET scanning in the HIV-HL

needs to be further studied. If PET is to be utilized, a baseline study is mandatory, since early PET interpretation is based on a site-to-site comparison of FDG uptake both before and after chemotherapy. Pitfalls are numerous in these patients in whom HIV-associated immunodeficiency predisposes to infection, as does the use of aggressive immunosuppressive chemotherapy regimens. PET imaging requires cautious reading and pertinent clinical correlation to avoid misdiagnosing benign disease as malignant. This can pose a problem, for example, when hypermetabolic foci seen in the lung or esophagus, which are common sites of HIV-and/or chemotherapy-promoted infections. Nodal FDG uptake can be observed in lymphoma, various infections (e.g., *Mycobacterium avium* intracellular, *Mycobacterium tuberculosis*, Herpes simplex virus, among others) and other AIDS-related malignancies such as Kaposi sarcoma. In addition, stimulation of bone marrow following treatment with granulocyte colony stimulating factors induces a striking increase in FDG uptake in bone marrow. To take into account the possibility of minimal residual uptake, a semi-quantitative approach has recently been proposed for interim PET interpretation in the context of an international protocol for advanced-stage HL.

Finally, PET is useful for an accurate initial staging and it should be recommended to monitoring treatment response. PET scans have prognostic value, and a negative scan is frequently associated with a favorable outcome. Significance of residual uptake at sites of disease needs further evaluation (e.g., biopsy). However, the use of FDG in the follow-up of HIV-HL patients who achieved CR cannot be routinely recommended and further studies are warranted to assess the possible value of such scans.

Conclusions

The outcome of patients with HIV-HL has improved in recent years with better combined antineoplastic and antiretroviral approaches. The main important challenges for the next years are: (a) to better understand the pathogenesis of HD and the ways that HIV interacts with this process;

(b) assess in a randomized trial whether ABVD is the standard regimen in HIV setting; (c) to validate the role of PET scan both in the staging and in the evaluation of response; (d) to better understand the interactions between chemotherapy and antiretroviral therapy in order to reduce the toxicity of both approaches; (e) to evaluate the use of new drugs (i.e., bortezomib) in this setting; (f) to evaluate the long-term toxicity of the treatment in cured patients.

Cross-References

- ▶ [Burkitt and Burkitt-like Lymphoma](#)
- ▶ [Diffuse Large B-cell Lymphoma](#)
- ▶ [Epstein-Barr Virus \(EBV\)](#)
- ▶ [HIV-Associated Cancers](#)
- ▶ [Primary Effusion Lymphoma](#)

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Host Genetics and Genomics

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Definition

HIV host genetic research seeks to comprehensively describe human genetic influences on HIV/AIDS. The rationale behind host genetic and genomic analyses, the different study designs, and the proven associations between DNA polymorphism and HIV infection outcomes are discussed in this section.

Heritability of Susceptibility to HIV

Susceptibility to HIV infection varies considerably between individuals, owing in part to the virus itself and to other environmental influences. It has long been known, however, that differences in HIV control are also partially heritable. The strongest evidence comes from in vitro studies of

immortalized B lymphocytes from extended families, which demonstrated that more than half of the observed difference in cell susceptibility to HIV-1 is due to the individual genetic background (Loeuillet et al. 2008). Heritability is also demonstrated by rare case studies involving related individuals: for example, identical twins concomitantly infected with the same retroviral strain had very comparable clinical histories, while a third brother, contaminated with a different HIV-1, had a markedly different disease course (Draenert et al. 2006).

Genetic Analysis

Overview

Studies of the host genetic factors contributing to HIV susceptibility and disease progression have generally focused on either quantitative measurements of disease progression (e.g., set point viral load, CD4+ T cell decline, or time to AIDS) or a binary classification usually involving extreme phenotypes (e.g., elite control of viremia or exceptional resistance against HIV acquisition in highly exposed individuals). The goal of such investigations is to uncover genetic variants (either individually, combined on haplotypes, or interacting) whose presence significantly correlates with the quantitative phenotype or, in the case of a binary phenotype, is found at significantly different frequencies between cases and controls. Such genetic studies have uncovered strong influences on disease outcome mediated by the class I HLA genes (in particular *HLA-B*) and *CCR5* (discussed in detail below). However, many other reported associations in numerous genes have either failed replication in subsequent studies or have not undergone sufficient investigation so as to be considered bona fide genetic influences on HIV control.

Candidate Gene Genotyping

Among the most common approaches for detecting genetic associations with polygenic traits in the pre-genomic era were candidate gene studies. As suggested by the term “candidate gene,” this approach involves some a priori knowledge of gene function and a presumed

importance to the trait of interest. Such studies are attractive since they require very little in terms of technology and investment (targeted genotyping or sequencing), once patient cohorts have been built and characterized. In HIV infection, commonly interrogated classes of candidate genes include innate and adaptive immune mediators (such as cytokines, chemokines, and their receptors), HIV dependency factors (i.e., host genes required for viral replication), and factors that restrict either HIV or other related viruses in vitro or in vivo (e.g., *APOBEC3G* and *TRIM5*).

One of the earliest examples was the description of the impact on HIV disease of a 32-base pair deletion in the *CCR5* gene (*CCR5Δ32*), a receptor for the chemokines CCL3, CCL4, and CCL5, acting as the main HIV co-receptor on CD4+ T cells. *CCR5Δ32* is overrepresented in its homozygous form among exposed uninfected individuals and associates with delayed disease progression in heterozygous individuals (Dean et al. 1996). However, despite this early success, few other genetic variants identified through candidate gene studies have stood up to multiple rounds of replication by subsequent investigations.

The main failures of this method are the generally small sample sizes, the lack of correction for multiple testing, and in particular the inability to account for non-phenotypic factors associating with genotype that can be misinterpreted as true associations (such as differences in ethnic background between cases and controls or cryptic relatedness between study participants). An example of such a spurious association is the reported link between the $-46C/C$ genotype in the Duffy antigen receptor for chemokines (*DARC*) gene (known to influence susceptibility to *Plasmodium vivax* malaria) and HIV resistance (He et al. 2008). Subsequent investigations into this gene demonstrated that the frequency differences observed in the initial study were likely due to differing proportions of African and European ancestries between cases and controls (the $-46C/C$ variant has been driven to fixation in multiple African populations) rather than to a true association with HIV resistance (Julg et al. 2009; Walley et al. 2009). Ultimately, targeted candidate gene studies have given way to genome-wide

approaches where methods have been developed to account for such confounders.

Genome-Wide Association Analysis

Facilitated by the complete sequence of the human genome, extensive catalogues of human variation and laboratory and statistical techniques to produce and analyze massively dimensional data sets, genome-wide association studies (GWAS) have become the leading tool for interrogating the genetic underpinnings of complex traits. These studies aim at testing all common variants (defined as those present in the population at $>5\%$ frequency) for association with a trait of interest by assuming that all common variants, even if not directly tested, are captured through linkage disequilibrium (LD). Success of GWAS requires large cohorts of well-characterized individuals in order to have sufficient statistical power to detect the often-modest effect sizes of disease-associated variants. Since the publication of the first GWAS in 2005, more than 1,000 associations have been reported with hundreds of traits (a catalogue of which can be found at www.genome.gov/gwastudies). In practice, GWAS use microarrays to genotype patient samples and controls (or populations with a measured quantitative trait) at hundreds of thousands to millions of single nucleotide polymorphisms (SNPs). Tests for association are most commonly performed individually for each variant by regressing phenotype (binary or quantitative) on SNP dosage (i.e., the number of variant alleles present) plus covariates. The linear (quantitative) and logistic (binary) regression approaches have been widely adopted as these models can flexibly include covariates to correct for confounders. These covariates generally include principal components calculated from the genome-wide SNP data to control for population stratification (Price et al. 2006). Due to the large number of tests being performed (approximately 1×10^6 independent tests), the community has adopted a statistical threshold of p-values below 5×10^{-8} (referred to as genome-wide significance) in order to claim discovery. In almost all cases, independent replication in a separate sample (either through direct genotyping or meta-analysis) is also required.

There have been multiple GWAS performed in cohorts with HIV phenotypes, primarily in individuals of European ancestry, and mostly focusing on HIV viral load or disease progression (CD4 decline or time to AIDS). Almost all of them have described the same signals located in the major histocompatibility complex (MHC) region, identifying it as the major genetic determinant of disease outcome in the genome-wide context (Fellay et al. 2007; The International HIV Controllers Study 2010). Similar results have been observed in the MHC region of African American populations, although the precise SNPs identified differ from Europeans (due to differential LD between tag SNPs and causal variants between ethnic groups) (Pelak et al. 2010; McLaren et al. 2012). Additionally, a small number of GWAS have been performed on cohorts of HIV-exposed uninfected individuals. None of these have identified genetic signals that pass the statistical thresholds required to claim genetic discovery (Petrovski et al. 2011; Lingappa et al. 2011). Of note, the sample sizes of the GWAS performed to date in the HIV field are small in comparison to other complex traits. Thus, only very strong effect sizes have been detected. Meta-analyses of existing GWAS data sets will be required to determine if further common genetic variants with smaller effect sizes contribute to susceptibility to HIV acquisition and/or disease progression.

Exome and Whole Genome Sequencing

Moving beyond GWAS, which are limited to testing relatively common variants, advances in sequencing technologies and reduction in costs now make it possible to interrogate all classes of genetic variants in a single experiment. Currently, the favored study design involves sequencing the approximately 2% of the human genome that is known to code for genes, i.e., exome sequencing. In addition to providing genotypes on common variants located in genes (also provided by GWAS), low frequency polymorphisms and some forms of structural variation (e.g., small insertion/deletions) are also obtained. Several genes underlying Mendelian diseases have been identified using this approach, and it is now being applied to

common traits. In general, these studies focus on analysis of rare, coding mutations that are presumed to have larger effects on phenotype than more common variants (as severely deleterious mutations are selected against reaching high frequency by evolutionary pressure). However, to detect individual rare variants as associated with a particular phenotype would require sample sizes that are much larger than those currently available. Thus, techniques to increase power for detection of signals in exome data are receiving much attention. These include using extreme phenotypes (i.e., individuals presumed to be highly enriched for causal mutations), variant collapsing methods (i.e., combining all variants in a gene rather than testing them individually), and empirical weighting based on functional impact or evolutionary context. Use of these methods in combination has been successful in increasing power of variant detection (Emond et al. 2012).

As sequencing costs continue to decline, the focus will move from exome to whole genome sequencing. The advantage of this approach over exome sequencing is that it provides complete characterization of variation in all genetic regions in a sample. However, the drawbacks include increased cost and computational load, as well as multiple testing burden. Additionally, strategies to enhance power through collapsing variants in noncoding regions of the genome are less clear than in exome data sets and will require further development. Although there are no published studies to date, both exome and whole genome sequencing approaches are currently being applied to cohorts with HIV phenotypes to address the role of rare genetic variants on influencing HIV outcome.

Phenotypes and Cohorts for Genomic Analysis

Genetics of Resistance Against HIV Infection

An understanding of host genetic resistance against HIV infection is of key importance to identifying novel prophylactic drug targets or correlates of protection for rational vaccine design. Individuals that were repeatedly exposed to HIV

but remained seronegative have been compared to HIV-infected cohorts or to large numbers of samples collected from the general population, in an attempt to detect significant differences in allelic frequency of genetic variants, suggesting a protective or deleterious role in HIV acquisition. Exposed uninfected subjects have been described in various high-risk populations: examples include hemophiliacs exposed to contaminated batches of factor VIII concentrates in the early days of the pandemic, men who have sex with men reporting high-risk behavior, and highly exposed female sex workers (Telenti and McLaren 2010).

Genetics of Control of Viral Replication

Viral load during primary infection and when a patient has reached the set point are strong predictors of disease progression. Set point viral load (spVL), the stage of infection where viral load is relatively stable prior to progression to AIDS, shows substantial variation in the population. Genetic studies of viral load have used both spVL (\log_{10} transformed) (Fellay et al. 2007) and an extreme phenotype approach comparing HIV controllers to progressors (The International HIV Controllers Study 2010). Controllers are a subset of HIV long-term non-progressors that maintain low virus load in the absence of therapy (Deeks and Walker 2007). This group can be further subdivided into viremic controllers, those with spVL < 2,000 copies per mL of plasma, and elite controllers, those with spVL < 50 copies/mL. Although it has been hypothesized that the genetic influences mediating extreme viral control may differ from those that impact set point in the general population, this has not been observed. In fact, for those genetic variants that are reproducibly associated with HIV progression (Table 1), a strong correlation between the magnitudes of effect on spVL and controller/progressor status is observed (Fellay et al. 2009). This suggests that at least the common genetic influences are shared between these phenotypic measurements. Whether there are further modifying genetic influences that contribute to viremic/elite control in addition to these known effects is a topic of ongoing investigations.

Genetics of Disease Progression and AIDS

In addition to viral load, other laboratory or clinically measurable outcomes have been used as phenotypes in genomic analyses of the natural history of HIV disease, most notably the speed of disease progression as measured by the rate of CD4+ T cell decline or the time from seroconversion to an AIDS-defining illness. Here again, extreme phenotype study designs have been applied: individuals with sustained levels of CD4+ T cells despite years of infection (long-term non-progressors) and, inversely, patients presenting a very rapid immune deterioration and progression to AIDS (rapid progressors) were carefully selected from large clinical cohorts for GWAS or sequencing analyses.

Host Genetics in HIV Vaccine Trials

The tools of human genetics can also be used to explore the interindividual differences in response to immunogens, particularly in the context of randomized vaccine trials. In the HIV field, a GWAS assessed the host genetic determinants of HIV-specific responses to the T-cell vaccine tested in the Step trial (Fellay et al. 2011). Even if no polymorphism reached genome-wide significance, single nucleotide variants located in the MHC showed the strongest association with response to the HIV-1 Gag protein. These SNPs were markers for HLA-B alleles already known to associate with differences in HIV control, indicating that the host immunogenetic background plays a role in the host immune response to T-cell vaccines and confirming the existence of a connection between natural viral control and vaccine response.

Confirmed Human Genetic Determinants of HIV Control

As mentioned above, several variants previously identified by candidate gene studies and GWAS have failed to be widely replicated. In the two largest GWAS of viral control, the authors attempted to replicate >100 variants with reported effects on HIV disease outcome (The International HIV Controllers Study 2010; Fellay

Host Genetics and Genomics, Table 1 Single nucleotide polymorphisms reproducibly associated with HIV disease outcome

Variant	Gene class	Implicated gene	Description	Impact	References
rs333	Chemokine receptor	CCR5	32-base pair deletion in exon 4 resulting in the loss of expression on cell surface	HIV resistance (homozygous) delayed progression (heterozygous)	(Dean et al. 1996)
rs1800024			C > T transition mutation in the promoter region of CCR5. In high LD with rs1799864		Delayed progression
rs1799864		CCR2	Valine to isoleucine amino acid change at position 64 (V64I). In high LD with rs1800024 in CCR5		(Smith et al. 1997)
rs2395029	HLA	HLA-B	G allele is a near-perfect proxy for classical HLA-B*57:01 allele	Low viral load/ delayed progression	(Fellay et al. 2007)
rs4418214			C allele lies on haplotypes containing either HLA-B*57:01 or 27:05		(The International HIV Controllers Study 2010)
rs2523608			G allele is in high LD with HLA-B*57:03 and other protective alleles in African Americans		(Pelak et al. 2010; McLaren et al. 2012)
rs9264942		HLA-B/C	C allele tags haplotypes carrying multiple protective HLA-B alleles and a microRNA binding site (rs67384697) that modulates HLA-C expression level		(Fellay et al. 2007; Fellay et al. 2009)
rs67384697		HLA-C	Insertion/deletion polymorphism that disrupts a miRNA binding site in the 3' UTR of HLA-C, modulating expression level		(Kulkarni et al. 2011)

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et al. 2009). With the exception of a small number of variants in the MHC region, in *CCR5* and in *CCR2*, all failed to be replicated. Although differences in study phenotypes, in ethnic background of study participants, or in circulating viral subtype may be proposed to explain some of this lack of replication, it is most likely that the majority of reported associations were falsely positive. Similarly, several large-scale studies of exposed uninfected individuals have failed to discover or replicate variants associated with resistance to infection, with the exception of *CCR5*Δ32 (Telenti and McLaren 2010). Thus, the number of variants that have been reproducibly associated with resistance to HIV infection or various disease

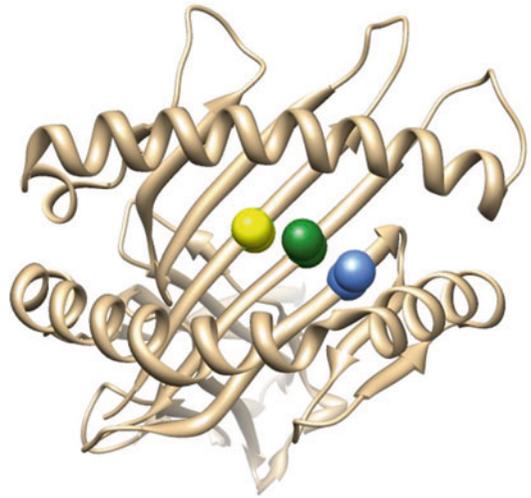
outcomes is small (a list of these is provided in Table 1).

In addition to *CCR5*Δ32 (discussed above in the “Candidate Gene Genotyping” section), two variants have been consistently replicated in the *CCR5/CCR2* region. The *CCR2* V64I variant (rs1799864) causes a valine to isoleucine amino acid change in the *CCR2* protein (thought to be a minor co-receptor for HIV entry) and associates with better viral control (Smith et al. 1997). A promoter variant in the *CCR5* gene (*CCR5* C927T or rs1800024) has also been shown to associate with delayed HIV disease progression. However, strong linkage disequilibrium exists between *CCR2* V64I and *CCR5* C927T in

Europeans (the predominant population in which they have been studied), preventing inference as to which is actually driving the observed impact on disease outcome.

Variants in genes of the MHC have the strongest effects on HIV control, likely mediated through binding and presentation of HIV peptides to the host immune system. Multiple classical HLA alleles with positive and negative influences on disease progression have been identified in several ethnic groups (discussed in detail in a separate entry: “► [MHC Locus Variation](#)”). GWAS have also identified multiple unlinked SNPs in this region that are strongly associated with disease outcome (Table 1) (The International HIV Controllers Study 2010; Fellay et al. 2009). The two most commonly described SNPs are rs2395029, a near-perfect proxy for the classical *HLA-B* allele 57:01, and rs9264942, located 35 kilobases upstream of *HLA-C* (often called *HLA-C* -35). The latter SNP has been identified as an expression quantitative trait locus influencing *HLA-C* mRNA levels. Further investigation has suggested that this *HLA-C* expression effect is mediated through an insertion/deletion polymorphism (rs67384697) in the 3' UTR of *HLA-C*, which is highly correlated with rs9264942 (Kulkarni et al. 2011). Additionally, strong evidence for association at rs4418214 in individuals of European ancestry and rs2523608 in African Americans has also been reported (Pelak et al. 2010).

Recently, attempts have been made to map specific amino acid residues carried by protective and non-protective classical *HLA-B* alleles (the gene that most strongly influences HIV control). These studies have shown the strongest associations map to amino acid residues in the peptide-binding groove, the domain of the protein responsible for selecting peptide epitopes to present to cytotoxic T lymphocytes (The International HIV Controllers Study 2010; McLaren et al. 2012; Kløverpris et al. 2012). Interestingly, three of the top amino acid residues are adjacent to one another and located in the beta sheets lining the bottom of the peptide-binding groove, suggesting an important functional role for this domain in particular (Fig. 1). These and other amino acid positions in the binding groove of HLA-B have



Host Genetics and Genomics, Fig. 1 Ribbon diagram looking into the peptide-binding groove of HLA-B showing spatial arrangement of top amino acid residues associated with modulating HIV outcome. Variations at positions 9 (yellow), 97 (green), and 116 (blue) lying at the bottom of the peptide-binding groove have been identified as key mediators controlling viral load in African (position 9 (Kløverpris et al. 2012)), African American (positions 97 and 116 (McLaren et al. 2012)), and European (position 97 (The International HIV Controllers Study 2010)) populations (Figure was modified from PDB entry 2BVP and prepared with UCSC Chimera)

been shown to explain the majority of the signal at the SNPs described above (The International HIV Controllers Study 2010).

Although variation in *CCR5/CCR2* and in HLA genes are reproducibly associated with HIV outcome, it remains to be determined if they account for the entirety of the host genetic influence. Meta-analyses of GWAS and large-scale sequencing studies are now seeking to detect common polymorphisms of more modest effect as well as rare variants, which are more likely to play a direct causal role. The combination of these approaches should finally delineate the impact of human genetic diversity on HIV control.

Clinical Implications of Host Genetic Determinants

The recent advances in the human genomics of HIV infection have improved understanding of

the natural history of the disease and opened new avenues in translational science. The inclusion of human genetic variation in the design of vaccine trials and of population-based in vitro experiments will allow for more informed analyses, stratified by genotypes of interest. More directly relevant to clinical care, personalized medicine based on genetic knowledge has the potential to benefit patients by allowing doctors to individually adjust diagnostic procedures and treatments. It will be essential, however, to explain the nature and the relevance of the genetic findings to the public and to healthcare providers: a limited number of variants have excellent predictive values (a clear example from the HIV pharmacogenetic field is the causal link between HLA-B*57:01 and hypersensitivity to abacavir), but most of them are poorly predictive for any given individual, even when they have significant effects at the population level. Genetic scores will be developed to help clinical decision-making, but they will never replace a global clinical assessment.

Conclusion

Recent developments in genomics have changed the way information about human DNA variation is used to understand and treat disease. The identification of associations between human genetic variants and HIV-related outcomes has the potential to benefit individuals and populations worldwide: a better comprehension of basic pathogenic processes can lead to the development of new drugs or vaccine strategies, and knowledge of individual genetic variation will pave the way toward more personalized medical care.

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Housing as HIV Prevention

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Definition: Housing as HIV Prevention

The co-occurrence of homelessness and HIV infection has long been recognized. However, evidence is beginning to show that housing status is not simply a correlate, but is strongly implicated

in multiple causal levels of risk for infection and continued transmission of HIV. Effectively addressing HIV risk requires attention to contextual or structural factors that shape or constrain individual behavior, which directly or indirectly affects an individual's ability to avoid exposure to HIV, or for HIV-positive individuals to avail of health-promoting and risk-reducing resources. A growing evidence base identifies housing status as a key structural factor influencing HIV vulnerability, risk, and health outcomes. Housing occupies a strategic position as an intermediate structural factor, linking broader societal processes of economic, social, and cultural exclusion to the more immediate physical and social environments within which individuals carry out day-to-day life (Aidala and Sumartojo 2007). The pressure of daily survival needs, limited opportunities to form stable partner relationships, high rates of transactional sex, exposure to violence, substance use as a way to cope with stress or mental health issues, and other conditions of homelessness make homeless and unstably housed persons especially vulnerable to HIV infection. Homelessness or inadequate housing itself appears to place persons at risk of HIV infection, and among persons already disproportionately impacted by HIV/AIDS (e.g., men who have sex with men (► [HIV Prevention for MSM](#)), persons of color, homeless youth, injection drug users, and victimized women (► [HIV Prevention and Women](#))), the lack of stable housing greatly amplifies their vulnerability for HIV and transmission to others. Research has also demonstrated that receipt of housing assistance has an independent, direct impact on reduction of drug and sexual risk behaviors as well as improving access to treatment (Access to Care) for HIV-positive persons and viral suppression which results in an additional prevention impact by reducing HIV transmission. The evidence on housing as prevention indicates that housing interventions improve the health and quality of life of people living with HIV/AIDS and also reduce new HIV infections, thereby contributing to both primary and secondary HIV prevention. As a strategic target for intervention, housing addresses the more proximal consequences of a multitude of broader social

and economic factors that affect HIV prevention and health care.

Housing and HIV Epidemiology

Since the beginning of the epidemic, lack of housing has been significantly associated with the risk for HIV exposure and transmission. Multiple studies have documented the dramatically higher prevalence of HIV/AIDS among homeless people compared to the general population. Among homeless persons in the USA, an estimated 3 to 20% are living with HIV (Robertson et al. 2004). The prevalence of HIV/AIDS is three to more than ten times higher among persons who are homeless or unstably housed (Housing and Homelessness) compared with persons with stable and adequate housing, though results vary by the population and geographic area studied (Culhane et al. 2001; Wolitski et al. 2007). A recent review of studies primarily in other developed nations found lower rates than in the USA, but prevalence ratios nonetheless indicate that HIV is much more common among the homeless in other nations as well (Beijer et al. 2012).

HIV/AIDS contributes substantially to excess mortality among homeless persons. For example, the rate of new HIV diagnoses among users of the New York City's homeless shelter system is over 16 times the rate compared to the general New York City population, and the death rate due to AIDS-related causes is seven times higher (Kerker et al. 2005; see also Chueng and Hwang 2004).

Housing and Risk for HIV Infection

Evidence has demonstrated that housing status is strongly associated with HIV-risk behaviors. Persons who are homeless or unstably housed are more likely to use drugs, use needles, share needles, have unprotected sex and multiple partners, and engage sex for money, drugs, or a place to stay (Survival Sex). The people most at risk of HIV – men who have sex with men, persons of color, unattached youth, injection drug users

(Injecting Drug Users (IDUs), Epidemiology of HIV/AIDS), and impoverished women – are significantly more likely to become HIV infected if they lack stable housing (Bruneau et al. 2011). A surveillance study of HIV infection among heterosexuals in 24 US cities found that the odds of testing positive for HIV are almost twice as high for residents of high-risk neighborhoods who experience homelessness compared to persons in the same neighborhoods with more stable housing (CDC 2011). Proven HIV-risk reduction interventions – including counseling, needle exchange, and other behavioral interventions (► [Behavior Aspects of HIV Treatment as Prevention](#)) – are considerably less effective for participants who are struggling with housing issues (Des Jarlais et al. 2007). Housing instability creates layers of chaos and unpredictability in the daily lives, environments, and habits of the homeless that creates multiple contexts of risk and limits access to risk reduction resources. Studies have confirmed a “dose relationship” such that homeless individuals are at increased risk for HIV compared to the unstably housed, and both homeless and unstably housed individuals have greater risk for HIV than those with stable secure housing (Aidala et al. 2005).

Housing Is HIV Prevention

Stable housing is an effective strategy for reducing an individual's risk for HIV and continued transmission of HIV to others. Longitudinal studies show a clear association between change in housing status and risk behavior change (Behavior Change). Formerly homeless persons who improve their housing status reduce risk behaviors by as much as half, while persons whose housing status worsens have higher rates of risky behavior (Aidala et al. 2005). Change in housing status is associated with risk reduction controlling for substance use, mental health issues, and receipt of health, behavioral health, and supportive services.

Housing assistance is a promising secondary prevention intervention. “Prevention for positives” is critical for protecting the health of people

living with HIV/AIDS (PLWHA) and reducing the continued spread of HIV. HIV infection increases risk for housing loss. An estimated 50% of all PLWHA will experience homelessness or housing instability during the course of their illness (HUD 2012). The HIV epidemic is increasingly concentrated among low income, racial/ethnic, and sexual minority communities and/or transient populations who may face limited housing options. However, regardless of prior housing resources, medical conditions and medical costs often lead to housing problems for persons with chronic illness. Illness limits employment and income drops, while housing costs continue to rise. Stigma (Stigma and Stigmatization) and discrimination associated with HIV can also limit housing options. For many, mental illness and substance abuse further challenge the ability to secure and maintain housing.

The lack of stable housing is associated with lack of treatment success. Homeless PLWHA compared to those who are stably housed are more likely to delay entry into care and to remain outside or marginal to HIV medical care. They are less adherent to treatment regimens and less likely to achieve viral suppression. Homeless/unstably housed PLWHA whose housing status improves over time are more likely to report continuous care and care that meets clinical practice standards than those who continue to experience housing instability. They are more likely to return to care after dropping out (Aidala et al. 2007; Kidder et al. 2007). Those who receive housing assistance are more likely to reduce risk behaviors, to engage in medical care, and to enjoy better health (Kidder et al. 2007, Buchanan et al. 2009). Access to housing improves access and adherence to antiretroviral medications, which lowers viral load and reduces the risk of transmission to uninfected persons (Leaver et al. 2007). Housing status is a more consistent predictor of health-care access and treatment outcomes than individual characteristics, insurance status, substance abuse and mental health comorbidities (Mental Health or Substance Abuse), or behavioral health service utilization. A systematic review of the literature identified 102 studies published between 1996 and 2012 that examined the effects of housing

status on health-related outcomes in PLWHA. Overall, findings from the included studies show that homelessness and unstable housing consistently contribute to poorer access to health services, ART adherence (► [Antiretroviral therapy \(ART\), Prevention of HIV](#)), and health outcomes and to increased HIV-risk behaviors. Almost all studies (87%) that investigated health outcomes among PLWHA found a negative association with homelessness or unstable housing, although some studies were underpowered to show statistical significance (Aidala et al. 2012). A more comprehensive review including later publications to 2014 reports the same pattern of findings regarding the association of unstable or inadequate housing and worse HIV engagement with care and health outcomes (Aidala et al. 2016).

Several local demonstration projects (e.g., Schwarcz et al. for San Francisco) and two large random assignment studies – the Chicago Housing for Health Partnership (Buchanan et al. 2009) and the HUD-CDC Prevention’s three-city Housing and Health Study (Wolitski et al. 2010) – provide the strongest evidence that providing housing assistance improves access and adherence to HIV care, reduces viral load, and reduces high-risk behavior associated with HIV transmission. These were both “housing first” models, providing PLWHA direct access to permanent housing without prior or ongoing demonstration of sobriety or commitment treatment, although services were available.

Housing Interventions Work and Are Cost-Effective

The Housing and Health (H&H) Study assessed the impact of immediate access to rental assistance vouchers on the medical care, health, and risk outcomes of homeless or unstably housed adults living with HIV/AIDS. The H&H Study included 630 HIV-positive participants in three cities (Baltimore, Chicago, and Los Angeles), half of whom were randomly assigned to receive a rental assistance voucher. The comparison group was referred to existing community resources in the attempt to secure housing. At the end of the

18-month study period, the majority of the intervention group achieved housing stability including those with long histories of chronic homelessness. Health outcomes improved dramatically with housing stability – including a 35% reduction in emergency room visits, a 57% reduction in the number of hospitalizations, and significantly improved mental health status. Even stronger differences were found in analyses that compared study participants who experienced homelessness during the follow-up period with those who did not. After controlling for socio-demographic variables, substance use, and physical and mental health status, those who continued to experience homelessness were 2.5 times more likely to use an emergency room, were 2.8 times more likely to have a detectable viral load at follow-up, reported significantly higher levels of perceived stress, and were more likely to report unprotected sex with a negative/unknown status partner (Wolitski et al. 2010).

The Chicago Housing for Health Partnership (CHHP) program provided housing and supportive service to persons with chronic medical illnesses including HIV who were homeless at discharge from hospitalization. The study examined medical care and health outcomes among formerly homeless patients randomly assigned to receive supportive housing compared to patients who were discharged to usual care in the community (most often emergency shelters). Among the one-third of CHHP study participants living with HIV/AIDS, those who received housing upon discharge from the hospital were almost twice as likely at 12 months to have an undetectable HIV viral load compared to HIV-positive participants who did not receive CHHP housing assistance (Buchanan et al. 2009). Overall, CHHP participants were three times more likely to achieve stable housing at 18 months than the usual care group and stability translated into significantly improved health outcomes. Controlling for a range of individual and service variables, housed participants had 29% fewer hospitalizations, 29% fewer hospital days, and 24% fewer emergency department visits than their counterparts (Sadowski et al. 2009).

Providing housing assistance for people living with HIV/AIDS both improved treatment

effectiveness and reduced costs associated with inappropriate care. Compared to usual care, the CHHP housing program generated average annual net public cost savings of over \$6000 per person (Basu et al. 2011). H&H Study cost analyses determined the cost utility of housing as an HIV-risk reduction and health-care intervention, considering the cost of the rent and services provided, HIV transmissions averted, medical costs saved, and quality-adjusted life years saved. Findings indicate that housing interventions are cost-effective if one out of every 64 HIV-positive persons receiving housing avoided HIV transmission to an HIV-seronegative partner. Considering comparative cost-effectiveness, with a cost per quality-adjusted life year (QALY) of \$35,000 to \$62,000, housing is a cost-effective HIV health-care intervention in the same range as widely accepted health-care interventions such as kidney dialysis (\$52,000 to \$129,000 per QALY) and screening mammography (\$57,000 per QALY) – and far less expensive than HIV pre-exposure prophylaxis (PrEP) (\$298,000 per QALY) (Holtgrave et al. 2007, 2013). The cost-effectiveness of housing as a platform to improve health outcomes and reduce health disparities has provided increasing justification for investing in housing interventions, which have been linked to reduced hospitalizations, emergency room visits, and other medical expenditures as well as improved quality of life for PLWHA. This is particularly relevant given that one in four homeless adults is hospitalized at least once in a year for largely preventable conditions. Forty percent of these adults are re-hospitalized within 14 months for the same condition, when they would have been better served by attention from outpatient services or ambulatory preventative care (Levinson and Ross 2007). Persons living in poverty without insurance and access to regular medical treatment tend to use costly emergency services more often.

Additional “housing first” randomized control trials provide clear evidence on the stabilizing effects of housing among homeless individuals with co-occurring substance use and mental health disorders, whereby providing safe housing spaces served to reduce risk behavior in substance

abuse and treatment nonadherence compared to “treatment first” options based on drug treatment, substance use counseling, and ambulatory medical service utilization (Burt 2002; Padgett et al. 2006). Housing first (HF) models developed in the USA are increasingly implemented in other national settings as well. See for example the *At Home/Chez Soi* national housing first program implemented by the Canadian Mental Health Commission whose evaluation concluded that HF rapidly ends homelessness, is associated with other positive outcomes, and is a sound public investment (Goering et al. 2014).

Conclusion: Policy Implications and Global Context

Accumulating evidence on the role of housing and other supportive services for people living with and at high risk of HIV/AIDS has influenced the policy landscape in the USA, resulting in several federal-level mandates issued by the White House, the Center for Disease Control (CDC), and the US Department of Housing and Urban Development (HUD) among other key governing entities and funders. The National HIV/AIDS Strategy (NHAS) for the USA released in 2010 set out three main goals: (1) to reduce new HIV infections, (2) to increase access to care, and (3) to improve health outcomes for PLWHA and to reduce health disparities related to HIV. NHAS calls specifically for increased housing support as a means by which to improve health outcomes and reduce health disparities for PLWHA (White House Office of National AIDS Policy 2010). HUD has committed to using housing as a platform to improve health in general and improve HIV outcomes in particular by expanding the reach of the Housing Opportunity for People Living with AIDS (HOPWA) program. HOPWA is a federal program that supports states, municipalities, and nonprofit organizations to develop and manage housing units and provide rental assistance and/or short-term payments in order to prevent homelessness among PLWHA (HUD 2012).

Internationally, the HIV and housing service sectors have increasingly worked together to

address the complex relationships between housing conditions, housing rights, and HIV/AIDS. The human settlement challenges in overcrowded slums with inadequate water, sanitation, and basic services are intensified by the impact of HIV. Risk of HIV infection is especially higher in settings where women have limited housing rights. Research has shown that women who have secure access to land and housing are better able to avoid relationships that increase their risk for HIV infection and to better manage their HIV if infected. Gender norms (Gender Roles) and traditional practices in nations that limit women’s access to housing both generate and sustain women’s disproportionate susceptibility to HIV infection (COHRE 2013).

The World Health Organization has included housing as one of the fundamental determinants of health. The right to adequate housing is defined as human right by the Office of the United Nations High Commissioner for Human Rights. UN-HABITAT has an initiative to incorporate programs to address AIDS in rights and development programs particularly in urban areas around the world. Another source of resources, research, and advocacy is the Centre on Housing Rights and Evictions (COHRE), an international human rights organization whose mission is to ensure the full enjoyment of the human right to adequate housing among all populations. COHRE has a program specifically to address women’s housing rights in the context of HIV/AIDS (COHRE 2013). The Interagency Coalition on AIDS and Development (ICAD) and Rooftops Canada (ICAD 2010) provide further resources and references on housing issues as they intersect with HIV/AIDS in developing nations.

Cross-References

- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Behavioral Aspects of HIV Treatment as Prevention](#)
- ▶ [HIV Prevention and Women](#)
- ▶ [HIV Prevention for MSM](#)

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Human Papillomavirus (HPV)

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Definition

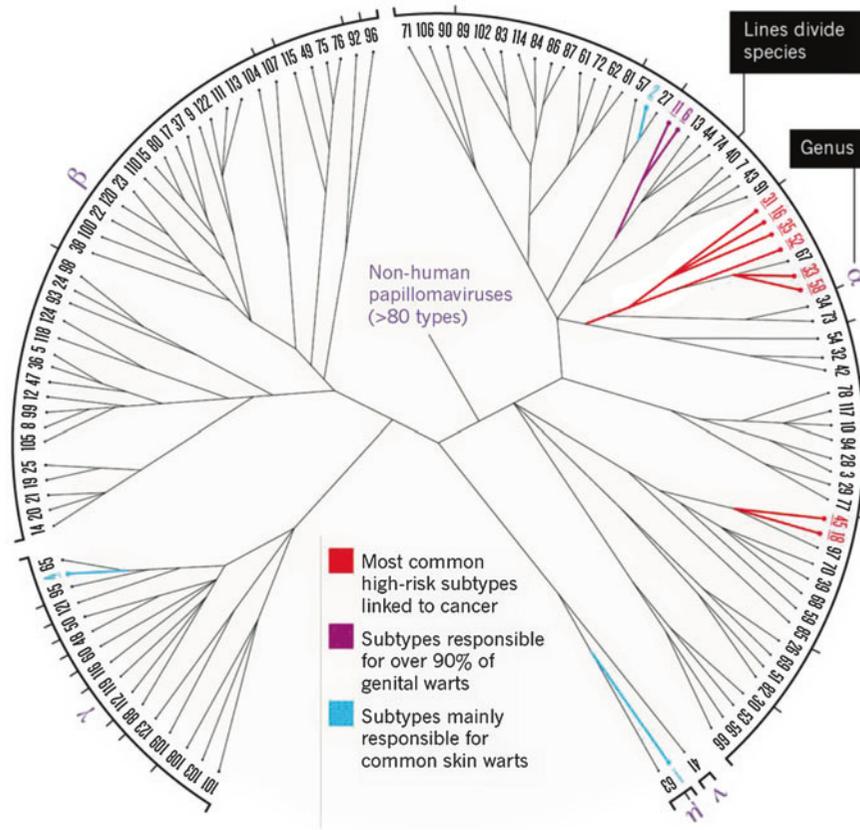
Human papillomaviruses (HPV) are a family of small DNA tumor viruses with a size of ~52–55 nm. The family consists of ~200 different genotypes; many of the types cause benign warts or papilloma, while a small fraction of oncogenic or “high-risk” types can cause invasive cervical cancer or other tumors. HPV infects keratinocytes in the basal layer of stratified squamous epithelia and replicates in the nucleus of infected keratinocytes along with keratinocyte differentiation. The viral genome in size of ~7.9 kb encodes six early,

nonstructural regulatory proteins (E1, E2, E4, E5, E6, and E7) and two late structural proteins (L1 and L2). E6 and E7 are two oncoproteins responsible for the viral oncogenesis of high-risk HPVs, including HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, HPV73, and HPV82. L1 is a major structural component of viral capsid, and its self-assembly in vitro into a viral-like particle (VLP) provides the basis of prophylactic vaccines against infections of several HPV types. In addition to cervical cancer, high-risk HPVs are associated with the development of various anogenital cancers and certain head and neck cancers.

Classification of Human Papillomaviruses

HPVs are a family of ~200 different genotypes, with 155 genotypes being completely sequenced. Papillomaviruses were originally classified as members of the family *Papovaviridae*, but reclassified in 2002 as an independent family, *Papillomaviridae*. The family *Papillomaviridae* currently contains at least 29 genera, and human papillomaviruses are grouped into five (alpha, beta, gamma, mu, and nu) genera (Fig. 1). The classification of papillomaviruses depends on the most conserved L1 open reading frame (ORF) by genotyping. Different genera share less than 60% nucleotide sequence identity in the L1 ORF. A new type of papillomavirus is given when its DNA sequence of L1 ORF differs by more than 10% from the closest known HPV type. Otherwise, a subtype indicates the difference between 2% and 10% and a variant less than 2%. HPV types are numbered in the order of their discovery. HPVs in the alpha genus cause mucosal and a fraction of cutaneous HPV lesions, while HPVs in the beta, gamma, mu, and nu genera cause cutaneous lesions.

HPVs are also grouped clinically as high-risk (oncogenic) types, which are frequently associated with invasive cervical cancer, and low-risk (non-oncogenic) types, which are found mainly in genital warts. Fifteen HPV types (types 16, 18,



H

Human Papillomavirus (HPV), Fig. 1 Classification of papillomaviruses (Adapted with permission from *Nature* (Crow 2012))

31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) are high risk. Twelve HPV types (types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and 89) are low risk (Munoz et al. 2003). Among the high-risk HPVs, HPV16 and HPV18 are the principal causes of cervical cancer, with a combined, worldwide relative contribution of ~70% of invasive cervical cancer.

General Properties of Human Papillomaviruses

HPV Virions and Genome Structure

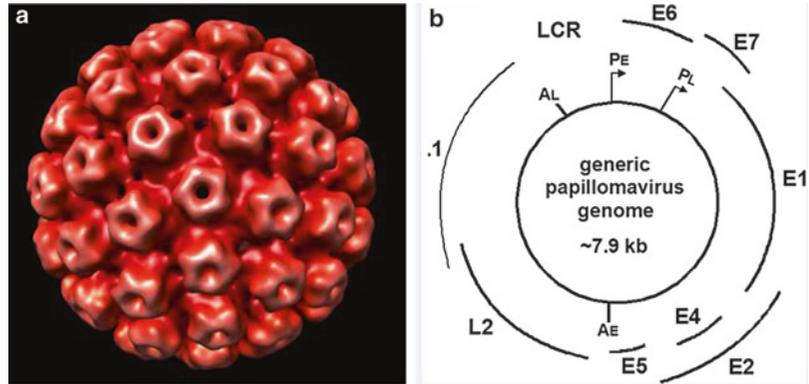
A mature HPV particle or virion contains a double-stranded, circular genome in the size of ~7.9 kb covered by a shell or capsid. HPV particles do not have an envelope or a lipid membrane outside of its capsid and thus is resistant to ether

treatment. An HPV capsid is composed of 72 pentamers, of which 60 are hexavalent and 12 are pentavalent (Fig. 2a). These pentamers are made up by two viral structural proteins, L1 and L2. Viral L1 (~55 kDa) is the major capsid protein and represents approximately 80% of the total viral protein. Viral L2 (~70 kDa) is a minor capsid protein. Each viral pentamer is comprised of five copies of L1 attached by one L2 from the inside of the pentamer. This is particularly true for the pentamers on the L2 vertices of icosahedral capsid shell. HPV virions are extremely stable and tolerate high temperature, low pH, proteases, and desiccation.

The HPV genome can be divided into three major regions: early, late, and a long control region (LCR), also called the noncoding region (NCR). The three regions are separated by two polyadenylation (pA) sites: an early pA (AE) site and a

Human Papillomavirus (HPV), Fig. 2

HPV genome structure and virion particles. (a) HPV11 VLP visualization of cryoEM reconstruction (<http://nramm.scripps.edu/?p=1042>). (b) HPV genome in a circular episomal form, with relative positions of viral early (*PE*) and late (*PL*) promoters and early (*AE*) and late (*AL*) polyadenylation signals



late pA (AL) site (Fig. 2b). The early region encodes six ORFs (E1, E2, E1[^]E4, E5, E6, and E7) and this region of HPV16, HPV18, and HPV31 also encodes an E2[^]E8. The late region lies downstream of the early region and encodes L1 and L2 ORFs. The LCR region (~850 bp) has no protein-coding function, but bears the origin of DNA replication and transcription factor-binding sites important for RNA transcription (Zheng and Baker 2006).

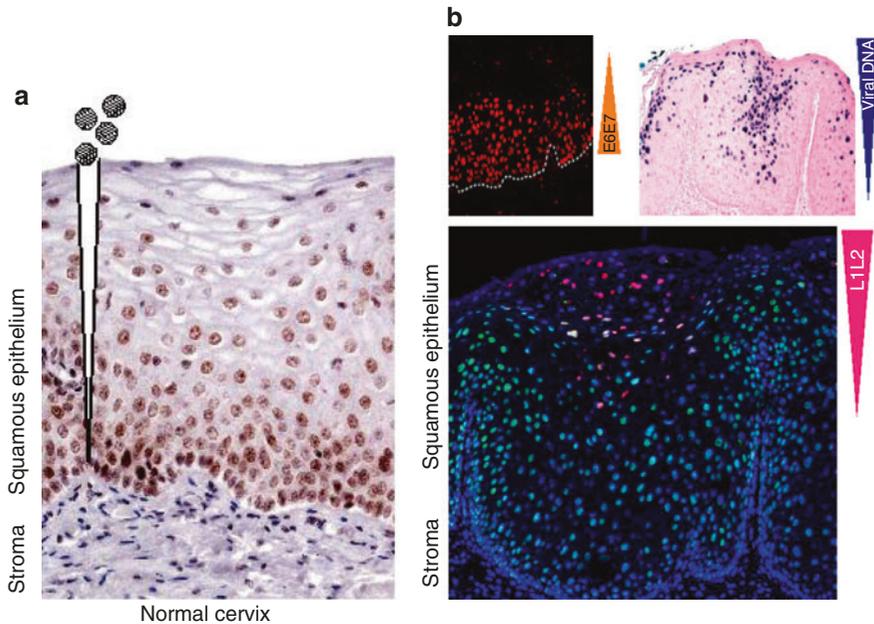
HPV Life Cycle

HPV depends on keratinocyte differentiation to complete its life cycle (Fig. 3). HPV infection is cell type-specific and requires the access of viral particles to human basal cells on the basal lamina through micro-wounding induced by scratching, sexual intercourse, etc. Initial attachment of HPV virions to the host cells takes place by the interaction of viral major capsid protein L1 with the host receptor, heparan sulfate proteoglycan. This interaction leads to a conformational change and exposes viral minor capsid protein L2 for furin/proprotein convertase digestion. Subsequently, exposed L2 (aa 108–120) interacts with the S100A10 subunit of annexin A2, a secondary cell receptor, followed by viral entry of the infected cells by endocytosis (Raff et al. 2013). L1 dissociation from the L2-viral genome, which is facilitated by cyclophilin B, results in viral particle uncoating and leaves the L2-associated viral genome in the endosome to interact with the retromer complex and to be transported to the nucleus via the trans-Golgi network. Once in the nucleus, L2 localizes the

viral genome to PML bodies for the transcription of early genes and establishment of infection.

HPV DNA replication requires the A + T-rich origin of DNA replication in the LCR and two viral DNA-binding proteins, E1 as a DNA helicase and E2 as an accessory factor to E1 (McBride 2008). It occurs bidirectionally in the nuclear replication foci during two different stages of the viral life cycle. The initial DNA replication takes place in an average of once per cell cycle in the G2 phase in the infected basal cells and allows the cells to maintain 50–100 copies of virus genome per cell. DNA repair plays a role in the promotion of HPV DNA amplification. The interaction of E1 with a cellular protein p80/UAF1 is required for efficient maintenance of the viral episome in undifferentiated keratinocytes. E2 binds to the viral genome and tethers it to mitotic chromosomes through mitosis by interaction with Brd4 on mitotic chromosomes to ensure partitioning of replicated viral episomes to the nuclei of both daughter cells (McBride 2008).

Viral vegetative DNA replication takes place in highly differentiated, upper layer spinous keratinocytes no longer undergoing cellular DNA synthesis. This stage of viral DNA replication is robust and presumably undertaken by a rolling circle replication mechanism, leading to amplification from a low, stable copy number to several thousands of viral genome copies per cell to be packaged into progeny virions. Viral DNA amplification is required for HPV late gene expression. Virions assemble into paracrystalline



Human Papillomavirus (HPV), Fig. 3 Keratinocyte differentiation-dependent HPV life cycle. **(a)** Infection of the cervical basal keratinocytes by viruses (*circles, top left*) is initiated through a microtrauma usually during sexual intercourse. The panel is modified with permission (Jia et al. 2009). **(b)** Expression of viral E6 and E7 is initiated in the infected basal cells (*orange color on the top left* for MCM7 as a surrogate for viral E6 and E7). Viral DNA

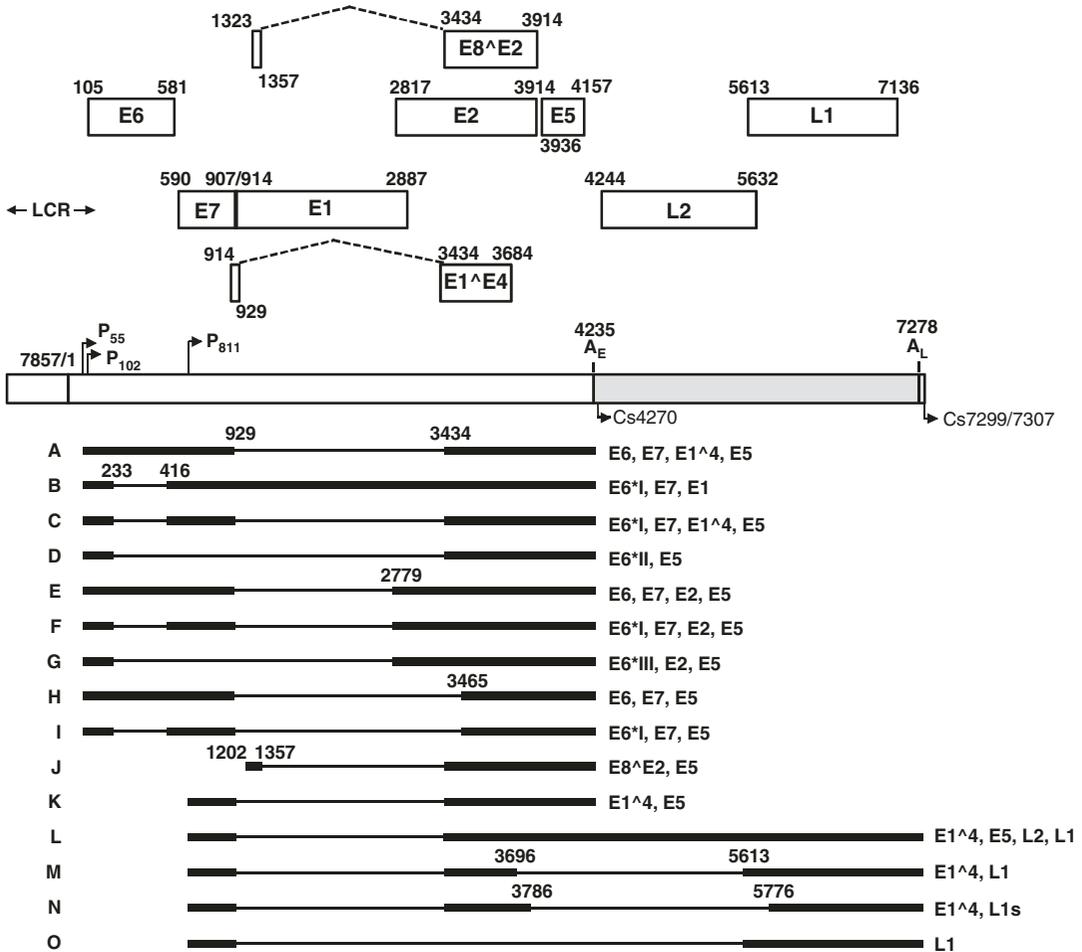
replication takes place in the spinous and granular keratinocytes under intermediate or high differentiation (*navy blue color on the top right* for viral DNA). However, viral L1 and L2 (*red color for L1 in the bottom*) become detectable only in the granular and cornified keratinocytes under terminal differentiation (This panel is adapted with permission (Zheng and Wang 2011))

arrays in the granular layer and egress from the cornified layers of the epithelium.

HPV Genome Expression

HPV transcribes its RNAs from one strand of its genome in one direction and starts from two major promoters named by their transcription start site position in the virus genome. A viral early promoter upstream of the E6 ORF and a viral late promoter residing in the E7 ORF are responsible for the expression of viral early and late ORFs, respectively. The viral early promoters P97 in HPV16, P99 in HPV31, and P55/P102 (previously named as P105) in HPV18 are three most studied early promoters. Their activities are tightly controlled by *cis*-elements in the LC-R. These *cis*-elements, including consensus E2-binding sites, interact with cellular transcription factors and the viral transactivator/repressor E2 protein and regulate the transcription from

each viral early promoter in undifferentiated keratinocytes. The resulting early primary transcripts (pre-mRNAs) carry all the early ORFs, which span three exons and two introns and undergo alternative RNA splicing and polyadenylation using an early pA signal (Fig. 4). The viral late promoters P670 in HPV16, P811 in HPV18, and P742 in HPV31 are three studied late promoters. Their activities can be induced only in differentiated keratinocytes during vegetative virus replication. Because a viral late promoter is positioned in the E7 coding region (Fig. 4), transcription from the late promoter has to bypass the early pA site to allow expression of the late region. As a result, a true late pre-mRNA is a chimeric transcript of the early and late regions, with the early region in its 5' half and the late region in its 3' half. This late pre-mRNA can be processed either into an early region transcript which is cleaved and polyadenylated at the



Human Papillomavirus (HPV), Fig. 4 Genome structure and transcription map of HPV18. The *bracket line* in the *middle* of the panel represents a linear form of the virus genome for better presentation of head-to-tail junctions, promoters (*arrows*), early (A_E) and late (A_L) pA sites, and mapped cleavage sites (CS). The ORFs (*open boxes*) are diagrammed above the *bracket*, and the numbers above each ORF (E6, E7, E1, E2, E5, L2, and L1) are the positions of the first nucleotide (nt) of the start codon and the last nt of the stop codon assigned to the HPV18 genome. The E1^ΔE4 and E8^ΔE2 ORFs span two exons with the nt positions

indicated. Because the first AUG of E1^ΔE4 and E8^ΔE2 is positioned in the first exon, formation of an intact E1^ΔE4 or E8^ΔE2 ORF requires RNA splicing (*dashed lines*). LCR is a long control region. Below the *bracket line* are the RNA species derived from alternative promoter usage and alternative RNA splicing. Exons (*heavy lines*) and introns (*thin lines*) are illustrated for each species of RNA, with the mapped splice site positions being numbered by nt positions in the virus genome. Coding potentials for each RNA species are shown on the right (Adapted with permission (Wang et al. 2011))

early pA site (transcript K) or a late region transcript which is cleaved and polyadenylated at the late pA site (transcript L–O) (Fig. 4). The alternative splicing of HPV early and late transcripts produces various species and sizes of mRNA transcripts with multiple coding potentials (Fig. 4 transcripts A–O). Thus, any given HPV transcript, no matter whether it is an early or late

transcript, could be bicistronic, tricistronic, or polycistronic and contains two or more ORFs (Zheng and Baker 2006; Wang et al. 2011). Conversely, a particular ORF can be a part of multiple mRNA species. Thus, to determine which transcript encodes which viral proteins has been a challenge, and we know very little about which protein is translated from which transcript.

Because E6, E1, and L2 ORFs span over an intron region, the expression of these three proteins requires the retention of its corresponding intron. RNA splicing promotes expression of E7 from viral early transcripts in high-risk HPVs and E4 from viral late transcripts.

Viral Proteins

The HPV genome encodes eight major viral proteins: E1, E2, E4, E5, E6, E7, L1, and L2. L1 and L2 are structural proteins for virus capsid formation. The other six viral proteins are nonstructural and are responsible for virus replication and pathogenesis. E6 and E7 are viral oncoproteins responsible for cell transformation and tumorigenesis.

E1

E1 (~68 kDa) is a site-specific DNA-binding protein for the viral origin of DNA replication. Although relatively little is known about how E1 protein is produced in HPV-infected tissues, E1 is biochemically a well-studied protein. E1 can be subdivided into three functional regions: a C-terminal helicase/ATPase domain, a central origin-binding domain, and an N-terminal regulatory region. E1 serves as a DNA helicase and opens the DNA duplex to initiate viral DNA replication. E1 by itself has low binding affinity for the viral origin of replication, which contains specific, palindromic E1-binding sites. However, binding of E2 protein to specific sites adjacent to the E1-binding sites helps recruit E1 in a cooperative manner. When loaded onto the origin, the E1/E2 protein complex recruits host replication factors such as topoisomerase I, DNA polymerase alpha/primase, replication protein A, and Brd4 to initiate viral DNA replication (McBride 2008). E1 protein is dispensable for maintenance of replication, but is essential for the initial and productive replication of HPV16 DNA. Overexpression of E1 blocks S-phase progression and triggers an ATM-dependent DNA damage response in viral DNA replication foci during the initial viral DNA amplification.

E2

E2 (~42 kDa) is a viral transcription factor in addition to its role in viral DNA replication. E2

contains two defined functional domains. The N-terminal domain is crucial for transcriptional activation, whereas the C-terminal domain possesses the DNA/RNA binding and dimerization properties of the protein. These two domains are linked by a hinge region. E2 interacts with E1 and enhances the binding affinity of E1 to the origin of replication. E2 functions as a transcriptional activator or repressor to regulate viral early promoter activity through the consensus E2-binding sites, upstream of the viral early promoter. However, E2's transcriptional repression occurs only in cells harboring integrated, but not episomal HPV16 DNA. E2 binding to the viral genome is important for tethering viral genome to mitotic chromosomes throughout mitosis (McBride 2008). E2 also plays roles in apoptosis, ubiquitination, and intracellular trafficking. E2 regulates RNA processing including RNA splicing and RNA polyadenylation.

E4

E4 (~10 kDa) is the most abundantly expressed HPV late protein and accumulates in the cytoplasm of differentiating cells in the upper epithelial layers. E4 is expressed as an E1^{E4} protein in which the N-terminal 5-amino acid (aa) residues are derived from the E1 ORF spliced to the E4 ORF. HPV16 E4 expression and cleavage of its N-terminal 17-aa residues by calpain lead E4 to multimerization, formation of amyloid-like fibers, and disruption of the normal dynamics of the cyokeratin networks. E4 mediates cell cycle arrest in G2 by sequestering Cdk1/cyclin B1 onto the cyokeratin network to prevent the accumulation of active Cdk1/cyclin B1 complexes in the nucleus. The E1^{E4} protein of HPV16 may also regulate gene expression at the posttranscriptional level by interacting with a DEAD box containing RNA helicase and SR protein kinase 1 (SRPK1).

E5

E5 is a small (~10 kDa), hydrophobic, oligomeric channel-forming membrane protein, which is localized in the endoplasmic reticulum (ER) and the nuclear envelope. Assembled hexameric E5 channels in membranous environments have a

defined luminal diameter and stoichiometry. The N terminus of E5 is restricted to the ER lumen, while its C terminus is exposed in the cytoplasm to mediate interactions with cytoplasmic and ER proteins. The C terminus of E5 also induces koilocytosis, the structural cellular changes that are characteristic of papillomavirus infection. HPV16 E5 is also an oncoprotein. High levels of E5 expression in the mouse skin in the presence of persistently provided exogenous estrogen induce epithelial hyperproliferation, resulting in spontaneous tumor formation and increased dysplastic disease in the cervical epithelium. E5 induces anchorage-independent growth, enhances immortalization of human primary keratinocytes by E6 and E7, and causes cell-cell fusion. E5 activates epidermal growth factor receptor (EGFR) signaling pathways and enhances mitogen-activated protein kinase (MAPK) activity, but attenuates TGF β 1/Smad signaling and downregulates the expression of MHC class I.

E6

E6 is an ~18 kDa nuclear oncoprotein that interacts with hundreds of cellular proteins. E6 inactivates cellular p53 family proteins (p53, p63, and p73) essential for cell cycle control, DNA repair, and cell adhesion (Moody and Laimins 2010). The complex of E6 with E6-associated protein (E6-AP) functions as a ubiquitin-protein ligase in the ubiquitination of p53. E6 contains two hypothetical zinc fingers involved in zinc binding and three nuclear localization signals (NLS) as well as a PDZ-binding site in the N terminus. E6 dimerizes through its N-terminal domain and this self-association promotes polyubiquitination of p53 by E6-AP. The basic-hydrophobic pocket of E6, which composes of two zinc domains and a linker helix, interacts with an acidic LxxLL motif of host proteins to exercise E6 transformation and degradation activities (Zanier et al. 2013). Besides its ability to immortalize and transform cells, E6 regulates gene expression at transcriptional and posttranscriptional levels through interaction with other transcription factors/coactivators and splicing factors, as well as its direct interaction with DNA and RNA. High-risk E6 enhances telomerase activity and activates several signaling

pathways, including Akt, Wnt, Notch, and mTOC1, but inhibits apoptosis, keratinocyte differentiation, and the interferon response. High-risk E6 regulates the expression of a subset of cellular miRNAs (Zheng and Wang 2011). In E6 transgenic mice, E6 oncoprotein, in the absence of E7, synergizes with estrogen to induce cervical cancer after 9 months.

E7

E7 is a ~16 kDa nuclear protein. The N-terminal half of E7 contains conserved regions (CR) that have sequence similarity to other viruses. These conserved regions have sequence similarity to a portion of the CR1 and the entire CR2 in adenovirus E1A and SV40 T antigen. E7 contributes to the binding and degradation of pRB and its related pocket proteins p107 and p130 through cullin 2 ubiquitin ligase complex associated with ZER1. A conserved Leu-X-Cys-X-Glu (LXCXE) motif in CR2 is sufficient for the association of E7 protein with pRB. The C-terminal half of E7 contains a CR3 region with a zinc-binding domain; this region contributes to the degradation of pRB and to E7 dimerization and transformation activities. E7 interacts with many cellular transcription factors/coactivators and participates in epigenetic reprogramming. In addition to its cellular transformation activities, oncogenic E7 also plays a role in the viral life cycle and deregulates the cell cycle by stabilizing p21 and upregulating p16 expression. Oncogenic E7 interacts with the centrosomal regulator gamma-tubulin and induces mitotic defects and aneuploidy, leading to centrosome abnormalities and chromosomal instability. In transgenic mice, the continuous expression of E7 is required for the maintenance of cervical cancers and precancerous lesions even in the presence of viral E6 (Jabbar et al. 2012). E7 downregulates the expression of MHC class I and prevents the recognition of HPV-induced lesions by cytotoxic CD8 T cells.

L1

L1 (~55 kDa) is a major structural component of viral capsid. Both HPV16 and HPV18 L1 mRNAs initiate their translation from an initiation codon right at the splice junction of exon 2 and exon 3 of

the viral late mRNAs and encode corresponding 506-aa HPV16 L1 and 508-aa HPV18 L1 (Fig. 4), both of which are shorter than the one originally predicted in the GenBank database (Wang et al. 2011). L1 self-assembles into pentameric capsomers with a hollow channel at the center through a five-fold central axis when five L1 monomers come together and assemble in a symmetrical manner. Thus, its tendency to form a pentavalent structure is directly reflected in the star-shaped motif visible as a result of each capsomer. Purified capsomers can form capsids, which are stabilized by disulfide bonds between neighboring L1 molecules through their C termini. The C-terminal half of L1 also contains activities for HPV DNA binding and nuclear localization. The N-terminal half of L1 interacts with L2. L1 capsids assembled *in vitro* are the basis of prophylactic vaccines against infections of several HPV types (Schiller et al. 2012). Although most portions of L1 are well conserved between types, the surface loops of L1 can differ substantially, probably reflecting a mechanism for evasion of neutralizing antibody responses elicited by previous papillomavirus infections.

L2

L2 (~70 kDa) is a minor capsid protein which exists in an oxidized state within the papillomavirus virion, with the two conserved cysteine residues forming an intramolecular disulfide bond. A single molecule of L2 interacts with an L1 pentamer via a C-terminal L1-binding domain. L2 is not required for capsid formation, but participates in the encapsidation of the viral genome. Up to 72 molecules of L2 can be incorporated per capsid, one beneath the axial lumen of each L1 capsomer. Both C- and N-terminal NLSs of L2 bind to viral DNA during capsid formation and are important for nuclear localization of the DNA particle. L2 also interacts with cellular proteins during the infectious entry process. After the initial binding of the virion to the cell, the N terminus of L2 containing a consensus furin cleavage site (RxKR) is cleaved by a cellular protease, furin. The N-terminal L2 has a conserved transmembrane domain with three GxxxG motifs to facilitate homotypic and heterotypic interactions

between transmembrane helices for vDNA translocation across the endo-/lysosomal membrane. Disruption of some of these GxxxG motifs has been shown to result in noninfectious viruses. L2 interacts with members of the T-box family, TBX2 and TBX3, and represses transcription from the long control region of HPVs. A small N-terminal portion of L2 is well conserved between different papillomavirus types. Experimental vaccines targeting these regions may offer protection against a broad range of HPV types.

HPV Infections and Transmission

HPVs can cause benign and malignant tumors in persistently infected skin and mucosal tissues anywhere in the human body. Benign tumors induced by low-risk HPVs are also called papilloma or warts, and these are in general harmless. However, when such a papilloma occurs in the larynx or upper airway, it could be life threatening as exemplified by recurrent respiratory papillomatosis (RRP), a juvenile disease predominantly caused by HPV6 and HPV11 infection. RRP tends to recur and has the potential to spread throughout the respiratory tract. Benign tumors in the genital area are called condyloma acuminatum or genital warts, of which around 96–100% are caused by low-risk HPV6 or HPV11 infection. Although sexual contact as a risk factor in the development of cervical cancer was described in 1842 by Domenico Rigoni-Stern and the infectious nature of human warts was established in 1907 by Giuseppe Ciuffo's self-inoculation experiments with a cell-free extract of common warts, a landmark breakthrough on HPV cervical infection as a cause of cervical cancer was not achieved until 1983 when HPV16 DNA was discovered in ~60% of cervical cancer samples by Harald zur Hausen and colleagues in Germany. Since then, the role of various HPV genotypes in cervical cancer has been extensively studied, with HPV16, HPV18, HPV31, HPV33, HPV35, HPV45, HPV52, and HPV58 together contributing up to 91% of invasive cervical cancer and HPV16, HPV18, and HPV45 contributing up to

94% of cervical adenocarcinomas. Cervical cancer (see ► [Cervical Cancer and HIV](#)) is the second most common cancer among women worldwide. Approximately 500,000 incident cases and 320,000 cases of attributable deaths are predicted each year. More than 80% of cases arise in developing countries.

In addition to the cervix, high-risk HPVs also infect other anogenital areas and can lead to the development of anal, vaginal, vulvar, and penile cancers. Most anal cancers (~84%) are caused by HPV infection (see ► [Anal Cancer](#)). High-risk HPV infection of the oropharynx (the tonsils and the back of the tongue) may lead to the development of oropharyngeal cancer (see ► [Other HPV-Associated Cancers \(Oropharyngeal and Penile\)](#)). Oropharyngeal cancer is more common in men than women. The prevalence of HPV in oropharyngeal cancer is increasing, rising from 16.3% during 1984–1989 to 71.7% during 2000–2004. Epidermodysplasia verruciformis (EV) is an extremely rare autosomal recessive genetic hereditary skin disorder associated with a high risk of skin carcinoma and is characterized by high susceptibility to HPV5 and HPV8 of the skin. The role of human papillomavirus (HPV) in cutaneous squamous cell carcinoma (SCC) is elusive.

HPV infection is transmitted through skin abrasions (skin warts), by sexual intercourse, during passage through an infected birth canal (juvenile RRP), and probably in other ways. Women with multiple sex partners or a history of prostitution have an increased risk of infection with HPVs and an increased risk of cervical cancer. Women with history of cervical cancer (or precancer) also have an increased risk of anal cancer (see ► [Anal Cancer](#)). Receptive anal intercourse increases the risk of anal cancer in both men and women. Oral HPV infection is related to oral sex behavior. Most HPV infections are transient and have either no viremia or only a minimal viremic phase. Thus, HPVs are not disseminated in general to other sites by blood in the course of HPV infections. However, detection of HPV DNA in human peripheral blood mononuclear cells and the findings of papillomavirus productive infection in the lymphocytes, placenta, and bovine fetal tissues

indicate that hematogenous and vertical spread of HPV should be carefully investigated.

Pathogenesis and Immune Responses of HPV Infections

After HPV enters epithelial cells in the wound basal layer, virus infection is established by the initiation of viral gene expression and then DNA replication. The HPV life cycle is tightly linked to cell differentiation, and the time from infection to release of virus can be approximately 3 weeks. Viral early gene expression and initial viral DNA replication happen in undifferentiated cells in the lower layers of the infected skin or cervix, while late gene expression and vegetative DNA replication occur in highly differentiated cells in the upper layers of the infected skin or cervix (Fig. 3). Although HPVs do not induce a lytic virus infection or cytolysis/cell death, viral early gene expression causes cell cycle interruption, apoptosis, keratin network collapse, DNA damage, genome instability, viral genome integration into the host genome, and cell immortalization and transformation. The viral late gene expression leads to the maturation of virus particles which appear as aggregates in the infected cell nuclei.

Even though most HPV infections are asymptomatic and cause no clinical problems, chronic or persistent cervical or anal HPV infection may result in histologic changes, with the infected cells displaying koilocytosis and nuclear inclusion bodies. The presence of koilocytes is a characteristic of HPV infection in all precancerous lesions and can be found in cervical smears. The period between infection and first appearance of lesions is highly variable and can range from weeks to months. HPV-induced histologic changes are classified as cervical intraepithelial neoplasia (CIN) grade 1, 2, or 3 on the basis of increasing degree of abnormality of cell growth in the cervical epithelium (see ► [Cervical Cancer and HIV](#)). The likelihood of spontaneous clearance versus progression to cancer in the absence of treatment varies for CIN1, CIN2, and CIN3. Histologic CIN1 or cytologic low-grade squamous intraepithelial lesion (LSIL) indicates

abnormal cell growth that is confined to the basal 1/3 of the epithelium, usually clears spontaneously (80% of cases), and rarely (<1%) progresses to cancer. About 10–20% of women with CIN1 progress to CIN2 or CIN3, which corresponds to cytologic high-grade squamous intraepithelial lesion (HSIL). CIN2 and CIN3 have a lower percentage (~40%) of spontaneous clearance and a higher (~10%) percentage rate of progression to cancer if not treated (Schiffman et al. 2007). CIN2 and CIN3 are distinguished by the extent of neoplasia, which is confined to the basal 2/3 of the epithelium in CIN2 and more than 2/3 in CIN3. CIN3 may involve the full thickness of the epithelium and is sometimes referred to as “cervical carcinoma in situ.” Expression of viral E6 and E7 in the infected epithelial cells inhibits cell differentiation and induces cell immortalization and transformation, resulting in disruption of virus productive life cycle and leading to viral genome integration into the host genome. Although the integration of host genome is random in general, the circular HPV genome is commonly disrupted at the E1 or E2 region for the integration. Thus, the degree of E1 or E2 integrity would reflect HPV genome status in cervical cancer tissues.

Because HPVs cause infections that are largely if not exclusively limited to the epithelium, they are largely shielded from the host immune response. To date, the correlates of immunity to HPV infection remain elusive. The majority of HPV infections are transient and cause no clinical problems. Most of new HPV infections clear within 1–2 years. The median duration of new infections is 6–18 months (Schiffman et al. 2007). However, the women with HIV/AIDS or immunosuppression take longer time to clear their HPV infections. Not all infected persons have antibodies. Approximately 60% of women with incident HPV infections may have antibodies. The median time to seroconversion after a new infection is approximately 8 months. Among serum antibodies against many different viral products, the best characterized and most type-specific antibodies are those directed against conformational epitopes of the L1 protein.

HPV inhibits innate immunity and evades the host immune system. HPV infection suppresses interferon (IFN) synthesis and signaling and the expression of MHC class I in the infected cells, resulting in reduced recognition of the infected cells by CD8⁺ T cells or exclusion of CD8⁺ T cells from the dysplastic epithelium of HPV16-associated cervical lesions. HPV-specific CD4 T cells isolated from lymph node biopsies of cervical cancer patients also suppress proliferation and cytokine (IFN-gamma, IL-2) production by responder T cells. However, T cells do play a role in preventing persistent HPV infection and inducing wart regression. Healthy individuals with HPV infections display E6-specific memory T-helper cells in their blood. The majority of subjects clearing HPV16 display an HPV16 E6-specific cytotoxic T-lymphocyte (CTL) response. Failure of this response is associated with the development of cervical cancer. A significant CD8⁺ T-cell tumor infiltration with a higher CD8⁺/CD4⁺ and/or CD8⁺/regulatory T-cell ratio prevents the tumor cells from metastasizing to the tumor-draining lymph node. Moreover, T-cell defects due to a mutation in a *ras* homologue gene family member H (RHOH) gene and MST1, EVER1, and EVER2 deficiencies also lead to persistent HPV infections.

Diagnosis of HPV Infections

Exfoliated Cell Cytology and Tissue Biopsy

Pap smear, also called a cervical smear or smear test, is a screening test to check for changes in the cervical cells from the outer opening of the cervix; it is done to identify individuals who can benefit from procedures to prevent progress to cervical cancer (see ► [Cervical Cancer and HIV](#)). The cytologic changes that can be observed include atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells, cannot exclude HSIL (ASC-H); low-grade squamous intraepithelial lesion (LGSIL or LSIL); high-grade squamous intraepithelial lesion (HGSIL or HSIL); squamous cell carcinoma; and atypical glandular cells not otherwise specified (AGC or AGC-NOS). Dysplasia seen on a biopsy of the

cervix is grouped histologically into three categories: CIN1, mild dysplasia; CIN2, moderate to marked dysplasia; and CIN3, severe dysplasia to carcinoma in situ.

Electron Microscopy

HPVs cannot be grown by conventional tissue culture methods. HPV infection can be diagnosed by electron microscopy, in which virus particles in clinical specimens can be visualized either by negative staining or thin-sectioning techniques. In thin sections of human skin warts, papillomavirus particles can be found in aggregates in an infected nucleus.

Viral Antigen Detection

HPV infection can also be diagnosed by detection of HPV proteins in infected tissues or exfoliated cells. In general, HPV L1 is detectable in cervical smears in most high-risk HPV-associated LSIL, but becomes undetectable in most of HSIL cases.

Viral E2, E4, and E7 proteins have been detected from formalin-fixed paraffin-embedded cervical cancer tissues or cervical smears. Type-specific HPV E4 and E7 antibodies are particularly useful in distinguishing HPV type-specific infection of HPV16, HPV18, HPV58, and other genotypes by immunohistochemistry, ELISA, or Western blot.

Viral DNA and RNA Detection

Qualitative or semiquantitative DNA or RNA tests for the diagnosis of HPV infection have been developed that are based on the sequence conservation and variation of viral L1-coding regions. To date, four FDA-approved HPV DNA tests, one FDA-approved RNA test, and two Europe-approved HPV DNA tests are commercially available. These include (1) Hybrid Capture 2 (HC2) HPV DNA Test (Digene Corp., Gaithersburg, MD), (2) Cervista HPV HR test (Hologic, Bedford, MA), (3) Cervista HPV 16/18 test (Hologic, Bedford, MA), (4) cobas 4800 HPV Test (Roche, Pleasanton, CA), (5) APTIMA HPV assay (Gen-Probe, San Diego, CA), (6) LINEAR ARRAY HPV Genotyping Test (Roche Molecular Systems Inc, Alameda, CA), and

(7) INNO-LiPA HPV Genotyping Extra (Innogenetics, Ghent, Belgium).

The HC2 is an in vitro nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of 13 high-risk types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and 5 low-risk types of HPV (6, 11, 42, 43, 44). This DNA assay was the first FDA-approved HPV DNA test and has become the standard in many countries and widely used in clinical studies. The HC2 distinguishes between the low-risk and high-risk groups, with the detection limits of ~5,000 genome copies, but cannot determine the specific HPV genotype.

The Cervista HPV HR test and the Cervista HPV 16/18 test are two HPV DNA tests for high-risk HPV infections, but have no genotyping capability. The Cervista HPV HR test is an in vitro diagnostic test for detection of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in cervical specimens. The Cervista HPV HR test uses the Invader chemistry and is a signal amplification method for detecting specific nucleic acid sequences.

The cobas 4800 high-risk HPV Test uses fluorescence signal to detect nucleic acids amplified by using real-time PCR methodology and detects 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). The cobas 4800 system, consisting of two separate instruments, integrates sample preparation, amplification and detection, and result management. It has high throughput (designed to process up to 280 samples per day) and is automated.

The APTIMA HPV assay detects viral E6/E7 mRNA transcripts of 14 high-risk HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), but has no genotyping capability. The APTIMA HPV assay is a transcription-mediated amplification-based assay, and the amplicons are then detected by hybridization protection with chemiluminescent-labeled single-stranded nucleic acid probes that are complementary to the amplicons.

The LINEAR ARRAY HPV Genotyping Test is used in the European Union for detection and genotyping of 37 high- and low-risk HPVs (6, 11,

16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39, and CP6108). The test utilizes the amplification of target DNA by PCR with PGMY09/11 primers and then hybridizes the amplicons to multiple HPV genotype-specific probes fixed on a membrane strip.

INNO-LiPA HPV Genotyping Extra uses the principles of reverse line blot hybridization. The test amplifies HPV DNA with SPF10 primers at the L1 region and then hybridizes the amplicons to the probes fixed on membrane strips in sequence-specific lines. The INNO-LiPA test detects and distinguishes 28 low- and high-risk HPVs including 18 high-risk-HPVs (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82), 7 low-risk HPVs (6, 11, 40, 43, 44, 54, 70), and 3 additional genotypes (69, 71, 74).

Serology

HPV serology is a valuable tool to study immune status prior to and after HPV vaccination. Although most HPV infections are cleared spontaneously within 2 years, the majority of infected subjects develop and maintain serum antibodies to viral L1 for up to 5 subsequent years. Serology is not commonly used for clinical diagnosis because more than 40% of women do not seroconvert after HPV infection. The standard methods are VLP ELISA and pseudovirion neutralization assays. Both methods have similar sensitivity and specificity in the detection of serum L1 antibodies in the majority of infected subjects. There is recent evidence that antibody to HPV16 E6 can be predictive of subsequent development of oropharyngeal or anal cancer with a high degree of specificity, and this will be an important area for future research.

Detection of Cellular Surrogates of High-Risk E6 and E7 in Infected Tissues

A few cellular biomarkers of high-risk HPV E6 and E7 expression have been used to indicate viral oncoprotein expression in precancer lesions or cancers. These include cell cycle inhibitors (p16Ink4a and p18Ink4c), MCM7

(minichromosome maintenance protein 7), cyclin E2, and a subset of miRNAs (Zheng and Wang 2011).

Epidemiology and Prevention of HPV Infections

The global prevalence of HPV infection among women with normal cervical cytology is around 11–12%. The prevalence of any HPV is about 26.8% in females aged 14–59 and 44.8% among women aged 20–24 years in the United States. The majority of HPV infections are transient and asymptomatic; 70% of new HPV infections clear within 1 year, and approximately 90% clear within 2 years. The median duration of new infections is 8 months. Up to 50% of young women with one type of HPV infection may acquire another type of HPV infection within a few years. Although coinfection with multiple high-risk HPVs may lead to an increased risk of CIN2/CIN3, persistent infection with any high-risk HPV is the most important risk factor for cervical lesions and cervical cancer. The risk of cancer development varies by HPV type, with HPV16 being more oncogenic than other high-risk HPV types. Factors associated with cervical cancer (see ► [Cervical Cancer and HIV](#)) also include an increased number of lifetime sexual partners, increased age, other sexually transmitted infections, immune suppression, and other host factors. There are also epidemiology reports of smoking being associated with increased cervical cancer, although it is not clear if this may be due to association with other risk factors. The time between initial HPV infection and development of cervical cancer is usually decades. Many aspects of the natural history of HPV remain to be understood, including the role and duration of naturally acquired immunity after HPV infection.

Because HPV is the most common sexually transmitted infectious agent, condom use and circumcision are two common practices to prevent sexual partners from HPV transmission. Pap smear screening is widely used in developed countries to check for changes in the cervical cells from the outer opening of the cervix and to

prevent progress to cervical cancer by treatment of these lesions. While likely, it is not known if screening for and treatment of anal HSIL will similarly prevent anal cancer, the US National Cancer Institute has recently initiated a large randomized clinical trial to study this issue. Two FDA-licensed prophylactic HPV vaccines, Gardasil from Merck (USA) and Cervarix from GlaxoSmithKline (UK), are very safe and effective in preventing new or persistent HPV infections and reducing HPV infection-induced cervical, vulvar, vaginal, anal, and penile lesions. Their use will be an important public health measure to prevent HPV-associated cancers (Schiller et al. 2012). Both vaccines are comprised of viral DNA-free, L1 VLP. Cervarix contains L1 VLP from HPV16 and 18 produced in insect cells, while Gardasil is comprised of L1 VLP from HPV6, 11, 16, and 18 produced in yeast. Because it contains L1 from HPV6 and 11, Gardasil immunization also provides protection against genital warts. In the presence of adjuvant aluminates, both the quadrivalent Gardasil and the bivalent Cervarix are highly immunogenic and excellent HPV type-specific protection is provided to girls and boys at age 11 or 12 who receive all three vaccine doses (0, 1, and 6 months). Catch-up vaccination with either one of HPV vaccines is approved by the US FDA for young women through age 26 or young men through age 21 (see ► [Cervical Cancer and HIV](#)).

Due to the limited inclusion of HPV types, the current vaccines do not provide cross-immune protection against other types of high-risk mucosal HPVs. However, there is evidence to suggest that HPV L2 might work as a pan-HPV vaccine against different HPVs. A small N-terminal portion of L2 is well conserved among different HPVs, and experimental vaccines targeting these conserved domains offer protection against a broad range of HPV types (Jagu et al. 2013).

Most of therapeutic vaccine approaches target high-risk HPV E6, E7, or both to control disease progression in preexisting HPV infections and lesions. To date, these studies have provided only a gleam of success. An experimental HPV16 E6 and E7 synthetic long peptide vaccine can increase the number and activity of HPV16-

specific CD4⁺ and CD8⁺ T cells in patients with vulvar intraepithelial neoplasia or cervical cancer. HPV VLP entry into NK cells triggers cytotoxic activity and cytokine secretion, although VLP does not activate Langerhans cells. A therapeutic HPV16/HPV18 DNA vaccine with optimized E6 and E7 codons stimulates high titers of anti-E6/E7 antibodies and high levels of cellular immunity by both CD8⁺ and CD4⁺ T cells (Bagarazzi et al. 2012).

Conclusion

HPVs have been well recognized as a group of small DNA tumor viruses that are highly transmissible through sexual or close contact. In the past decades, HPV infection has been found in association with the development of various human cancers, including cervical, anogenital, oropharyngeal, and even some skin cancers. Although there is more to be learned about the basic biology of HPV, the translational research on HPVs has remarkably advanced our understanding of HPV epidemiology, disease progression, clinical diagnosis, and cervical cancer prevention. Today, L1-based HPV vaccination has become a general practice and has been mandated in many countries to protect girls and boys from HPV infections and HPV-related precancerous lesions and cancer.

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Identification and Validation of HIV Cofactors

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Definition

Viruses in general and HIV in particular have a limited genetic content. To accomplish their life cycle, lentiviruses make therefore use of host proteins, the so-called cellular cofactors, to complement the limited set of viral proteins carried in the viral particle. Interactions between host cell and viral proteins during different stages of lentiviral infection can provide attractive new antiviral targets (see entry ► [Cellular Cofactors of HIV as Drug Targets](#)). The interaction between HIV envelope proteins and the CD4 receptor and either CXCR4 or CCR5 coreceptor is the best known virus-host interaction (see entries ► [CXCR4, Coreceptors](#) and ► [Attachment/Binding](#)). This interaction resulted in the development of the HIV fusion inhibitors enfuvirtide (targeting GP41) and maraviroc (targeting CCR5-GP120 interaction). Other host proteins that have been put forward as

potential drug targets are DDX3 (see entry ► [DDX3, Cofactors, and RNA Export](#)) and cofactors of HIV integration and nuclear import. Since these last cofactors were identified and validated by a systematic approach, those will be described in more detail as to make general recommendations about cofactor identification and validation. Validated cofactors will be prioritized in terms of their potential to serve as new drug targets.

The insertion of lentiviral cDNA in a host cell chromosome is a step of no return in the replication cycle, after which the host cell becomes a permanent carrier of the viral genome and a producer of lentiviral progeny. Integration is carried out by integrase (IN), an enzyme playing also an important role during nuclear import. Plenty of cellular cofactors of HIV-1 IN have been proposed. To date the lens epithelium-derived growth factor (LEDGF/p75) is the best studied cofactor of HIV-1 IN (see entry ► [Role of LEDGF/p75 in Cell Biology and Disease Pathogenesis](#)) (Cherepanov et al. 2003). Moreover, small molecules that block the LEDGF/p75-IN interaction have recently been developed for the treatment of HIV infection (Christ et al. 2010). The nuclear import factor transportin-SR2 (TRN-SR2) has been proposed as another cofactor of HIV IN-mediating nuclear import of the virus (see entry ► [Role of Transportin-SR2 \(Transportin-3, TRN-SR2, TNPO3\) in HIV Replication](#)) (Christ et al. 2008). Using both IN cofactors as examples, the approaches to be taken to identify and validate novel cofactors as new antiviral targets will be

described. Similar approaches may be undertaken for other HIV proteins such as reverse transcriptase (RT) or protease. In fact high-throughput screens based on yeast two-hybrid screening (Rain et al. 2009) or co-immunoprecipitation (Jager et al. 2012) have been undertaken to identify novel cofactors of HIV. For each of the candidate hits, a thorough validation has to be undertaken to verify if the host protein is important for HIV replication to understand its role in the replication cycle and to determine whether the interaction between the viral protein and the cofactor can be considered a potential new antiviral target. It follows that identification without validation provides only a small step forward in the better understanding of the disease.

Identification and Validation of Integrase Cofactors as Novel Antiviral Targets

Farnet and Bushman noticed that a factor important for integration activity *in vitro* was removed upon gel filtration of HIV-1 PICs in the presence of high salt (Farnet and Bushman 1997). The activity could be restored by addition of protein extracts from uninfected human SupT1 cells. This observation opened a new field in retrovirology focused on so-called cellular cofactors of retroviral integration. Purified proteins from diverse sources could rescue the intermolecular integration activity of retroviral PICs isolated from infected cells and salt stripped of associated host factors. The factor was identified as the high mobility group chromosomal protein A1 (HMGA1; HMG I(Y) protein). HMGA1 is a nonhistone DNA-binding protein involved in the regulation of inducible gene transcription and microRNA expression in both benign and malignant neoplasias. The same complementation method led to the discovery of another cellular cofactor of HIV, barrier-to-autointegration factor (BAF) (Lee and Craigie 1998). By combining antibodies against known viral and cellular PIC components (MA, Vpr, Ku-80) with anti-BAF antibodies, human BAF was proven to be a component of the PIC (Lin and Engelman 2003). The functional co-immunoprecipitation strategy was

based on examining different fractions obtained from HIV-1-infected C8166 T cells for the presence of integration activity, viral IN, and endogenous BAF (Lin and Engelman 2003). Although BAF was suggested to protect retroviral DNA from auto-integration and also to promote the association of PICs with target DNA, knockdown of BAF by siRNA in HeLaP4 cells was later found not to affect HIV-1 replication. Validation of the role of cellular cofactors in lentiviral infection thus requires multiple independent experimental approaches.

The initial discoveries of HMGA1 and BAF were followed by a systematic search for cellular cofactors of lentiviral integration. The increasing interest in the interactomics of HIV integration and replication resulted in algorithms for the identification and proper validation of cofactors (Fig. 1). The discovery of novel HIV-1 cofactors as potential antiviral targets can be accomplished by different techniques and is often based on the search for specific and direct protein interaction partners by yeast two-hybrid (Y2H) screen or high-throughput co-immunoprecipitation (co-IP) followed by mass spectroscopy. Alternatively, full-genome RNA interference (RNAi) screens can be used to identify genes/proteins involved in HIV integration/replication.

Physical protein-protein interactions between viral protein and cofactor (Y2H and co-IP) need validation in a phenotypic assay. After specific RNAi-mediated depletion of the specific host factor, the impact on HIV replication is determined. If depletion of the candidate cofactor, verified by RT-QPCR and Western blotting, has no deleterious effect on HIV replication, the cofactor can be dismissed as an important cofactor of HIV replication. If depletion results in a stimulation of HIV replication, the binding partner may represent a restriction factor. In parallel colocalization of viral protein (IN) and host protein in the cell can be verified by microscopy. Phenotypic assays measure single and multiple rounds of infection in both laboratory-immortalized cell lines (e.g., HeLaP4) and primary CD4⁺ T cells and macrophages. In the authors' expertise multiple-round replication represents the best assay system to validate cofactors. The use of multiple siRNAs

interactor 1 (INI1)/hSNF5 and transportin-SR2 (TRN-SR2) were identified as IN cofactors (Christ et al. 2008; Kalpana et al. 1994).

Three RNAi-based whole-genome screens for HIV infection in mammalian cells were reported in 2008 (Brass et al. 2008; Konig et al. 2008; Zhou et al. 2008). Drawbacks of these screens are the use of HeLa or HEK293T cells that are not natural host cells of HIV-1 infection. VSV-G pseudotyping of HIV may confound interactions with natural host factors during early steps of the replication. The use of mutated or cell line adapted viruses in the screens can be another source for false-negatives and positives. The necessity of proper validation of potential cofactors derived from siRNA screens is underlined by comparison of the results of two large siRNA screens performed for HIV. Brass et al. (Brass et al. 2008) identified 284 genes, whereas Zhou et al. (2008) picked up 232 genes. Only 15 genes overlapped between both studies (Zhou et al. 2008). LEDGF/p75 was not identified in either of them.

Nuclear import is an important step in lentiviral infection. The classical technique to study nuclear import of cellular proteins with recombinant import factors is based on digitonin-permeabilized cells. The method was also adapted to study nuclear import of snRNA and DNA. This technique is of limited use for the study of lentiviral nuclear import since NLSs of individual viral proteins can be masked within the PIC, and the data obtained for isolated proteins do not need to fit the real situation during viral infection. There are now better approaches available for studies of lentiviral nuclear import (and early postentry steps in general) based on advances in fluorescence microscopy: real-time *in vivo* tracking (Arhel et al. 2006) and the so-called PIC import assay (Christ et al. 2008; Albanese et al. 2008). The PIC import assay is based on fluorescently labeled viral particles containing IN fused to eGFP (HIV-IN-eGFP) *trans*-incorporated in the particle through a fusion with HIV-1 Vpr. In 2008, transportin-SR2 (TRN-SR2, TNPO3) was independently identified as a cellular cofactor of HIV-1 replication in two siRNA screens (Brass et al. 2008; Konig et al. 2008) and as an HIV-1 IN

binding partner by Y2H screening (Christ et al. 2008) (see entry ► [Role of Transportin-SR2 \(Transportin-3, TRN-SR2, TNPO3\) in HIV Replication](#)). Although its exact role in HIV-1 infection has not been fully clarified, several independent studies confirmed TRN-SR2 as a genuine cellular cofactor to the extent that it is now being used as a positive control in HIV-1 interaction studies.

After validation of the interaction between host factor and viral protein, drug discovery can be initiated, facilitated by high-throughput screening (HTS) and high-content screening (HCS) technologies developed since the 1990s, as, for example, amplified luminescent proximity homogeneous assay (AlphaScreen[®]) technology, high-throughput FLIM for protein-protein interaction screening, enhanced chemiluminescence, fluorometric microvolume assay technology (FMAT[™]), LEADSeeker[™], scintillation proximity assays (SPA), etc. These screening technologies allow screens to be performed efficiently, cost-effectively, and with low amounts of material. Nowadays there is a trend to move from labeled reporter assays toward label-free assays. If structural biology approaches (crystallography, NMR, SAXS, etc.) can reveal the interface of the PPI aided by site-directed mutagenesis to corroborate the hot spots of the interaction, structure-based drug design can be embarked upon. For the discovery of LEDGINS, AlphaScreen[®] technology and structure-based drug design were used.

Conclusion

This review highlights the importance of research on cellular cofactors of HIV replication as potential targets for anti-HIV drugs. The interaction between LEDGF/p75 and IN is crucial for HIV replication, and the rational design of LEDGINS as novel antivirals represents an important achievement in translational research. Efficient targeting of host-virus PPIs expands the possible arsenal of targets beyond HIV-encoded enzymes. This novel paradigm can be extended to other viral diseases. Increased understanding of the

virus-host interactome can be the basis for plenty of future antivirals. Since PPIs have pivotal roles in virtually all physiological and disease-related intracellular macromolecular complexes, development of SMIPPIs can benefit many therapeutic areas. While the example described here is particularly relevant to the field of virology, applications of SMIPPI technology to other fields will increase as knowledge on the role of PPIs in human diseases expands.

Since the nuclear import of PICs still represents a black box in the knowledge of HIV infection and since IN plays an active role at this stage, study of the IN interactome may also shed light on this process. The discovery that the importin TRN-SR2 is a binding partner of integrase can provide the lever to open this box. Research on HIV nuclear import not only provides insights in basic virology but has also great potential for drug discovery especially since nuclear import is a bottleneck in HIV replication. There is increasing evidence that lentiviral chromosomal target site selection for integration is linked to nuclear import of PICs. Moreover, proper illumination of the lentiviral route to the nucleus and of the impact on integration site selection will aid the design of safer gene therapy approaches.

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Identifying Opportunities to Block HIV-1 Transmission in the Female Genital Tract

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Definition

HIV-1 transmission is both inefficient and heterogeneous, and host factors that prevent and/or facilitate viral acquisition in HIV-uninfected individuals hold the key to better biomedical prevention. This review will highlight some of the factors influencing HIV transmission via the female genital tract.

Background to the Problem

HIV is predominantly transmitted during sexual intercourse (► [HIV-1 Sexual Transmission](#)), with young women having the highest risk of HIV infection in parts of the world that are severely impacted by the pandemic (Abdool Karim et al. 2011b). Despite the size of the current HIV pandemic, HIV is relatively inefficient at infecting susceptible target cells across the genital mucosa of women, with estimated per-coital transmission rates as low as 1 per 1,000 contacts (Powers et al. 2008). This suggests that natural barriers to HIV infection within the mucosal surfaces of the female genital tract are generally effective. To gain entry via mucosal surfaces, HIV must first transverse several layers of hostile territory prior to finding enough suitable target cells for it to eventually establish a lifelong infection.

While HIV transmission via mucosal surfaces is generally inefficient (HIV-1 Transmission Dynamics), specific failures of mucosal defenses must nevertheless occur to account for the more than two million successful HIV transmission events that take place annually. Various cofactors for HIV transmission, such as other coincident sexually transmitted infections, undermine mucosal defenses and consequently amplify the per-contact risks of HIV acquisition. This review focuses on what is known about male-to-female HIV transmission, the reasons why most potential transmission events are “normally” blocked, and how biomedical augmentation of natural mucosal defenses against HIV might offer women protective immunity from infection via their genital tracts.

While this review will focus primarily on factors that influence HIV acquisition in women, it is clear that the concentration of HIV in blood of their HIV-infected sexual partners is the best-described predictor of HIV transmission risk. This has been defined in prospective studies of HIV serodiscordant couples where higher virus loads in blood and in genital secretions from the HIV-infected partner predicted similarly higher risk of HIV acquisition in the uninfected partner (Baeten et al. 2011). Further compelling support for this relationship has come from several studies which showed that antiretroviral drug treatment of HIV-infected partners almost completely protected their HIV-negative partners from infection (Cohen et al. 2012; ► [Antiretroviral Therapy \(ART\), Prevention of HIV](#)). Despite the reported efficacy of such a prevention strategy, several operational barriers to the widespread use and timing of antiretroviral drug therapies exist in HIV-infected individuals such that there remains a need for continued commitment to the development of alternative strategies to protect HIV-uninfected individuals.

Although the anatomical features of mucosal surfaces that influence the risk of HIV acquisition or the precise behavioral factors that impact the health and integrity of these surfaces are not completely understood, both of these approaches

represent critical targets for the development of better vaccines and biomedical prevention strategies.

Preventing HIV Exposure

All the most effective methods to completely block HIV infection involve avoiding exposure. These methods include having only one faithful HIV-uninfected partner, consistent and correct condom use, and/or remaining sexually abstinent. Because sustainably modifying sexual behavior has proven difficult (Behavior Change), the development of new and/or refined biomedical approaches to minimize HIV exposure is urgently needed. Many of these interventions (such as medical male circumcision, topical or oral pre-exposure prophylaxis, early treatment of HIV-infected partners in discordant relationships, and HIV vaccines) have shown partial efficacy in preventing HIV infection in randomized clinical trials. Although partial efficacy may suggest imperfect protection, an alternative interpretation is that some individuals are more difficult to protect than others, either due to higher levels of HIV exposure or the presence/absence of host immune factors that modulate HIV risk. While the extent of HIV exposure is partially partner dependent (e.g., higher partner HIV viral loads, the frequency of unprotected sexual intercourse, or number of different partners), the risks of HIV infection once a person is exposed are likely to also be determined by a complex combination of host factors (including an individual's genetic makeup, their immune status, genital health, and a range of other factors). Understanding the heterogeneity in HIV risk at an individual level may hold the key to understanding how transmission can be successfully blocked.

How Does HIV Gain Entry?

When a woman is exposed to HIV during heterosexual intercourse, initial contact between HIV and the female genital mucosa will usually occur

in the presence of semen (► [HIV Prevention and Women](#)). HIV in semen is a heterogeneous combination of cell-free viral particles and HIV-infected target cells from the male partner (such as CD4+ T cells that are present in semen) (► [HIV-1 Transmission; Influence of Bodily Secretions](#)). While the proportionate role of cell-free versus cell-associated HIV contributing to male-to-female transmission is unclear, it is likely that both mechanisms are involved. Plasma viral loads in male partners accurately predict the concentrations of cell-free HIV present in semen, and conditions such as urethritis in males (inflammation of the urethra) increase both the concentration of immune cells contained in semen and HIV transmission risks to sexual partners. Despite semen having well-described anti- and pro-inflammatory effects on cells of the immune system (many of which are aimed at facilitating fertilization of female partners), most studies of HIV transmission have ignored the role of semen in determining transmission rates. Improved systems to study cell-to-cell HIV transmission are needed to dissect its role in genital HIV transmission and pathogenesis (Abela et al. 2012), and the design of strategies to block HIV transmission should consider both the cell-free and cell-associated viruses that are contained within the semen.

The female reproductive tract can broadly be divided into (1) the upper reproductive tract which consists of the uterus, fallopian tubes, and ovaries; (2) the lower reproductive tract which consists of the vagina and ectocervix; and (3) the transformation zone between the ectocervix and endocervix which signifies the intersection of the upper and lower reproductive tracts. Among the best defenses against HIV infection within the female genital tract are the extracellular components of the innate immune system, which include the physical barrier presented by the vaginal mucous layer, the antimicrobial molecules within this layer, and the relatively acidic pH within the vaginal lumen (which is regulated by healthy commensal microflora). Secretory cells in the endocervical canal, which are arranged in the gland-like crypts, produce this mucous. The mucous barrier is made up of

glycoproteins that constitute a structural framework that not only prevents contact between the cervicovaginal epithelium and pathogens from the vaginal vault (by steric hindrance) but also physically binds to growth factors and immunological proteins such as protective antibodies. Vaginal mucous is composed of commensal and other bacteria present in the vault, host cells that have been shed, inflammatory cells and their secreted products, and serum transudate.

The majority of HIV exposures are effectively blocked or neutralized by these innate defenses, which provide an impermeable barrier to the virus. When HIV breaches the initial mucous barrier, an important unresolved question is how the virus is then able to breach the underlying epithelial cell barrier. The importance of the epithelial barrier in defense against HIV infection is evidenced by the increased risk for HIV transmission when this barrier is disturbed (e.g., genital ulcers, bacterial vaginosis, or abrasions in the vaginal and cervical epithelial layers).

The cervical transformation zone between the ectocervix and endocervix has been identified as the main portal of HIV entry during successful transmission because it is lined with only a single layer of columnar epithelial cells – a barrier that is presumably less formidable than the multilayered stratified epithelia found in the vagina and ectocervix. The transformation zone also contains higher concentrations of HIV target cells than the vagina and ectocervix. The importance of the transformation zone during HIV transmission is further supported by the fact that women with cervical ectopy (immature formation of the ectocervix with columnar cells from the endocervix appearing at the ectocervix) are more susceptible to HIV infection than healthy women. Despite the relatively increased susceptibility of the transformation zone to HIV infection, it is important to note that it is certainly not the only route by which male-to-female HIV transmission occurs. The surface area of the vaginal lumen is substantially larger than that of the cervix and micro-abrasions in the multilayered vaginal epithelial barrier are likely to also account for a portion of

HIV entries. It is also worth noting that women who have had hysterectomies (who do not have uteruses) can become HIV infected and that cervical barrier methods such as diaphragms have failed to prevent HIV acquisition.

Once HIV has breached the epithelial and mucosal barriers of the genital mucosa (► [Mucosal Immunity to HIV-1](#)), it must successfully infect particular target cell types in order to establish an infection. Since there are a couple of likely target cell types that HIV is capable of infecting, it is currently uncertain which of its potential target cell types are the most important in this regard. HIV can infect a variety of immune cells that express the primary entry receptor CD4 and the chemokine coreceptors CCR5 and CXCR4. Such cells include CD4+ T cells, dendritic cells, macrophages, and monocytes. Within the genital mucosa, such cells are typically located in the submucosal layer beneath the epithelium. In addition to becoming productively infected (which means that infected cells have replicating virions within them), it has also been demonstrated that HIV can be “captured” by dendritic cells that do not themselves become infected during this process but can facilitate the infection of other cells of the immune system (► [Dendritic Cell Interaction with HIV-1 Envelope Glycoprotein – Implications for Prevention of Transmission](#)).

Studies performed in vitro and in primate models of vaginal transmission suggest that CCR5+ CD4+ T cells are the major initial target of HIV (Hladik et al. 2007). Although these cells are present in the submucosa to protect against potential invaders, HIV directly infects these cells and uses them to gain direct access to draining lymph nodes that in turn contain very high concentrations of HIV-susceptible target cells. Movement of HIV from the genital mucosa to draining lymph nodes occurs within 1 week of an initial mucosal surface breach and marks the point at which the irreversible establishment of latently infected cell populations occurs (Li et al. 2009). Any additional natural or medically medicated barriers to HIV infection must therefore act prior to HIV becoming fully established in the mucosa and spreading to the draining lymph nodes.

Factors That Influence Mucosal HIV Transmission

For the sake of understanding how the establishment of HIV-infected cell populations might be effectively blocked during transmission events, it is worthwhile considering the many factors that could influence this process. For example, in order to migrate into tissues such as the genital mucosa, immune cells need to specifically exit blood vessels that penetrate the high endothelial tissues – a process known as extravasation. Extravasation is achieved when immune cells circulating in blood (in high endothelial venules) recognize specific chemical signals (such as inflammatory or chemotactic cytokines) within blood plasma or receptor molecules on the surfaces of blood vessel epithelial cells that allow the immune cell to move out of the endothelial venule into tissue. The specific movement of T cells to mucosal surfaces occurs mostly in response to inflammatory cytokine gradients. High concentrations of inflammatory cytokines at mucosal sites (such as those produced in response to sexually transmitted infections or physical trauma during sex) create a gradient that allow the migration of high numbers of activated CD4+ CCR5+ immune cells into the genital mucosal tissue. The initiation of a productive HIV infection requires the presence within the genital submucosa of adequate numbers of susceptible CD4+ target cells (► [CD4+ T Cell Depletion](#)). Since genital tract inflammation is associated with increased rates of T-cell recruitment to the genital mucosa, it is perhaps unsurprising that such inflammation is associated with an increased risk of HIV transmission.

In addition to specifically recruiting HIV-targeted cells to the genital mucosa, genital inflammation may also interfere with the innate barrier function of the mucosa (► [Immune activation and HIV transmission](#)). Specifically, by inducing alterations in the epithelial cell layer, the mucous layer, and the composition of the genital microbiome, inflammation can directly impact the ease with which HIV is able to penetrate into the submucosal layers of the genital tract. A better general understanding of the causes and consequences of genital inflammation could

therefore yield crucial insights into how to best preserve the effective natural barriers against HIV incursion of healthy mucosal surfaces.

Sexual Transmission Usually Involves Only a Single HIV Variant

The difficulty HIV faces in breaching mucosal barriers during transmission is perhaps highlighted by the finding that as many as ~80% of HIV infections are initiated by a single-virus variant implying that many infections might be initiated by just a single-virus particle (Keele et al. 2008). Since individuals who are chronically infected with HIV typically harbor thousands of distinct HIV variants, it is particularly noteworthy that they will generally only transmit a single variant. Such an observation is consistent with both the low transmission probabilities implied in HIV-1M epidemiological studies and the small genital tissue infection foci seen in primate studies during the first few days of infection. It remains unknown both why ~20% of HIV transmission events involve the transmission of multiple HIV variants (i.e., multiple distinct virions) and whether there are particular genetic features of the virus variants that make it through the transmission bottleneck that predispose them to establishing a new infection.

Blocks to Transmission

Medical interventions aiming at HIV prevention might seek to either preserve or promote the integrity of the natural mucosal barriers or treating the medical conditions that have caused the damage in the first place (Preventing HIV-1 Transmission Through Novel Strategies).

Promoting the Integrity of the Natural Mucosal Barrier

Although ongoing infections with sexually transmitted microbes including Herpes simplex virus-2 (HSV-2) (► [Risk of Sexual HIV-1 Transmission](#):

Co-infections Associated with Risk), *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* and disruptions of natural commensal flora such as bacterial vaginosis are known to increase HIV transmission risks, several well-conducted clinical trials aimed at treating such infections and promoting overall genital health have failed to convincingly reduce rates of HIV transmission. The reasons for this remain unclear, but it may be that damage caused to the genital mucosa and other immunological effects of sexually transmitted infections persist for long after these infections are resolved.

While certain sexually transmitted infections and bacterial vaginosis are known to increase risk for HIV transmission, defining characteristics of the genital mucosa that are associated with better protection is important. The international definition for a healthy female genital tract is one harboring predominantly *Lactobacillus* species, having a pH between 3.5 and 4.5, and having no bacterial vaginosis, candida, or STI. Lactobacilli species have been shown to lower vaginal pH by the production of lactic acid and H₂O₂, resulting in a low and protective pH. Furthermore, lactic acid is more effective than acidity alone as a microbicide against HIV or against pathogens such as *Neisseria gonorrhoeae*. When these species of *Lactobacillus* are displaced by a diverse mix of anaerobic bacteria, the vaginal pH increases (above the optimal range which is ≤ 4.5) and bacterial vaginosis results.

There are several modes of action by which Lactobacilli could alter the vaginal microenvironment and reduce risk of inflammation and HIV susceptibility. These include: (i) antagonism of pathogens by competition for binding or nutrients, (ii) stimulating the production of antimicrobial factors, (iii) modulation of epithelial barrier integrity, (iv) epithelial or immune cell function via production of cytokines and other stromal factors, and (v) generation of tolerizing DCs and regulatory T cells. While antibiotic treatment of sexually transmitted infections and bacterial vaginosis is commonly implemented to improve vaginal health, alternative approaches that have been considered include probiotics. Convincing clinical efficacy of probiotic applications has been

documented for various conditions, including prevention of antibiotic-associated diarrhea, necrotizing enterocolitis, irritable bowel syndrome, and reduction of respiratory tract infections. Since the vaginal microbiota of women with bacterial vaginosis has been found to contain a reduced number of lactobacilli, lactobacilli administered orally or intravaginally have been tested for their effectiveness in colonizing the vagina and curing women with bacterial vaginosis or at least preventing its recurrence. Probiotics delivered topically do alter the vaginal flora by suppressing the overgrowth of pathogenic phyla and reestablishing vaginal homeostasis. Topical application decreases culture-positive bacterial vaginosis in pregnant women, and oral probiotics given alone or in combination with oral antibiotics fair better than antibiotics alone in decreasing culture-confirmed bacterial vaginosis and associated bacteria.

Treating STIs or Dampening Genital Tract Inflammation to Reduce HIV Risk

Several large randomized control trials have shown that neither the prevention nor aggressive treatment of sexually transmitted infections was effective in reducing HIV incidence. Unfortunately these trials did not also directly test whether direct management of genital tract inflammation had any impact on HIV transmission. Since inflammation is a critical initial stage during the initiation of immune responses at mucosal sites, an important consideration in HIV prevention strategies should be the safest and most effective approaches to modulating inflammatory responses in the genital tract.

The rationale for the management of genital inflammation in the context of reducing HIV transmission risks has perhaps been best illustrated in a nonhuman primate study (Li et al. 2009). It was found that topical application of glycerol monolaurate during vaginal exposure to SIV decreased concentrations of important chemokines in genital secretions. This led to reduced degrees of target cell recruitment in treated animals, which in turn was associated

with enhanced protection from SIV infection. While glycerol monolaurate is a relatively non-specific anti-inflammatory molecule, other anti-inflammatories that are safe for use on mucosal tissues are in early phases of preclinical development. Anti-inflammatory molecules that specifically block only the components of normal immune responses that are exploited by HIV to access and infect suitable target cells will likely be the best candidates for inclusion within anti-inflammatory formulations specifically geared to reducing HIV transmission risks (► [HIV Infection, Immune-Based Interventions](#) for).

Passive or Vaccine-Induced Antibodies to Protect Against HIV Entry

Taking lessons from other viral infections for which effective vaccines are available (human papillomavirus and hepatitis B virus), the best-established correlates of vaccine-induced protection are neutralizing antibodies (► [Preventing HIV-1 Transmission Through Vaccine-Induced Immune Responses](#)). Neutralizing antibodies generally bind to the outside of an invading pathogen and prevent its entry into host cells. In the case of HIV, there is evidence from nonhuman primate studies that both neutralizing antibodies and T cells can protect against high-dose vaginal virus challenges. Neutralizing antibodies that are able to neutralize a broad array of HIV strains only develop in ~20% of HIV-infected individuals and usually only after years of chronic antigenic stimulation. There is concerted effort to characterize what these antibodies recognize, how they develop, and where they originate from as this will give important insight into how the epitopes of HIV vaccines should be designed so as to achieve similarly broadly neutralizing vaccine antibody specificities.

Certain well-characterized HIV-specific broadly neutralizing antibodies isolated from HIV-infected individuals have been tested for their ability to protect primates against SIV infection after passive immunization (Hessell et al. 2009; BrNAb Responses; HIV-1 Transmission and Effects of). Even when administered

intravenously, no SIV infection was noted within vaginal tissues, suggesting that antibodies were able to reach mucosal sites in sufficient numbers to provide protection.

The RV 144 “Thai” trial recently demonstrated 31% protection against HIV infection and was the first partially protective vaccine tested in humans. Although being protective, RV 144 did not elicit neutralizing antibody responses either systemically or at the genital mucosa. Despite this, the best correlate of vaccine efficacy in this trial was shown to be the ability to stimulate HIV-specific immunoglobulin G antibodies that bound to a specific part of the HIV envelope (the variable loops 1 and 2 or V1V2 region of HIV envelope). This specific V1V2 part of the HIV envelope is biologically important since it is involved in attachment of HIV to target cells (Haynes et al. 2012). This suggests that the V1V2 region of the HIV envelope that was bound by these antibodies might be a crucially important target for future vaccines. Despite being unable to block cell entry, non-neutralizing antibodies, such as those induced during the RV 144 Thai trial, were effective at binding to HIV and clearing HIV-infected cells. Because no mucosal samples were collected from individuals vaccinated during the RV 144 Thai trial, it is unknown whether HIV-specific antibodies identified in blood were also present at mucosal surfaces and/or in genital secretions.

Harnessing T Cells to Protect Against HIV Infection

While T cells that are reactive against HIV-derived epitopes (small 8–12 amino acid long fragments of viral proteins) are thought to reduce disease severity (► [HIV & SIV, CD8+ T Cell Responses](#) to), their role in preventing new HIV infections is less clear. Such cells facilitate viral clearance either directly by killing virus-infected cells or indirectly by producing soluble cytokines that enhance the ability of virally infected cells to destroy intracellular virions. For nonpersistent viral infections (such as influenza), vaccine-elicited T cell responses are believed to provide protection even in the absence of appropriate

antibodies. Several vaccine vectors aimed specifically at eliciting HIV- or SIV-specific T cell responses have been developed and tested in pre-clinical and clinical trials. Preclinical studies have shown that these T cell-based vaccines are able to decrease SIV viremia postinfection. However, no solely T cell-based vaccine has been shown to prevent SIV or HIV infection in preclinical or clinical trials. In fact, the only vaccine that has recently shown to provide complete protection of nonhuman primates from SIV challenge was the one utilizing the live, replicating cytomegalovirus-based delivery vector, which elicited lifelong anti-SIV effector memory mucosal T cells in all animals receiving the vaccine (Hansen et al. 2009).

Advances in the understanding of T-cell memory have revealed additional T-cell subsets such as tissue-resident memory T cells that may be crucially important components of any fully protective vaccine-induced anti-HIV cytotoxic T lymphocyte response. It may be that the imperfect protection provided by first-generation anti-SIV cytotoxic T lymphocytes eliciting vaccines is attributable to their failure to induce sufficient numbers of memory T cells at mucosal sites of initial infection foci so that these can be controlled prior to the occurrence of systemic viral dissemination.

One of the most effective vaccine strategies yet tested in nonhuman primates has employed the use of live attenuated SIV variants (► [HIV-1 Prevention Using Live-Attenuated Vaccines](#)). These studies have provided valuable data on the relative benefits of vaccine-induced anti-HIV T-cell and antibody responses. Specifically, a recent study evaluated 11 different potential correlates of protection following vaccination with a live attenuated SIV variant and surprisingly found that only SIV-specific T-cell responses in lymph nodes were protective against vaginal SIV challenge (Hansen et al. 2013). Interestingly, as is the case with “wild-type” HIV variants, this vaccine strain preferentially infected T follicular helper cells within lymph nodes. Unfortunately further studies focusing on cytotoxic T lymphocyte responses at

mucosal surfaces are required to determine exactly how such live attenuated SIV vaccines are capable of providing sterilizing immunity against vaginal SIV challenges.

Despite the successes of certain anti-SIV cytotoxic T lymphocyte-inducing vaccines that have been tested in nonhuman primates, it is important to point out here that far fewer vaccine candidates have so far been tested in humans. Also, the results of clinical trials evaluating these vaccine candidates have been altogether both less compelling and less predictable than those tested in primates (McElrath et al. 2008). Finally, the successes of many promising anti-SIV vaccines, such as those based on attenuated virus variants, will likely never be translated into humans due to legitimate concerns that attenuated HIV variants could potentially revert to pathogenic variants that cause AIDS-like illnesses in vaccinated individuals.

Antiretroviral Therapy Can Prevent HIV Infection

The precedent for using antiretroviral drugs in HIV-negative individuals to prevent them from acquiring HIV comes from several very successful mother-to-child transmission trials in HIV + pregnant women (► [Pre-Exposure Prophylaxis \(PrEP\)](#)). Clinical trials have clearly demonstrated the plausibility of using antiretroviral drugs to protect women from HIV infection when taken either orally or in a topical microbicide formulation (Abdool Karim et al. 2010).

It is noteworthy, however, that certain other trials have failed to demonstrate that preemptive antiretroviral treatments provide HIV-negative women with any protective benefits. Although it is currently unclear why some trials have indicated protection and others not, it is likely that differences between the trials with respect to overall treatment adherence, routes of drug delivery, and antiretroviral concentrations achieved at mucosal surfaces could in part account for the differences in their outcomes. For example, overall protection in the 1% tenofovir microbicide trial

(CAPRISA 004) was 39% across all treated women, although a sub-analysis of women who were regular gel users and had >1,000 ng/ml tenofovir in their cervical secretions showed 79% protection (Abdool Karim et al. 2011a). The major conclusion of successful trials is that they are able to prevent HIV infections when antiretroviral drugs reliably reach the sites of HIV exposure.

Since these trials all showed only partial efficacy, it is clear that HIV transmission events can occur even in the face of what would normally be considered protective antiretroviral drug concentrations. It is hoped that a better understanding of the infections that occurred in HIV-negative women treated with antiretrovirals will both inform the design of better drug formulations and delivery mechanisms and illuminate the reasons why, even when present at high concentrations at the correct anatomical sites, antiretrovirals are only sometimes protective.

The Value of Knowing Your Epidemic

Information about the prevailing conditions within the genital milieu in which HIV transmissions are occurring is invaluable when deciding on what prevention modalities would work best.

Conclusion

HIV prevention remains a major public health priority that will likely only be effectively addressed by novel biomedical developments. Detailed analyses of the early stages of HIV and SIV infections have revealed that within healthy genital tracts, there exist various highly effective natural barriers to HIV transmission. Despite this, the pandemic persists and millions of new infections continue to occur annually. Thorough understanding of how the natural barriers to HIV infection result in per-contact transmission rates being lower than 1% and biomedical augmentation of these barriers so as to further reduce these

rates have started to show promise. Further work is, however, clearly needed to translate promising prevention strategies into practically viable treatments. Determining the best ways to combine and synergistically deploy multiple partially protective pharmaceuticals so as to decrease per-contact transmission rates by an order of magnitude or more will likely be a major research priority over the coming decade.

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Immune Activation and HIV Transmission

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Definition

Transmission of HIV is dependent upon target availability at the portal of entry. Activated T cells are the preferential targets for HIV. The interaction between host immune system and viral invader triggers a cascade of inflammation that fuels the infection. Factors influencing T cell recruitment and activation at mucosal sites affect HIV acquisition. Low basal levels of immune activation have been observed among HIV-exposed seronegative (HESN) individuals. These individuals show reduced susceptibility to HIV infection, and the study of their natural correlates of protection has highlighted the importance of immune activation in HIV transmission.

Immune Activation Drives HIV Replication

The immune system is conditioned to respond to “danger” signals from foreign pathogens and inflammatory stimuli. In response to the signals, an activation cascade is triggered and several factors from the innate and adaptive immune systems begin their journey. This cascade of signaling events governs CD4+ T-helper cell fate and function and plays an important role in tailoring the immune response. By targeting CD4+ T cells, the human immunodeficiency virus (HIV) has

evolved one of the most efficient strategies to hijack immune defense. Mainly transmitted through unprotected sexual contact, HIV infects local populations of CD4⁺ T cells at the site of mucosal entry, before becoming rapidly disseminated to the lymphoid organs. The rapid CD4⁺ T depletion in mucosal sites, hallmark of HIV infection, contributes to disorganizing the immune system and causes a progressive loss of immune functions. Given that HIV preferentially targets and replicates in activated CD4⁺ T cells (Card et al. 2013), immune activation fuels the infection rather than contributing to its elimination. Hence, immune activation not only drives progression to AIDS (Miedema et al. 2013) but also impacts individual susceptibility to acquire HIV.

The activation program of T cells is triggered upon antigen recognition by the T cell receptor (TCR). Mature antigen-presenting cells (APC) present antigens to naïve or recirculating antigen-experienced T cells and provide proper co-stimulatory signals. This is followed by a remodeling of the metabolic program resulting in proliferation and expression of activation markers, effector molecules, and factors involved in energy production. Once activated, T cells offer the ideal cellular environment for massive production of HIV virions. HIV integrates into transcriptionally active units of the cell chromosome and pirates several host factors, including transcription factor NF- κ B, to promote its own replication. Host inflammatory response also directly encourages the replication process by triggering signaling pathways involved in NF- κ B nuclear translocation and initiate HIV long terminal repeat (LTR) transcription (► [Cellular Cofactors for HIV Transcription](#)).

Activation status governs cell susceptibility to HIV infection. Expression of early and late activation markers on the cell surface can be monitored and reflects HIV capacity to establish a productive infection. In peripheral blood mononuclear cells (PBMC), expression of high levels of HLA-DR, CD25, CD69, or CCR5 by CD4⁺ T cells correlates with higher infection and replication rate *in vitro* (Card et al. 2013). HLA-DR and CD25 are late activation markers expressed by memory cells with antigenic activity, while

CD69 is an early activation marker expressed after TCR engagement. Thus, antigen-experienced cells are preferentially targeted by HIV. *In vivo*, effector memory T cells (T_{EM}) are the first cells depleted during the course infection. Sexual transmission of HIV is the result of a single virus using CCR5 to mediate its entry into the cell and initiate productive infection. Similar genetic bottleneck has also been observed during HIV transmission from the mother to the infants. Most T_{EM} cells express HIV co-receptor CCR5 and reside in mucosal sites under constant microbial pressure where they secrete immune effectors and offer the perfect phenotype for HIV infection and replication (selection of CCR5 using viruses; transmission; overview 385719).

Unlike T_{EM} cells, antigen-experienced central memory T cells (T_{CM}) reside in lymphoid organs and harbor a resting or quiescent phenotype. This nondividing subset is refractory to HIV production and is consequently latently infected (Zack et al. 2013). Low transcriptional and metabolic activity, low or absent expression of CCR5, and presence of host restriction factors render the cellular environment hostile to viral production and contribute to the formation of a viral reservoir (Zack et al. 2013). Like T_{CM}, naïve T cells express low levels of CCR5 and harbor low metabolic and transcriptional program consistent with latent infection. Hence, HIV primarily targets activated CD4⁺ T cells with antigenic activity for replicative purposes but also takes advantage of the resting pool to keep dormant copies of its genetic material ready to be replicated upon activation signals.

Role of Activation in Early Transmission Events

Initial Cascade of Activation

Cell activation not only plays a major role in disease progression but also greatly affects the early events of HIV acquisition. CD4⁺ T cells with prior history of activation are the fuel of HIV infection and their presence at the portal of entry is a prerequisite for the rapid expansion of the local founder population that promotes viral

dissemination to lymphoid organs (Haase 2010). Our understanding of the key early events involved in the establishment of infection has been greatly improved by the study of the simian immunodeficiency virus (SIV) in macaque models of sexual transmission. The virus first gains entry through the epithelial layers of the female genital tract (FGT) by crossing the thick and hostile mucus layer. Epithelial cells recognize the “danger” signals contained in the infected inoculum and secrete immune mediators. The release of macrophage inflammatory protein-3 (MIP-3a/CCL20) at the entry site calls for the first wave of respondents. Plasmacytoid DCs respond to this chemical invitation by migrating to the genital epithelium where they also recruit T cells. By increasing the number of available targets, this inflammatory chain reaction initiated by innate mediators provides the favorable conditions for the growth of a local founder population. Once the founder population has reached a critical mass, the infection is spread systemically and crosses the point of no return (Haase 2010). Reservoirs are established and the overwhelmed immune system cannot contain the massive viral replication that takes place in the secondary lymphoid tissues.

In humans, the study of the genital mucosa, male and female, also points toward a similar sequence of early events. In the FGT, HIV can penetrate both the squamous stratified epithelium of the vagina and ectocervix and the single-layered columnar epithelium of the endocervix and upper tract. Cervical and foreskin ex vivo explant models have shown that HIV induces the expression of T cell attractant molecules that can recruit activated target cells at the portal of entry (Ganor et al. 2010). Although dendritic cells (DCs) and Langerhans cells (LC) were reported to be the first subsets to encounter and capture HIV (Ferreira et al. 2014), activated CD4+ T cells appeared to be better candidates for viral replication and establishment of a founder population (Haase 2010). Mucosae of the FGT and inner foreskin are a milieu rich in HIV-target cells. Compared to blood populations, cervical and foreskin T cells manifest the predominant inflammatory T_{EM} phenotype. They express high levels

of CCR5, activation marker CD69, and cytokines interleukin (IL)-17 and IL-22 (Prodger et al. 2011; McKinnon et al. 2011) which correspond to the cell phenotype preferentially depleted during acute HIV infection (Miedema et al. 2013) (HIV-1 sexual transmission; overview 385727). This milieu enriched in target cells only requires the right igniter to set in motion the cascade of inflammatory events that promote HIV infection.

Targets Availability

Hence, the targets availability is a key concept for HIV transmission. Infection is highly dependent on the availability of targets at the time of exposure and several evidences support this concept. Local application of an inhibitor of T cell activation, the glycerol monolaurate, prevents acquisition of SIV by decreasing activation and recruitment of target cells in the vaginal mucosa of macaques. On the other hand, the use of nonoxinol-9 in the first microbicide trials showed the opposite effect. Nonoxinol-9 induces an inflammatory response in the FGT and increased transmission rate instead of offering the expected protection (Card et al. 2013) (► [HIV Transmission Blocking Microbicides](#)). In men, HIV incidence rate positively correlates with foreskin’s size and removing this target-rich tissue by circumcision reduced the risk of acquiring HIV by 50–60% (Ganor and Bomsel 2010).

Sexually transmitted infections (STI) have also been under the spotlight of HIV transmission studies because they result in the disruption of the protective mucosal lining and induce an inflammatory response in the genital mucosa. Several epidemiological observations have highlighted the role of STI in increasing the risk of HIV acquisition two- to fivefold, through both vaginal and anal intercourse. The synergy between HIV acquisition and STI operates with several bacterial and viral pathogens, causing both ulcerative and non-ulcerative genital lesions, including genital herpes, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, infectious syphilis, and human papillomavirus (HPV). Deciphering the temporal relationship between STI and HIV acquisition has been challenging because STI and HIV share the same risks

factors. However, biological mechanisms such as disruption of the mucosal barrier by genital ulcers, induction of local inflammation, and recruitment of highly inflammatory leucocytes support a role for STI in facilitating HIV infection by recruiting and activating target cells at the portal of entry (Ferreira et al. 2014). Thus, factors that influence presence and activation of target cells at entry site seem to be critical to ensure the full sequence of events leading to HIV infection.

Acquisition of HIV and Immune Activation: Lessons Learned from HESN Individuals

Heterogeneity in HIV Susceptibility

Not all individuals are equally susceptible to HIV infection. Epidemiological observations have demonstrated the existence of a spectrum of susceptibility with a handful of individuals, about 10–15% of those exposed, harboring a surprising capacity to escape HIV infection despite multiple exposures (Fowke et al. 1996). These HESN individuals comprise individuals exposed through sexual behaviors such as female sex workers (FSWs) from high HIV prevalence areas, men who have sex with men, and serodiscordant couples, as well as individuals exposed through the bloodstream like drug-injecting users, hemophiliacs, and care workers with an accidental needle-stick exposure. Uninfected infants born from HIV-infected mothers are also considered as HESN and are exposed through the placenta during pregnancy or through intestinal mucosa when swallowing infected secretions, blood, and milk (Kaul et al. 2011). Mechanisms of protection are multifactorial and vary according to the route of exposure; however, several interesting lines of evidence indicate a similar pattern of reduced immune activation as one of the underlying mechanisms for HESN protection.

Reduced susceptibility to HIV infection has been associated by reduced systemic levels of T cells activation (lower frequency of CCR5, HLA-DR, and CD38 CD4⁺ and CD8⁺ T cells) in sexually and parenterally exposed individuals (Kaul et al. 2011; Card et al. 2013). In addition to

reduced T cell activation, dampened expression of inflammatory mediators or upregulation of factors involved in the control of immune activation has been observed in the blood and mucosal compartments of HESN individuals. A lower amount of TNF- α and reduced proportion of Th17 target cells was observed in the foreskin of men from discordant couples (Prodger et al. 2013), and cytokine gradient in Beninese FSWs suggests a T cell migration pattern from the FGT to the blood instead of a T cell recruitment pattern toward the FGT (Lajoie et al. 2013). Increased expression of factors involved in the control of immune activation such as Vitamin D receptor and IL-10 transcripts was also observed in plasmatic and vaginal compartments of serodiscordant couples (Aguilar-Jiménez et al. 2013). A similar observation was made for mother-to-child transmission. Higher levels of anti-inflammatory IL-10 protected against transmission, while elevated concentration of proinflammatory cytokines, especially interferon- γ (IFN- γ)-induced protein 10 (IP-10) in the placenta, was associated with HIV transmission during pregnancy (► [MTCT HIV-1 Transmission Routes and Mechanisms](#)). In addition, individuals with a polymorphism in the IL-10 promoter that increased IL-10 expression were less likely to acquire HIV (Boettler et al. 2012). Thus, low inflammatory signal in systemic and mucosal compartments along with lower T cell activation is a recurrent observation among individuals that remain uninfected despite frequent exposure.

However, immune activation could also be deemed essential to mount an immune response, and some studies have observed an opposite tendency looking at T and NK cell activation and Toll-like receptor (TLR) engagement (► [Natural Killer and Their Role in Preventing HIV Transmission](#)). HIV-specific T cell and B cell responses have been correlated with HESN protection through sexual, parenteral, oral, and placental routes (Piacentini et al. 2008). Yet, it is still unknown to what extent this response is involved in the protection against HIV acquisition. HIV-specific response is sporadic and may merely reflect exposure to HIV rather than a protective mechanism. Furthermore, waning of the HIV-specific

response in absence of ongoing exposure has been reported (Piacentini et al. 2008).

In summary, several observations from HESN's cohorts with various routes of exposure suggest that a combination of low baseline levels of T cell activation and dampened immune environment may be beneficial to protect individuals against HIV acquisition. Discrepancies exist among the results and reflect the important difference in HESN models and ethnicity as geographic localization was shown to influence the baseline levels of immune activation (Kaul et al. 2011). Looking at individuals prior to their seroconversion has helped reconcile these findings.

Immune Activation Prior to Seroconversion

Immune activation is a key driver of HIV disease progression and organ damage (Miedema et al. 2013). The pre-seroconversion immune activation was found to predict post-seroconversion disease progression in a prospective study from Amsterdam. This study of 102 individuals with well-documented seroconversion dates showed that the presence of pre-seroconversion low CD4+ T cell numbers or elevated levels of CD4+ T cell activation predicted the development of AIDS after acquisition of infection (Hazenberg et al. 2003). The role of pre-seroconversion inflammatory milieu in setting the stage for HIV acquisition has been supported by indirect evidence, with several studies establishing the role of STIs in augmenting HIV transmission.

Results from preexposure prophylaxis (PrEP) studies using both oral and topical formulations of antiretroviral drugs generated great enthusiasm for the prospect of HIV prevention with efficacy ranging between 39% and 74%. The analysis of CAPRISA 004 showed that women who acquired HIV had significantly higher systemic innate immune activation prior to infection, when compared to those who remained uninfected. Those that acquired HIV had a combination of systemically elevated levels of proinflammatory cytokines, platelets, activated NK cells, as well as more spontaneous cytotoxic T cell degranulation

in the blood (Naranbhai et al. 2012). It remains to be determined whether the heightened immune activation has any association with migration of lymphocytes to mucosal sites, thus presenting more targets at the site of mucosal entry.

Immune Quiescence: Lessons from the Female Sex Workers from Nairobi

Studying HESN individuals and seroconverters has brought convincing evidence that immune activation plays a significant role in HIV transmission. Some of the strongest indications come from the Pumwani cohort of female sex workers (FSWs), from Nairobi, Kenya. The Pumwani cohort was established in 1984 and has enrolled more than 4,000 women. Defying the odds imposed by the high HIV pressure of Nairobi's slums, the FSWs who remained seronegative (5–10%) were more likely to exhibit a true phenotype of resistance (Fowke et al. 1996). In the early years, HIV pressure was so strong that being engaged in sex work for 3 years or more would already imply a natural resistance to HIV infection for the ones remaining negative (Fowke et al. 1996). Now, successful prevention programs have considerably decreased HIV incidence in Nairobi's slums, and 7 years of commercial sexual activity is necessary to reach a similar level of pressure. Women who do not meet these criteria of exposure duration are considered to be at high risk of acquiring HIV and are often used as HIV-susceptible controls. Emerging lines of evidence collected by dozens of studies from the well-described Pumwani cohort point toward a central role of immune activation in HIV transmission. Low levels of immune activation, identified as immune quiescence, have been observed among resistant women and may be instrumental to limit targets availability and prevent HIV infection and dissemination.

Systemic Immune Quiescence

Systemic immune quiescence in HESN FSWs from the Pumwani cohort was observed on multiple strata, from the molecular signature to the cell phenotype. Reduced *ex vivo* frequency of

CD69+, CD4+, and CD8+ T cell was first reported in HESN individuals when compared to their HIV-susceptible counterparts. Along with lower T cell activation, HESN FSWs harbored higher frequency of CD4+ regulatory T cells (Tregs) expressing the forkhead box P3 (FOXP3) transcription factor that are involved in the control of immune activation. Higher Treg proportion and lower frequency of activated T cells correlate with HIV-reduced capacity to establish infection *in vitro*. Further evidence was provided when immune functions were compared between HESN and HIV-susceptible FSWs. Baseline cytokine production (TNF- α , IL-6, IL-1b, IL-10) was significantly lower in HESN individuals. Similar to what was observed among discordant couples from Abidjan, cell capacity to respond to mitogen stimulation was not affected in HESN individuals, suggesting that HESN individuals' immune system is not impaired in its capacity to respond to external threats (Card et al. 2013).

Further studies looking at the molecular signature by microarray analysis of peripheral immune cells confirmed the metabolic state of immune quiescence in HESN individuals. Several pathways involved in metabolism and immune functions are downmodulated in HESN individuals. Genes involved in TCR engagement, proteasomes, and glycolysis pathways are highly underexpressed in PBMC and CD4+ T cells from HESN individuals, while the mitochondrial oxidative phosphorylation pathway (OXPHOS) is the only pathway overexpressed (Card et al. 2013). Quiescent cells maintain low rates of glycolysis and predominantly oxidize glucose-derived pyruvate via OXPHOS pathway (Pearce et al. 2013). Low metabolic and transcriptional activity compromised the expression of factors essential for viral replication and render quiescent cells refractory to viral replication (Zack et al. 2013). The genetic expression profile of the blood immune cells indeed confirmed this quiescent phenotype observed in HESN individuals that may prevent productive infection of target cells.

Immune Quiescence in the FGT

The immune environment of the FGT also shows signs of immune quiescence among those FSWs

who remain uninfected. Significantly lower expression of NK and T cell recruitment factors IL-1 α , RANTES, monokine induced by IFN- γ (MIG), and IP-10 are observed in the FGT of HESN individuals along with lower frequency of HLA-DR expressing CD8+ T cells. Cytokine secretion capacities are also reduced in HESN individuals, and lower baseline levels of TNF- α and IL-10 as well as blunted production of IL-17, IL-22, IL-6, and IFN- γ transcripts upon superantigen stimulation are detected in cervical cells from HESN individuals. IL-17- and IL-22-producing cells are found in the FGT and are the preferential targets for HIV infection (Miedema et al. 2013; Card et al. 2013). Their reduced frequency corroborates a limited number of target cells in the FGT of HESN FSWs from the Pumwani cohort.

Immune quiescence also extends to innate functions in the FGT. Expression of various pattern recognition receptors (PPR) was measured in cervical mononuclear cells from HESN and HIV-susceptible FSWs. PPR include numerous receptors from different families (TLR, retinoic acid-inducible gene-1 (RIG-I)-like receptors (RLRs), C-type lectin receptors, Nod-like receptors, and DNA-dependent activator of interferon (IFN)-regulatory factors) and sense microbial products from the environment to activate the inflammatory response. Expression of TLR2, TLR7, TLR8, RIG-I, and MDA-5 was significantly reduced in cervical cells from HESN individuals. In addition, downmodulation of UNC93B in HESN individuals may blunt TLR trafficking within the cell. Nevertheless, HESN individuals' capability to respond to TLR stimulation is not impaired, and vigorous response to TLR7/TLR78 agonist stimulation is observed (Card et al. 2013).

In summary, baseline mucosal and systemic immune quiescence are observed among women that are protected against HIV acquisition despite years of sexual exposure. Accumulated evidence from the Pumwani cohort supports this phenotype on systemic and mucosal levels. Despite a lower baseline level of immune activation, HESN individuals possess potent immune capacity and are not immune compromised. A balance between

quiescence that prevents recruitment of target cells and immune responsiveness may be crucial for protection. But what exactly drives this phenotype in HESN individuals? Multiple genetic and environmental factors have been suggested.

To Be or Not to Be Activated: Factors Governing Immune Quiescence

Genetic Polymorphism in Interferon Signaling Pathway

Genetic polymorphism in interferon regulatory factor-1 (IRF1) correlates with reduced susceptibility to HIV infection in HESN individuals from the Pumwani cohort. IRF1 is a transcriptional activator and repressor involved in multiple biological processes, including regulation of innate and adaptive immune response. Three protective polymorphisms in the IRF1 gene have been identified and correlate with a ~60% reduction of basal and IFN-induced production of IRF1 protein and transient responsiveness to IFN- γ stimulation. Protective IRF1 genotypes also correlate with reduced ability to transactivate HIV LTR, suggesting reduced capacity to promote viral transcription. Transient kinetics and rapid silencing of the IRF1 response in PBMC have also been observed among HESN individuals who did not harbor protective IRF1 variants suggesting that more factors, still to be identified, are present in HESN individuals that may govern the kinetics of interferon response (Card et al. 2013). The down-modulation of IFN- γ responsive factors MIG and IP-10 in the cervix suggests that dampened interferon responsiveness can also be observed in the FGT (Card et al. 2013). Genetic polymorphism in these chemokines, however, was not shown to predict protection in a study combining data collected from 25 cohorts including over 6,000 patients and controls, suggesting that these genetic effects may be rare, of small magnitude, or specific for the different cohorts (McLaren et al. 2013).

Overexpression of Antiproteases

Overexpression of antiproteases with anti-inflammatory properties at the FGT could also

drive the low basal expression of inflammatory markers observed in the FGT of HESN individuals. Members of the serpin A family, cystatin B, Elafin, and A2ML1 were found to be overexpressed by proteomics approach in HESN individuals' cervical lavage, and Elafin has been identified as a correlate of protection in HESN individuals. Expression of antiproteases is usually induced by inflammatory cytokines and chemokines, and rapid expression of antiproteases in response to cytokine secretion may alleviate the inflammatory response in HESN individuals. Indeed, correlation between cytokines and antiproteases expression was strictly observed in HESN individuals, however, not in susceptible or positive FSWs. By preventing inflammation of the FGT, antiproteases probably also contribute to maintaining the integrity of the mucosal barrier, protecting against inflammation and disruption of the tight junctions (Kaul et al. 2011; Card et al. 2013).

Hormonal and Sexual Activity

Immune activation of the FGT can also be influenced by multiple factors. Hormonal cycle, microbiological environment, and sex work all have a recognized influence on the fluctuation of immune activation at the FGT. Human epidemiological studies showed that women using injectable contraception had doubling of the risk of acquiring HIV from their infected partners compared to those who used nonhormonal (Ferreira et al. 2014). The progesterone phase of the menstrual cycle or progesterone-based contraceptives have been suggested to create a window of opportunity for HIV infection by influencing cell susceptibility. Ex vivo HIV infection of biopsy explants has shown that tissues are more susceptible to productive infection when collected in the luteal (high progesterone) phase of the menstrual cycle and higher susceptibility to HIV infection correlated with progesterone levels (Ferreira et al. 2014).

Sexual activity also influences the level of T cell activation and the distribution and phenotype of the immune populations in the FGT. Contact between seminal fluid and cells at the FGT triggers a cascade of events involved in the

clearance of seminal debris, selection of fertilizing sperm, and induction and maintenance of immunological tolerance toward paternal antigens. Microbial and cellular products contained in the semen can trigger an antigenic response against bacterial, viral, or alloantigens. Sexual intercourse induced the secretion of proinflammatory cytokines that correlates with the recruitment of activated neutrophils, macrophages, DCs, and lymphocytes in the epithelial and stromal layers of the cervix. Continuous exposure to semen of several clients through sex work has been associated with alteration of the chemokines and cytokine profiles in the blood and the FGT of FSWs. T cell and monocyte attractant chemokine (MIP-3a; ITAC; MCP-1; IL-1, IL-6, and IL-8; TNF- α ; IP-10; MCP-1; MIP-1a and MIP-1b) were underexpressed in the FGT of FSWs when compared to low-risk non-sex worker populations. These alterations appear as early as 1 year after initiation of sex work and were more pronounced with accumulating years of sex work (Lajoie et al. 2013). Moreover, late seroconversion among HESN individuals from the Pumwani cohort has been correlated with reduction of commercial sexual activity. The high immune pressure maintained on the genital immune system may contribute to the induction of a tolerogenic mechanism that heightens the T cell activation threshold at the FGT.

A similar tolerogenic effect of sex work was observed among FSWs from Ivory Coast. Upon allo-stimulation, CD4⁺ and CD8⁺ T cells from FSWs expressed less CD69 on memory subsets compared to controls. Intrinsic capacity to respond to stimulation was not impaired among these HESN FSWs as stimulation with mitogen resulted in increased expression of CD69. In support of the effect of allo exposure, condom use was shown to impact the observed levels of systemic immune activation. Higher degree of condom use inversely correlated with expression of CD38 on CD4⁺ T cells of serodiscordant couples (Card et al. 2013; Lajoie et al. 2013). Induction of an active tolerogenic response by constant exposure to sexual antigens may contribute to keep on a leash immune activation at the FGT.

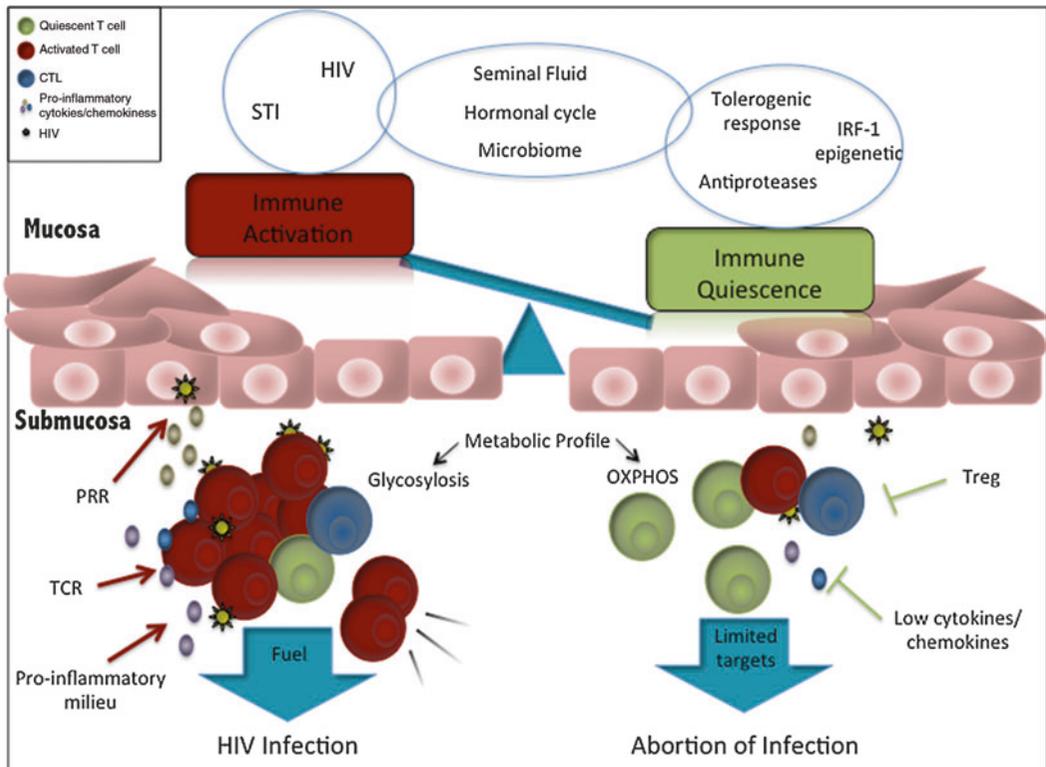
Regulatory T Cells

Tregs are well renowned for their implication in peripheral tolerance to self-antigen but also in induced tolerance to microbiome. Treg's role in HIV infection remains controversial. On one side, they inhibit HIV-specific T cell response and fuel the infection being targeted by HIV. On the other hand, Treg suppresses immune activation by direct contact with activated cells or through anti-inflammatory cytokine production. Tregs can also negatively impact HIV replication through cyclic adenosine monophosphate (cAMP) activity or by repressing HIV LTR transcription through Treg transcription factor FOXP3 (Card et al. 2013). Treg's role in HIV transmission has not been fully elucidated yet, but higher proportions of Treg that correlate with lower systemic T cell activation were observed in HESN individuals from the Pumwani cohort and in exposed-uninfected neonates (Card et al. 2013). Several gaps in knowledge remain in relation to the protective nature of Treg in HESN individuals. The nature of their antigenic specificity and their functional capacity has yet to be explored.

Vaccinate to Limit Immune Activation and Prevent HIV Transmission: Proof of Concept in the Monkey Model

Inducing immune quiescence at the portal of entry without impairing the cell capacity to mount an immune response seems to be an attractive strategy to prevent HIV acquisition among HESN individuals. The lesson learned from this group of individuals is now translated into preventive interventions. Induction of quiescence by tolerogenic vaccine has shown to be protective in monkey models.

In a first study, macaques were vaccinated with a live-attenuated simian/human immunodeficiency virus (SHIV) lentiviral vector producing continual low doses of virions and diminishing immune activation. Following an SIV challenge, FOXP3⁺ Treg populations expanded in the vaginal tract of immunized monkeys and this expansion correlated with lower immune activation at the genital tract. It was suggested that the quick resolution of the innate response collectively with



Immune Activation and HIV Transmission, Fig. 1 Immune activation and immune quiescence in HIV acquisition. HIV preferentially establishes infection in activated T cells. In the genital mucosa, factors triggering an inflammatory response such as sexually transmitted infections (STI) or HIV fuel the infection by activating and recruiting HIV targets cells at the portal of entry. Conversely, factors, genetic or environmental, favoring a quiescent phenotype at the genital mucosa may limit cell activation. Quiescent CD4⁺ T cells are susceptible to HIV infection but their low metabolic and transcriptional activity result in low level of viral replication. The absence of

inflammatory signals prevents the recruitment of targets cells. Low levels of productive infection in a quiescent environment may allow a sufficient opportunity to HIV-specific cytotoxic T cells (CTL) to clear the few infected cells leading to the abortion of infection. Some factors can favor the balance in both ways, according to inter-individual response to antigens (tolerogenic vs. inflammatory response) or cyclic expression of sexual hormones. PRR, pattern recognition receptor; TCR, T cell receptor; CTL, cytotoxic T cell; IRF-1, interferon regulatory factor-1; OXPHOS, oxidative phosphorylation pathway; Treg, regulatory T cells

the modest antiviral effector CD8⁺ T cell response elicited by SHIV immunization could eliminate the few HIV-infected cells at mucosal sites (Lajoie et al. 2013).

Similarly, protection against intrarectal SIV challenge was also achieved in macaques after oral immunization with a combination of heat-inactivated SIV and a digestive tract commensal bacterium that is known to induce immune tolerance (*Lactobacillus plantarum*). Induction of SIV-specific, histocompatibility complex (MHC)-Ib/E restricted CD8⁺ regulatory T cells potent at suppressing CD4⁺ T cell activation and viral

replication in SIV infected CD4⁺ T cells resulted in the protection of the immunized monkeys (Lajoie et al. 2013).

Thus, induction of a potent immunoregulatory mechanism through constant exposure to viral antigen or vaccination with a tolerogenic agent can protect against SIV infection by limiting T cell activation. Interestingly, even a modest CTL response, insufficient to control viral replication in other immunization models, in the context of controlled mucosal immune activation, was able to suppress viral replication. The best opportunity for the immune system to stop HIV is

to take advantage of its vulnerability, before the founder population is established. It was suggested that sufficient amounts of HIV-specific CTL that are able to kill infected cells when the first burst of local replication is not augmented by immune activation would lead to an aborted infection (Haase 2010). These studies support the observations made in HESN individuals and highlight the importance of controlling local immune activation without impairing immune response to prevent HIV transmission.

Conclusion

All together, the sequence of events involved in HIV acquisition is dictated by a fine equilibrium between immune activation and potent mechanisms of immune control (Fig. 1). Low levels of basal immune activation may prevent establishment of a founder population disseminating systemically, but sufficiently activated cells would ensure cells capacity to respond to the threat with a rapid control of inflammation. This judicious combination would warrant a sufficient number of HIV-specific CTL at the site of entry to prevent a burst in the initial replication. Uncontrolled immune activation would fuel the cascade of transmission events resulting in HIV acquisition, while maintaining a tight balance between basal quiescence and transient responsiveness with a quick return to basal state would favor a microenvironment refractory to HIV replication and prevent acquisition. Efforts should now be directed into translating what we know about immune quiescence and control of immune activation for developing new prevention strategies. The first attempts to develop tolerogenic vaccine in monkeys have so far been promising. Now it is time to see how we can translate that to humans.

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Immunogenetics of HIV-2 Infection

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Definition

Immunogenetics is the study of pathogen and host genetic factors that affect immunological responses leading to abnormal health conditions. The term also encompasses studies of the mechanisms of transmission of those factors from generation to generation in both pathogen and host. Over the past two decades, immunogenetics of infectious diseases has rapidly developed into a diverse field exploiting a range of methodologies to discover new genes and antigens that affect host immune responses. However, despite significant progress in the field and the use of new genetic tools (e.g., next generation sequencing) and study designs (e.g., genome-wide analyses) to tackle the ever-increasing burden of infectious diseases such as the Acquired Immunodeficiency Syndrome (AIDS) caused by the Human Immunodeficiency Virus (HIV), several important questions remain unanswered. For example, what are the immunogenetic variations in humans and in the virus that affect the differential susceptibility to infection? Which host genetic factors are involved in the heterogeneity of disease progression observed in humans? The latter is particularly important in HIV-2 infection because, while many remain healthy for decades, some people fail to control

viral replication and rapidly develop an immunodeficiency state similar to that seen in HIV-1. In this section we will consider the host genetic variations that influence susceptibility to HIV-2 and rate of progression of disease.

HIV-2

The second human immunodeficiency syndrome virus (HIV type 2) is structurally and genetically similar to HIV-1 with up to 60% homology in some genes (Guyader et al. 1987), is spread through same route as HIV-1, and can cause significant mortality and morbidity (Gilbert et al. 2003). However, HIV-2 is restricted to certain geographical areas (mainly West Africa and few European and Asian countries) for reasons that are not fully understood. One possible explanation could be the existence of host gene variants that favor or inhibit the spread of HIV-2 in affected individuals. The identification of new gene variants that modulate immune responses to HIV-2 could lead to identification of molecules or pathways that would serve as target for immunomodulatory or pharmacological interventions to curb the spread of the more virulent HIV-1 virus.

Genes That Affect HIV Infection and Disease Progression

A number of viral and host immune regulatory genes have been implicated in the control of HIV at different stages of infection and progression to AIDS (Table 1). These genes have been extensively examined in HIV-1 field but only a few studies have been performed in populations affected by HIV-2 (Diouf et al. 2002; Yindom et al. 2010).

HLA Studies

The human leukocyte antigens form a set of highly polymorphic genes located on chromosome 6 (6p21.31). HLA genes encode cell surface molecules which present short peptides derived

Immunogenetics of HIV-2 Infection, Table 1 Key genes affecting innate and acquired immune responses to HIV in humans

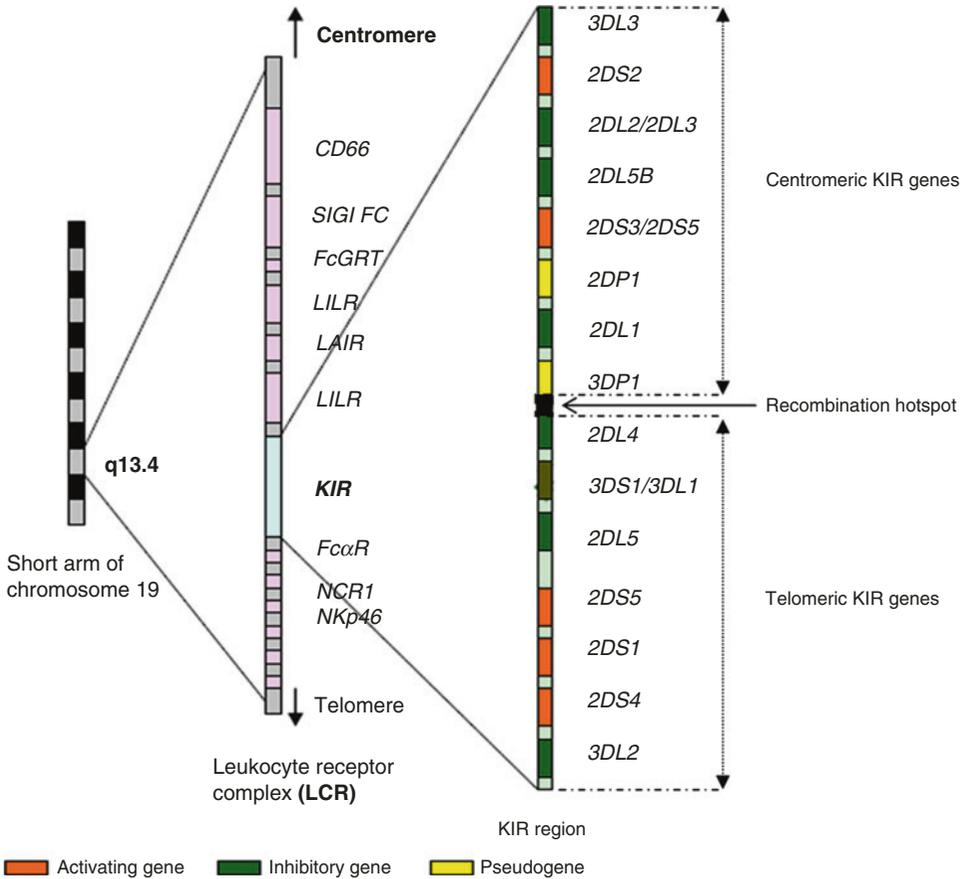
Gene	Allele	Mode	Effect		Mechanism of action
			HIV-1	HIV-2	
<i>HLA</i>	<i>A, B, C</i>	Homozygous	Accelerate AIDS	Not known	Decrease epitope recognition
	<i>B*27</i>	Co-dominant	Delay AIDS	Not known	Delay viral escape
	<i>B*57</i>	Co-dominant	Delay AIDS	Not known	Delay viral escape
	<i>B*35-PX</i>	Co-dominant	Accelerate AIDS	Not known	Not clear
	<i>B*1503</i>	Co-dominant	Not known	Accelerate progression to AIDS	Not clear
<i>KIR</i>	<i>3DS1</i>	Epistatic with <i>HLA-Bw4</i>	Delay AIDS	Not known	Not clear
	<i>2DL2/2DS2</i>	Co-dominant	Not known	Protect against infection	Not clear

from intracellular proteins to T cells for recognition. This antigen presentation results in initiation of an adaptive type of immune response. Several studies have demonstrated the importance of certain HLA molecules in immune responses to viral infections (Carrington and O’Brien 2003; Trachtenberg et al. 2003). Immunological evidence from HIV-2 studies in West Africa suggests that specific immune responses to HIV-2 can occur in vivo (Rowland-Jones et al. 1995; Leligidowicz et al. 2007), but only two studies have investigated the role of *HLA* in HIV-2 infection (Diouf et al. 2002; Yindom et al. 2010). In one of these studies, HLA typing was performed using low-resolution genotyping techniques, and the authors reported that *HLA-B*35* was significantly associated with lack of p26 antibodies and higher risk of disease progression in 62 female sex workers. In the same study, *HLA-B*53* showed some beneficial effect in slowing progression to AIDS. The second study was carried out in a different population in another West African country using much more sensitive molecular techniques in a larger sample (n = 513 individuals). The authors found that a different set of *HLA* alleles were associated with susceptibility to HIV-2 infection and to disease progression. They reported a positive association between *HLA-B*0801* and HIV-2 acquisition. In the same study, analysis of 4–6 digits *HLA* alleles together with more than 20 years longitudinal data on CD4⁺ T cell counts and viral loads collected

from 136 chronic HIV-2 patients belonging to the same ethnic group revealed that specific *HLA* class I alleles could predict HIV-2 disease outcome. Individuals with *HLA-B*1503* allele had an overall lower CD4⁺ T cell counts and higher HIV-2 viral loads compared to people infected with HIV-2 over the same length of time but lacking this allele. The reason(s) behind this poor prognosis in people carrying *HLA-B*1503* is not known, and future studies are needed to unravel the functional mechanism behind this association.

KIR Studies

The killer cell immunoglobulin-like receptor (KIR) cluster is a family of 16 genes located within the leukocyte receptor complex (LRC) on human chromosome 19q13.4 (Suto et al. 1996) (Fig. 1). These genes encode receptors (type I glycoproteins) that specifically recognize HLA class I on the surfaces of other cells (targets). KIR molecules are expressed mainly by natural killer (NK) cells and some memory T lymphocytes (γδT cells). The function of these NK cell receptors is to help in the identification of abnormal body cells (also known as “non-self”) that could have been infected with intracellular pathogens or are in the process of undergoing transformation (tumorigenesis) (Naumova et al. 2007). KIR molecules do this by interacting with their ligands



Immunogenetics of HIV-2 Infection, Fig. 1 The genomic organization of KIR locus on human chromosome 19. KIR genes are tightly organized in a head-to-tail fashion within the leukocyte receptor complex (LCR) and occupy approximately 150 kb of genomic space. Inhibitory KIR genes are shown in *green boxes*; activating genes are

shown as *orange boxes*; pseudogenes are shown as *yellow boxes*; the recombination hotspot is shown as a *black box*; KIR genes vary in sizes and range between 10 and 16 kb and are mostly separated by a 2 kb intergenic space except for the 14 kb recombination zone upstream of KIR2DL4 and separating the telomeric KIR genes from centromeric KIR genes

(HLA class I molecules) and transducing signals to NK cells to either trigger inhibition of cytotoxicity or promote NK cell activation leading to the destruction of the target. The interactions between KIR and HLA class I molecules contribute to the modulation of immune responses against viruses, autoimmunity, and reproduction (Carrington and Martin 2006).

Like the *HLA* system, the *KIR* family shows extensive variation in gene content and allelic polymorphism, thereby distinguishing between individuals and populations (Yawata et al. 2002). KIR molecules are structurally similar to each other and have been classified based on the number of

extracellular domains (D0, D1, and D2) and the length of their cytoplasmic tail (long [2DL or 3DL] or short [2DS or 3DS]). Those with a long tail are “inhibitory” meaning that under normal circumstances, they prohibit NK cells from killing the target cells carrying the intact ligand, while those with a short tail are “activatory,” i.e., they instruct NK cells to lyse the target particularly when the intact ligand(s) for the inhibitory KIRs has(ve) not been found on the target cell. Lysis of any target therefore is tightly regulated by a fine balance between both the inhibitory and activatory signals.

On the basis of gene content, all *KIR* haplotypes have been classified in two groups, namely,

A and B. Group A haplotypes have a fixed gene content (*KIR3LD3*, *2DL3*, *2DP1*, *2DL1*, *2DL4*, *3DL1*, *2DS4*, and *3DL2*) with the only activating gene being *KIR2SD4*. In addition to having only 7–8 genes on the haplotype, group A genes are highly diversified at the allelic level. In contrast, group B haplotypes have variable gene content comprising both inhibitory and activating genes (4–16 genes) but with moderate or low allelic polymorphisms. Adding to the complexity is the fact that both A and B haplotypes can be divided into centromeric and telomeric parts based on the number of genes present upstream and downstream of the recombination hotspot located between two framework genes *KIR3DP1* and *2DL4* (Yawata et al. 2002).

Several epidemiological studies mostly from Western populations have demonstrated the importance of KIRs and NK cells in innate immunity of infectious diseases including HIV. These studies have implicated certain *KIR/HLA* compound genotypes with HIV outcomes. Only one of those studies was designed to look at the role of *KIR* genes on HIV-2 infection and disease progression in a community cohort of HIV-2 infected individuals in West Africa (Yindom et al. 2010). The study showed that *KIR* genes do not individually affect HIV-2 outcomes. Their effect was only noticeable in the presence of their ligands (HLA class I). The study reported that having *KIR2DS2/KIR2LD2* and at least one copy of HLA-C group 1 (C1) was beneficial or protective against HIV-2 infection. The authors found that in a community cohort of more than 500 participants from the same ethnic group (Manjaco in north western coast of Guinea Bissau), individuals with the compound genotypes *2DS2:HLA-C1/x* or *2DL2:HLA-C1/x* (where x is either C1 or C2) were more likely to be free of HIV-2. However, this finding needs to be confirmed in future studies in other HIV-2 cohorts.

Conclusion

Despite the interesting research opportunity offered to the scientific community by the nature of HIV-2 infection model which could offer

important insights into HIV-1 pathogenesis, only a few immunogenetic studies have been carried out thus far. There is a paucity of information on non-HLA-related genes in the field of HIV-2 immunogenetics. Several other genes are likely to be important including those affecting cell surface proteins involved in viral attachment and entry to cells and antigen processing; however, these genes have not yet been investigated. Funding opportunities for HIV-2 research are limited, but efforts to design and carry out good hypothesis-based immunogenetic studies in HIV-2 should be encouraged since this offers a natural model in humans where a potentially highly pathogenic immunodeficiency virus is normally well controlled by the host immune response.

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Immunological Responses to Antiretroviral Therapy

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Definition

Maintenance or restoration of immune function is a major goal of treating HIV infection with antiretroviral therapy (ART). Patients who achieve a normal CD4⁺ T cell count of >500/ μ L are unlikely to experience opportunistic infections and can expect a near-normal life expectancy, whereas patients whose CD4⁺ T cell count does not increase above 200/ μ L have substantially increased long-term mortality. Up to 36% of patients receiving ART never achieve a normal CD4⁺ T cell count and immune dysfunction may persist in those that do. Furthermore, immune reconstitution may, paradoxically, result in patients being more susceptible to some immunological disorders. Here, mechanisms of immune reconstitution and the causes, consequences, and management of persistent immune dysfunction and immune reconstitution disorders will be discussed.

Reconstitution of the Immune System Following Suppression of HIV Replication by ART

Depletion of blood CD4⁺ T cells is the most characteristic immune defect caused by HIV infection, and the CD4⁺ T cell count has been

used for many years to assess the severity of HIV disease and monitor immune reconstitution after commencement of ART. However, HIV infection affects many components of the immune system, and a greater understanding of the multiplicity of HIV-induced immune defects, and how these change on ART, provides a more comprehensive picture of immune reconstitution.

Bone Marrow, Thymus, and Lymphoid Tissue

Cells of the immune system are generated in the bone marrow and thymus, whereas immune responses against pathogens are generated in lymphoid tissue present in the spleen; lymph nodes and mucosal immune system, which includes pharyngeal lymphoid tissue (tonsils and adenoids); gut-associated lymphoid tissue (GALT); and bronchial-associated lymphoid tissue (BALT). Lymph nodes and GALT are prominent sites of HIV replication and both the bone marrow and thymus are also adversely affected by HIV infection. Replication of HIV in lymph nodes and GALT causes inflammation and fibrosis, which results in a persistent proinflammatory milieu and abnormal lymphoid tissue architecture that adversely affect mechanisms that normally maintain T cell numbers, T and B cell interactions and the maturation of B cell responses and antibody production (Estes 2013). Proinflammatory cytokines, such as IL-1 β , and interferon-alpha appear to play a prominent role in this process. These abnormalities improve when HIV replication is suppressed by ART but often do not resolve completely (Cha et al. 2014; Lederman et al. 2013). This may reflect persistence of low-level HIV replication consequent upon low penetrance of antiretroviral drugs into lymphoid tissues.

T Cell Numbers and Function

During early HIV infection, massive depletion of effector memory CD4⁺ T cells (expressing the CD4 and CCR5 molecules) occurs in the blood and lymphoid tissue, particularly the GALT, caused directly by HIV replication. Immune activation also contributes to CD4⁺ T cell depletion and becomes the dominant cause during chronic HIV infection. Plasma biomarkers of immune activation associated with CD4⁺ T cell depletion

include the proinflammatory cytokine TNF- α and the chemokine CXCL10 (also known as interferon-inducible protein 10 [IP-10]). Increased production of these molecules probably reflects immune activation and inflammation in response to HIV replication in lymphoid tissues (see “► [Chronic Immune Activation in HIV](#)”). Of the several subpopulations of CD4+ T cells that mediate immune responses against different types of pathogen, the type 1 helper T cell (Th1) subpopulation (characterized by IFN-gamma production) and the Th17 subpopulation (characterized by IL-17 production) are particularly affected by HIV infection (see “► [Th17 Cells](#)”). Depletion and dysfunction of Th1 CD4+ T cells is the major cause of increased susceptibility to systemic infections by opportunistic pathogens, whereas depletion and dysfunction of Th17 CD4+ T cells contributes to impaired immune responses against mucosal infections, including *Candida* sp. infection, and is a cause of increased translocation of microbial products from the gut, which also contributes to immune activation (Brenchley et al. 2006) (see “► [Microbial Translocation](#)”).

Depletion of CD4+ T cells from the blood and lymphoid tissues in HIV infection results from decreased production of naïve (antigen inexperienced) T cells in the thymus, decreased homeostatic proliferation of naïve and memory T cells in lymphoid tissue, and increased apoptosis and turnover of CD4+ T cells (see “► [Thymic Function](#)” and “► [T-Cell Homeostasis](#)”). After ART is commenced, numbers of CD4+ T cells in the blood and lymphoid tissues increase as a result of decreased immune activation, increased production of naïve T cells in the thymus (thymopoiesis), and improved homeostatic proliferation of naïve and memory CD4+ T cells, but all occur to a variable degree. Increased thymopoiesis reflects thymus regeneration during the first year of ART (Franco et al. 2002), which may persist on long-term ART, but is very variable and least likely to occur in older patients. Homeostatic proliferation of naïve and memory CD4+ T cells, regulated by interleukin (IL)-7, is impaired by ongoing immune activation, which is associated with decreased IL-7 receptor (R)

expression and abnormalities of the intracellular IL-7R signaling pathway. Proinflammatory cytokines, including IL-1 β , and IFN- α contribute to the defects of IL-7R signaling in patients receiving ART.

Up to 36% of HIV patients receiving effective ART exhibit persistent CD4+ T cell deficiency (CD4+ T cell count <500/ μ L) after 5 years of ART. Several factors are associated with poor recovery of CD4+ T cells on ART including coinfections with hepatitis B virus, hepatitis C virus, and cytomegalovirus (CMV), which probably contribute to immune activation; an age of greater than 50, which probably limits thymus regeneration; and a low nadir (lowest ever) CD4+ T cell count. About 40% of patients who commence ART with a CD4+ T cell count of <200/ μ L do not achieve a normal CD4+ T cell count, even after 10 years of ART (Kelley et al. 2009). The association of a low nadir CD4+ T cell count with poor CD4+ T cell recovery probably reflects irreversible damage to lymphoid tissues associated with advanced HIV infection.

Persistent CD4+ T cell deficiency is associated with an increased risk of infections, poor responses to vaccinations, and an increased risk of atherosclerotic vascular disease, cancer, and osteoporosis and fractures. While some of these conditions may be a direct result of the CD4+ T cell deficiency, it seems likely that others are a consequence of associated immune defects such as memory B cell deficiency or monocyte activation (see below).

B Cell Numbers and Function

B cells recognize microbial antigens via surface immunoglobulin (the B cell antigen receptor) and then present antigens to T cells via class II HLA molecules. Activated T cells express costimulatory molecules and secrete cytokines that induce B cells to differentiate into plasma cells and produce antibodies, or into memory B cells, which are crucial for recognizing antigens during subsequent infections with that microbe. Memory B cells are also generated after vaccination. Memory B cells and plasma cells are mainly produced in the germinal centers of lymphoid follicles in lymphoid tissue, including tonsils, lymph nodes,

and the GALT. This process is regulated by follicular helper T cells (T_{fh} cells), which are a subpopulation of CD4⁺ T cells that produce cytokines and expresses co-stimulatory molecules required for generating plasma cells and memory B cells (see “► [T Follicular Helper Cells in HIV Infection](#)”).

HIV infection induces B cell activation and the accumulation of “exhausted” subpopulations of B cells while reducing numbers of resting memory B cells (Moir et al. 2009). In addition, HIV infection of T_{fh} cells causes an increased number of dysfunctional T_{fh} cells. These abnormalities may reflect the adverse effects of lymphoid tissue inflammation and fibrosis on germinal center responses. The composite effect of these immune defects is increased production of immunoglobulins, particularly IgG, but a decreased magnitude and isotype diversity of IgG antibody responses. The latter defect manifests as serum IgG2 deficiency, which occurs in over 60% of HIV patients, and probably contributes to low opsonophagocytic antibody responses that increase the susceptibility of HIV patients to infections by encapsulated bacteria.

ART improves many aspects of B cell function, especially when commenced early in the course of the infection, but defects often persist, particularly in patients who were very immunodeficient before ART was commenced. Thus, numbers of circulating memory B cells may be persistently low, and antibody responses after vaccination with pneumococcal polysaccharides and influenza virus envelope antigens are subnormal. Evidence that persistent defects of opsonophagocytic antibody responses in HIV patients receiving ART are clinically significant is provided by the persistently higher rates of community-acquired pneumonia compared with the general population.

Vaccines recommended for patients with HIV infection induce neutralizing antibody responses against viruses (e.g., influenza virus, hepatitis B virus, human papillomavirus) or an opsonophagocytic antibody response against encapsulated bacteria (e.g., *Streptococcus pneumoniae*). Their efficacy can be optimized by vaccinating patients when they are receiving ART and by the use of modified dosing schedules, as exemplified by HBV vaccine.

NK Cell Numbers and Function

NK cells are lymphocytes with the specialized function of lysing virus-infected cells or cancer cells (cytotoxicity), though they are also capable of producing cytokines, such as IFN-gamma, that activate other cells. Activation of NK cells occurs by two mechanisms: firstly, as part of an innate immune response through the recognition of virus or tumor cell components on the surface of cells by an array of activatory or inhibitory molecules and secondly, as accessory cells in an antibody response by binding of antibodies to CD16 (FcγRIII) on the surface of NK cells. Interferon-alpha-dependant activation of NK cells contributes to natural control of HIV infection. Activation of NK cells by antibodies may also contribute to the control of HIV replication, as demonstrated by the findings of the RV144 HIV vaccine study.

NK cell numbers and function are decreased by HIV, and while these defects improve on ART, they may not return to normal (see “► [NKT Cells: Bridging Innate and Adaptive Immunity](#)”). Furthermore, activation of NK cells may persist in patients receiving ART. Persistent defects of NK cell responses might impair cancer immunosurveillance mechanisms and contribute to the increased risk of non-AIDS cancers in HIV patients receiving ART (Yanik et al. 2013).

Monocytes and Macrophages

Circulating monocytes and tissue macrophages primarily contribute to antimicrobial immune responses by phagocytosis and killing of microbes. This may occur as part of an innate immune response via various cell-surface and intracellular pathogen recognition molecules, or as accessory cells in adaptive immune responses resulting from activation by IFN-gamma produced by T cells via IFN-gamma receptors, or antibodies via Fc receptors.

Monocyte phagocytosis is decreased by HIV infection and improves on ART. HIV infection also causes monocyte activation, which can be assessed by assaying plasma levels of soluble (s) CD14 or sCD163 (see below). Monocyte activation may persist in patients receiving ART and contributes to the pathogenesis of atherosclerotic vascular disease and probably other serious non-AIDS events.

Dendritic Cells

The numerous types of dendritic cell (DC) recognize, process, and present microbial antigens to T cells. Plasmacytoid dendritic cells (pDC) form a distinct DC population that plays a dominant role in viral infections because they are equipped with pathogen receptor molecules for viruses and also produce large amounts of IFN- α . Blood pDC are depleted by HIV infection, in part because they migrate to sites of HIV replication, such as the GALT, where IFN- α production may contribute to immune activation as well as to control of HIV replication. Circulating pDC numbers increase after ART is commenced.

Additional Therapy to ART for Correction of Suboptimal Immune Reconstitution

Resolution of immune activation and inflammation in lymphoid tissue should maintain the architecture and cell function needed for the differentiation and interaction of lymphocytes. While suppression of HIV replication by ART alone has a beneficial effect, that effect is often incomplete. Therefore, additional therapies may be required to suppress immune activation and inflammation (Rajasuriar et al. 2013). Therapy for HBV or HCV infection, if present, may improve CD4+ T cell deficiency, but therapy for CMV infection, while effective, results in minor CD4+ T cell gains and is expensive. Atorvastatin and possibly other statins decrease monocyte activation, and preliminary evidence suggests that recovery of CD4+ T cells is better when statins are added to ART. Hydroxychloroquine also decreases immune activation in patients receiving ART but has not yet been shown to increase CD4+ T cell counts.

Recombinant (r) growth hormone increases thymus function and increases production of naïve CD4+ T cells but is very expensive and may cause adverse effects that include diabetes. Recombinant IL-2 therapy increases CD4+ T cell counts when added to ART, but this is not associated with a decreased incidence of infections, probably because the expansion of CD4+ T cells mainly affects a subpopulation of CD4+ T cells with a regulatory phenotype. Furthermore, adverse effects of rIL-2 therapy limit its value.

Preliminary data suggest that rIL-7 may be more effective and tolerable than rIL-2 therapy.

Other than vaccination, there are currently no therapies for increasing B cell function in HIV patients receiving ART. A small number of patients have a persistent defect of B cell differentiation that is usually associated with impaired production of IgG2 antibodies and may need immunoglobulin replacement therapy if they experience recurrent invasive infections with bacteria, usually pneumococci.

Effects of ART on Plasma and Cellular Markers of Immune Activation

HIV-induced activation of lymphocyte subpopulations and monocytes/macrophages often persists on ART (Lederman et al. 2013) and can be assessed by enumerating cells expressing activation markers in blood or tissues and by assaying plasma levels of molecules secreted by, or that have been cleaved from the surface of, those cells (Table 1) (see “► [Lymphocyte Apoptosis](#)”). Co-expression of CD38 and HLA-DR on T cells, particularly CD8+ T cells, is the most commonly used marker of T cell activation. Additional markers can be assessed to obtain information on T cell proliferation and turnover (Ki67),

Immunological Responses to Antiretroviral Therapy, Table 1 Cellular and plasma markers of immune activation most commonly used to assess HIV-induced immune activation and the effects of therapy on this

CD4+ and CD8+ T cells
Proportion of blood T cells co-expressing CD38 and HLA-DR
Proportion of blood T cells expressing Ki67, CD57, or PD-1
B cells and plasma cells
Serum levels of immunoglobulins or immunoglobulin free light chains
Monocytes and macrophages
Proportions of blood monocytes expressing CD16
Plasma levels of sCD163 or sCD14
Plasma levels of D-dimers
Proinflammatory cytokines and their receptors
Plasma levels of IL-6 (or CRP as a correlate) or sTNFR _{II}
Interferon-induced chemokines
CXCL10

senescence (CD57), and exhaustion (PD-1). Activation of B cells and plasma cells is most readily assessed by assaying serum immunoglobulins or free light chains. Monocyte activation may be assessed by enumerating the proportion of blood monocytes expressing CD16 or assaying plasma levels of sCD163 (a scavenger receptor) or sCD14 (a lipopolysaccharide receptor). Increased expression of tissue factor on activated monocytes and macrophages leads to activation of the coagulation system and increased plasma D-dimer levels, which has been associated with the occurrence of serious non-AIDS events, such as osteonecrosis. The activity of proinflammatory cytokines and chemokines is most readily assessed by assaying plasma levels of IL-6, CRP, TNFR2, and CXCL10. The use of ART will improve many markers of immune activation, but persistent abnormalities may persist and correlate with an increased risk of serious non-AIDS events and death (Lederman et al. 2013).

Immune Reconstitution Disorders

Reconstitution of the immune system after ART may, paradoxically, increase the susceptibility of HIV patients to particular immunological disorders. These disorders mainly occur in patients who were very immunodeficient prior to commencing ART. Three main categories of immune reconstitution disorder are encountered (Table 2).

Immune Reconstitution Inflammatory Syndromes and Related Disorders

Virtually any pathogen that can cause an opportunistic infection may be associated with an immune reconstitution inflammatory syndrome (IRIS) when immune responses against the pathogen are restored by ART (Table 3). An IRIS occurs in up to 40% of patients commencing ART, particularly those patients with a pretreatment CD4+ T cell count of <50/μL, and usually presents during the first 3 months of ART, mostly during the first few weeks. The infection by the pathogen may not be apparent before ART is commenced and is “unmasked” by the immune response against it (unmasking IRIS). In other patients, an

Immunological Responses to Antiretroviral Therapy, Table 2

Immune reconstitution disorders that may occur in HIV patients on antiretroviral therapy

Immunopathology resulting from the restoration of immune responses against opportunistic pathogens that presents as:

An immune reconstitution inflammatory syndrome (IRIS), in which the inflammation generated by the immune response is exaggerated and/or atypical and often causes significant morbidity and sometimes mortality

Disease that is indistinguishable from that usually caused by the pathogen, such as tuberculosis, herpes zoster, herpes simplex, or flares of hepatitis caused by HBV or HCV infection, during the first 3 months of ART

Autoimmune disease, mainly Graves' disease

Immune-mediated inflammatory disease, mainly sarcoidosis

IRIS occurs in patients who are receiving treatment, or have recently received treatment, for an opportunistic infection, which appears to paradoxically worsen when ART is commenced (paradoxical IRIS). An IRIS of the central nervous system is most likely to cause severe morbidity and death.

The hallmark of an IRIS is exaggerated or atypical inflammation associated with restoration of immune responses against a pathogen. While a high pathogen load is a common risk factor for all types of IRIS, the immune responses initiating the aberrant inflammation appear to differ with the type of pathogen. Inflammatory infiltrates containing CD8+ T cells are found in an IRIS associated with virus infections, such as JC polyomavirus (Fig. 1), whereas granulomatous inflammation or suppuration is typical of an IRIS associated with mycobacterial or fungal infections (Meintjes et al. 2008; Boulware et al. 2010). Antigen-specific Th1 responses have been associated with TB-IRIS, though their relationship with disease pathogenesis is unclear, but it has been difficult to demonstrate antigen-specific Th1 responses in patients with cryptococcal-IRIS (C-IRIS). There is, however, increasing evidence that innate immune responses and pro-inflammatory cytokines, particularly IL-6 and TNF-alpha, are produced in excess in TB-IRIS, MAC-IRIS, and C-IRIS and also data suggesting that chemokines might affect the trafficking of

Immunological Responses to Antiretroviral Therapy, Table 3 Opportunistic pathogens most commonly associated with an IRIS or associated disorders after commencing ART

Pathogen	Nomenclature or disease presentation
Mycobacteria	
<i>M. avium</i> complex	MAC-IRIS
<i>M. tuberculosis</i>	TB-IRIS
<i>M. leprae</i>	Leprosy reversal reaction after commencing ART
Bacille Calmette-Guérin	BCG-IRIS
Other nontuberculous mycobacteria	
Fungi and yeasts	
<i>Cryptococcus</i> spp.	Cryptococcal-IRIS (mainly meningitis)
<i>Pneumocystis jirovecii</i>	Pneumocystis-IRIS
Protozoans	
<i>Toxoplasma gondii</i>	Exacerbation or presentation of cerebral toxoplasmosis after ART
<i>Leishmania</i> spp.	
Viruses	
Cytomegalovirus (CMV)	CMV retinitis after ART CMV immune recovery uveitis
Varicella-zoster virus (VZV)	Cutaneous zoster after ART VZV-IRIS (usually CNS disease)
Herpes simplex virus (HSV) 1 and 2	Mucocutaneous herpes after ART (sometimes necrotizing or hemorrhagic) HSV-IRIS (mainly CNS disease)
Kaposi's sarcoma (KS) herpesvirus	KS-IRIS
JC polyomavirus	PML-IRIS
Hepatitis B virus	Hepatitis flare after ART
Hepatitis C virus	Hepatitis flare after ART
HIV	Exacerbation of HIV encephalitis after ART
Helminths	
<i>Strongyloides</i>	
Bacteria	
<i>Bartonella henselae</i>	

lymphocytes and myeloid cells to sites of inflammation (Chang et al. 2013). An animal model of MAC-IRIS has provided evidence of aberrant interactions between innate immune cells and T cells when ART is commenced in the context

of severe CD4+ T cell depletion and a high pathogen load, which leads to an exaggerated inflammatory response (Barber et al. 2012).

A severe IRIS can be life-threatening and may require the addition of immunomodulatory therapy to ART. Corticosteroid therapy is usually used but efficacy has only been demonstrated in a randomized controlled trial for TB-IRIS. Therapeutic inhibition of those proinflammatory cytokines or chemokines associated with disease pathogenesis (see above) is logical but not yet of proven benefit.

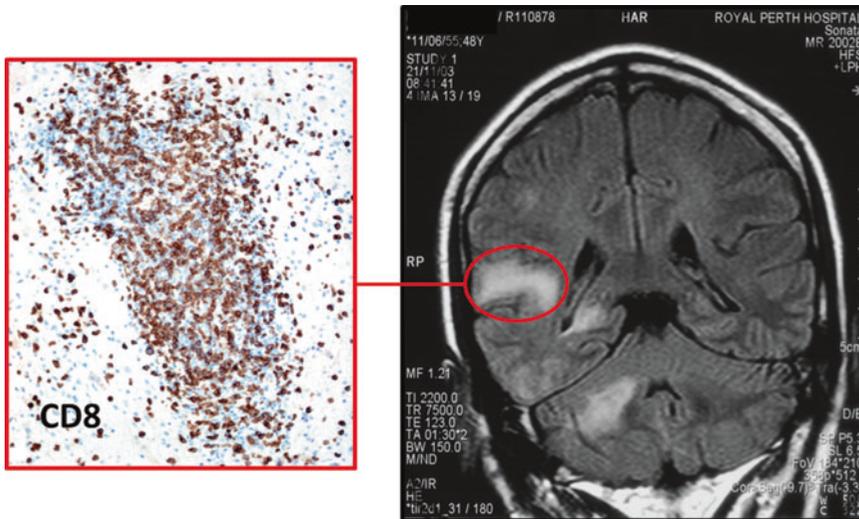
Autoimmune Disease Following Immune Reconstitution in HIV Patients

Several types of autoimmune disease have been reported in HIV patients who have experienced immune reconstitution on ART. Graves' disease of the thyroid is by far the most common (Crum et al. 2006) and often causes very severe thyrotoxicosis, which is commonly associated with severe extra-thyroidal manifestations, such as ophthalmopathy. Graves' disease occurs predominantly in patients who had a very low CD4+ T cell count at the time of commencing ART and who experience a substantial increase of the count on ART. Unlike the various types of IRIS, Graves' disease in HIV patients receiving ART presents at a median time of about 2 years after commencing ART and the immunopathogenesis therefore appears to be different. An acquired defect of central immune tolerance associated with higher numbers of circulating recent thymic emigrant naïve T cells may be one factor.

Systemic or cutaneous lupus has also been reported to present in HIV patients on ART. Those cases presenting during the first 3 months of ART have had serologic evidence of lupus before treatment, and it appears that the immunologic changes immediately after ART unmask subclinical disease. The other cases have presented after 9 months of ART, and the lupus may have resulted from an acquired disorder of immune tolerance during immune reconstitution.

Immune-Mediated Inflammatory Disease During Immune Reconstitution in HIV Patients

Immune-mediated inflammatory disease (IMID) results from immune responses that are apparently



Immunological Responses to Antiretroviral Therapy, Fig. 1 PML-IRIS of the brain after commencing ART for HIV infection. A biopsy of a brain lesion (*circled*) demonstrated large numbers of CD8+ T cells

not precipitated by an infection or the result of autoimmunity. Sarcoidosis is the most common type of IMID presenting as an immune reconstitution disorder in HIV patients (Foulon et al. 2004). It may present up to 3 years after commencement of ART. In some cases, the use of IL-2 or interferon-alpha (IFN- α) therapy appears to have been a precipitating factor. The immunopathogenesis of sarcoidosis in HIV patients receiving ART is unclear, but it is most likely to reflect an increased susceptibility to dysregulation of Th1 immune responses in the reconstituted immune system, which may be exacerbated by cytokine therapy that enhances Th1 immune responses, such as recombinant IL-2 or IFN- α therapy.

Conclusions

ART has substantially improved the health and longevity of people with HIV infection, but it does not completely resolve HIV-induced immune activation and immune dysfunction, particularly when commenced late in the course of HIV disease. Persistent deficiency and/or dysfunction of CD4+ T cells, memory B cells, NK cells, and monocytes and the associated abnormalities of cellular or plasma markers of immune

activation in patients receiving ART are associated with an increased susceptibility to infections and cancers and to serious non-AIDS events. Those patients who experience immune reconstitution from a state of severe immunodeficiency are also susceptible to immune reconstitution disorders, which include various types of IRIS and some autoimmune diseases and IMID. Most of these problems would be avoided by commencing ART before severe immunodeficiency develops. Additional therapies may be needed in patients who develop an IRIS or experience ongoing immune activation on ART. Research is required to elucidate the immunopathogenesis of these conditions so that treatments can be improved.

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target CD4⁺ T cells are eliminated by the immune system or as a result of viral infection after releasing progeny virus that propagates the infection. However, as a consequence of normal T cell memory, a subset of HIV-1 cells become infected and revert back to a resting, quiescent state. These cells contain an integrated HIV-1 provirus but are transcriptionally silent and represent the major barrier to HIV-1 eradication. The establishment of latency, the ability of latently infected cells to evade the immune response, and the reactivation and subsequent immune clearance of latently infected CD4⁺ T cells are all major immunological components of latent HIV-1 infection.

Introduction

Understanding latent HIV infection requires both an understanding of T cell-based immune memory in addition to the HIV-1 replication cycle. Latently infected cells contain a stably integrated HIV-1 provirus, are quiescent, and produce little to no detectable viral proteins, thus representing a huge hurdle for immune-based eradication strategies. Understanding the complex interactive between the host immune system and latent infection is likely necessary for complete clearance of HIV-1. Herein, the immunologic principles dictating the establishment, maintenance, and immune-based targeting of latently infected cells will be discussed.

Immunology of Latent HIV Infection

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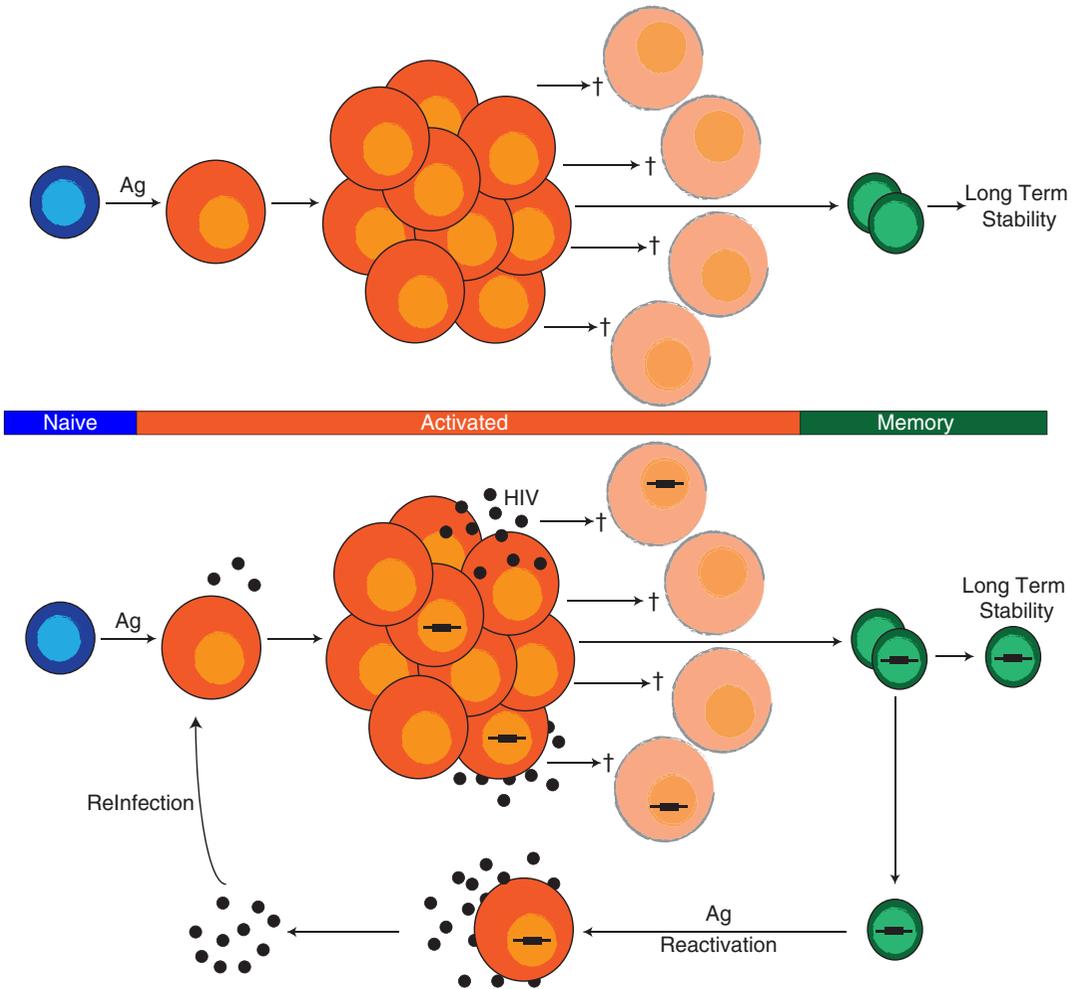
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Definition

Due to restrictions predicated by receptor and coreceptor binding, HIV-1 preferentially infects activated CD4⁺ T cells. Typically, HIV-1 infected

Establishment of the Latent Reservoir

Tropism constraints imposed by the surface glycoprotein gp120 dictate the infection of CD4⁺ T cells in HIV-1 infection. Consequently, the production of latent, HIV-1-infected cells is thought to be an inadvertent result of normal, T cell memory generation (Fig. 1). Typically, naïve CD4⁺ T cells encounter antigen that is presented by an antigen-presenting cell. This naïve T cell then undergoes clonal blast transformation which results in the generation of a pool of clonal effector cells in response to the original antigen. Once antigen has been cleared, the majority of these cells die, but a small fraction persist and revert to



Immunology of Latent HIV Infection, Fig. 1 Generation and maintenance of HIV-1 latency in CD4⁺ T cells. HIV-1 latency is a consequence of normal CD4⁺ T cell memory. Physiologically, naïve CD4⁺ T cells (*blue* cells) encounter antigen in the presence of APC cells and undergo blast transformation and clonal amplification as activated CD4⁺ T cells (*orange* cells). Once antigen has been cleared from the system, contraction occurs and the majority of active CD4⁺ T cells undergo death by apoptosis (†). A minority persist as memory CD4⁺ T cells

(*green* cells), in a long-lived pool of cells that can rapidly respond if exposed to the same stimulatory antigen. In the context of HIV-1 infection, HIV-1 preferentially infects activated CD4⁺ T cells. The majority of infected cells die via normal apoptosis or viral cytopathic effects, but a minority of infected cells revert back to a resting, memory state. These cells are long-lived, quiescent cells with a stably integrated provirus (*black bar*). If these cells reencounter antigen, they reactivate, thus allowing productive HIV-1 viral replication

a resting, quiescent state. A hallmark of the acquired immune response, these cells are primed to potently respond if restimulated with the same antigen (Dooms and Abbas 2006). While HIV-1 preferentially infects activated CD4⁺ T cells, resting CD4⁺ T cells can also be directly infected.

Because productive HIV-1 infection requires active proliferation of the target cell, HIV-1 reverse transcription and integration are not completed in most of these cells. Thus, the HIV-1 genome persists as an extrachromosomal form that retains the ability to integrate upon cellular

activation. These unintegrated genomes form the labile pre-integration latent reservoir (Bukrinsky et al. 1991; Zack et al. 1990) that is found in viremic patients. In contrast, infection of activated CD4⁺ T cells results in productive viral infection, and infected activated CD4⁺ T cells typically perish as a result of HIV-specific immune responses and/or viral cytopathic effects. However, it is possible that a small number of partially activated CD4⁺ T cells become infected and subsequently revert back to a fully resting, memory state. This process results in a stably integrated provirus, contained within a transcriptionally silent, long-lived memory CD4⁺ T cell (Han et al. 2007). These cells form the post-integration latent reservoir and are phenotypically similar to other resting CD4⁺ T cells.

The latent reservoir is established very early in the course of HIV-1 infection. Primary HIV-1 infection is typically characterized by high HIV-1 RNA levels, which decline to a stable set point at the onset of the acquired immune response. Over the course of years, CD4⁺ T cell counts progressively decline in the absence of antiretroviral therapy. AIDS is clinically defined when CD4⁺ T cell counts fall to less than 200 cells per microliter of blood, which occurs concomitant with the appearance of opportunistic infection due to immune system dysfunction. Effective therapy with combination antiretroviral therapy is thought to halt ongoing viral replication, and the implementation of therapy results in a dramatic decay of residual viremia to undetectable levels and a stabilization of CD4⁺ T cell counts (Perelson et al. 1997). With the advent of combination antiretroviral therapy, there was hope that HIV-1 infection could be eradicated. However, the establishment and persistence of the latent reservoir continues to be a barrier for HIV-1 eradication from infected individuals because treatment interruption rapidly leads to viral rebound. Current estimates, based on the half-life of infected, resting CD4⁺ T cells, predict that this stable reservoir will persist for greater than 70 years, thus mandating lifelong antiretroviral therapy (Siliciano et al. 2003).

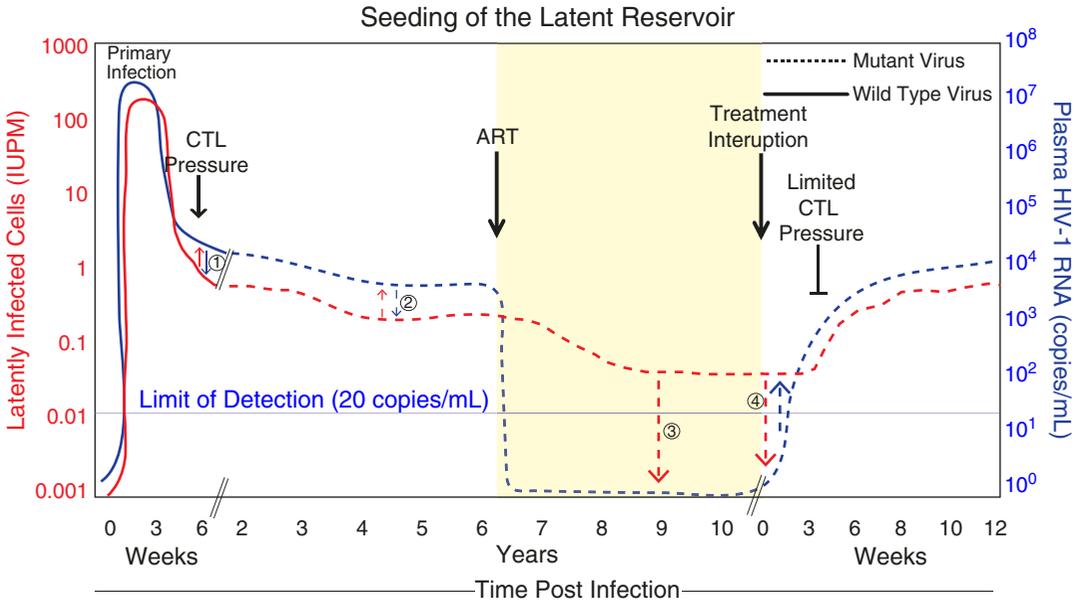
Seeding of the latent reservoir occurs early during viral infection (Chun et al. 1998). During acute infection, plasma HIV-1 RNA levels reach their peak, and the latent reservoir size is thought to be determined by the magnitude and duration of uninterrupted viral replication. Peak viral loads are reduced at the onset of the acquired immune response. The viral set point is a dynamic equilibrium that reflects viral fitness and suppressive activity of the immune system. Even in the face of immune pressure, high levels of viral replication allow the virus to escape both the cellular and humoral immune responses leading to the emergence of immune escape variants (Fig. 2). Early treatment with antiretroviral therapy has been demonstrated to reduce the overall latent viral burden (Jain et al. 2013), and potent immune responses early in viral infection have been hypothesized to reduce the initial seeding of the latent reservoir (Buckheit et al. 2013a).

Current antiretroviral therapies allow the reduction of HIV-1 replication to undetectable levels. However, due to latently infected cells, any cessation of antiretroviral therapy quickly results in a rebound in HIV-1 RNA levels, indicating that antiretroviral therapy alone is not sufficient to eradicate the latent reservoir.

Evasion from the Immune Response

Typically, HIV-1 elicits a strong antiviral humeral and cellular immune response. However, there is a lag between the initial infection event and the development of an acquired immune response and seeding of the latent reservoir occurs during this period. Thus, while there is strong evidence of selective immune pressure exerted on HIV-1, as evidenced by the development of escape mutation in both antibody and CD8⁺ T cell-restricted epitopes, the acquired immune response does not prevent the establishment of the HIV-1 latent reservoir early in viral infection (reviewed in Bailey et al. 2004).

From an immunologic standpoint, the latent reservoir presents a challenging barrier to HIV-1 eradication. Physiologically, the recognition of intracellular bacterial and viral infection requires



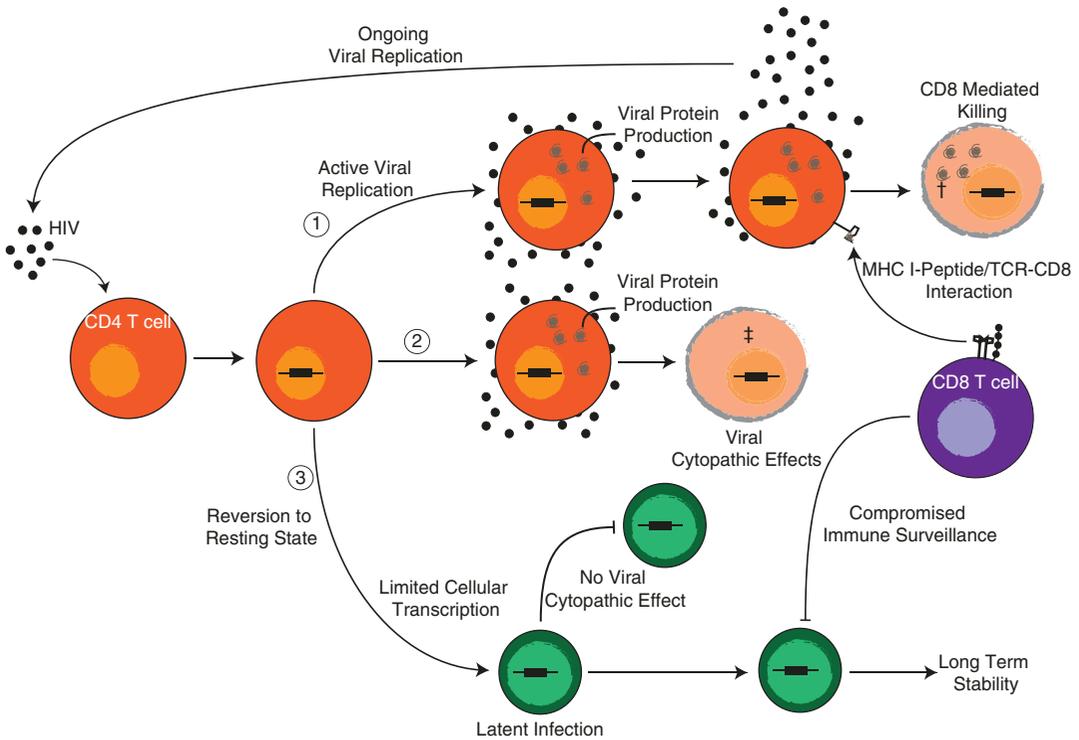
Immunology of Latent HIV Infection, Fig. 2 Immune pressure and seeding of the HIV-1 latent reservoir. The latent reservoir is seeded early in the course of HIV-1 infection. During primary infection, HIV-1 plasma RNA levels (blue line) peak, leading to the initial seeding of the latent reservoir (red line, measured in infectious units per million CD4⁺ T cells or IUPM). The number of latently infected cells, as measured on the Y axis, includes pre- and post-integration latency. The majority of latently infected cells measured during periods of high viremia are comprised of pre-integration, latently infected cells. Viremia is reduced by the emergence of the acquired immune response, and HIV-1 plasma RNA levels reach a dynamic set point level in the absence of antiretroviral therapy. Immune pressure results in the selection of immune escape variants (dotted lines), which ultimately circulate in the

periphery and seed the latent reservoir. During uncontrolled viral replication, there are exchange and mixing of the HIV-1 quasispecies between circulating and latent viral isolates (red and blue arrows, 1 and 2). Initiation of antiretroviral therapy (ART) reduced plasma HIV-1 RNA levels to undetectable levels (less than 20 copies per mL of blood). During the period of ART (yellow shading) ultralow-level circulating residual viremia is probably comprised of escape mutation variants released from the latent reservoir into the plasma (red arrow, 3). If treatment is interrupted, viral rebound occurs rapidly and free exchange between the replicating and latent pools is likely to continue (red and blue arrows, 4). Rebounded virus is comprised predominately of immune escape variants, resulting in reduced or eliminated cytotoxic CD8⁺ T cell responses (CTL)

the recognition of unique viral proteins. In some cases, this recognition occurs through the identification of unique viral RNA or DNA moieties, which activate intracellular pattern recognition sensors and downstream inflammatory effectors. Classically, virally infected cells are eliminated from the body by cytotoxic CD8⁺ T cells (Fig. 3). Viral proteins are degraded by the immunoproteasome, thus generating a series of cleaved viral proteins that are delivered to the assembling MHC class I complex. Processed peptides are then presented on the surface of the infected cell, and the cognate TCR-CD8 complex on cytotoxic CD8⁺ T cells recognizes

the peptide-MHC complex. Importantly, this surveillance mechanism requires the presence of viral antigens within infected cells (reviewed in (Janeway 2005)).

In contrast to activated CD4⁺ T cells, latently infected CD4⁺ T cells are quiescent and have dramatically reduced protein production. Transcription at the HIV-1 LTR requires the production of host transcription factors that are either reduced in resting CD4⁺ T cells or kept sequestered in the cytoplasm. For example, NF- κ B is kept inactive in the cytoplasm due to its interaction with I κ B. Upon cellular activation, I κ B is phosphorylated and degraded, thus allowing the translocation of



Immunology of Latent HIV Infection, Fig. 3 Cytotoxic CD8⁺ T cell response in latent and active HIV-1 infection. HIV preferentially infects activated CD4⁺ T cells (orange cells). Upon infection, HIV-1 integrates stably within the genome (black bar). The cell follows one of three distinct paths. 1 Active viral replication begins, resulting in high levels of virus production and intracellular viral proteins. These viral proteins are processed and presented extracellularly in an MHC-I peptide complex. This complex can be recognized by the TCR-CD8 complex of CD8⁺ T cells (purple cell), resulting in CD8⁺ T cell-mediated killing of these viral infected

cells (‡). Virus produced prior to killing can reinfect other susceptible CD4⁺ T cells. 2 Active viral replication begins, resulting in the production of high levels of viral proteins. The cell is killed as a result of viral cytopathic effects (‡). 3 The infected CD4⁺ T cell reverts to a resting, memory state (green cell) with limited host and viral cell transcription. Due to the lack of viral proteins, there is no cell death as a result of viral cytopathic effects. Additionally, the lack of viral proteins prevents detection by CD8⁺ T cells. Thus, these cells persist long term without productive immune surveillance

NF-κB to the nucleus. Additionally, the HIV-1 genome is kept silent due to the lack of tat and tat-associated factors that are similarly required for productive viral replication. These restrictions do not allow productive transcription of the HIV genome, and without viral protein production immune surveillance is compromised and infected cells are able to persist within the body without detection or elimination. Additionally, reduced viral protein transcription within latently infected cells will also reduce or eliminate cell death due to viral cytopathic effects (Fig. 3).

Furthermore, many circulating HIV-1 quasi-species are archived in the latent reservoir. As discussed previously, high levels of viral replication in the presence of a strong acquired immune response result in rapid escape from the immune system and consequentially the seeding of the latent reservoir with escape variant HIV-1 isolates (Queen et al. 2011). Thus, upon cessation of anti-retroviral therapy, the resulting rebound virus could already harbor immune escape mutations and could prevent a fully effective immune response (Fig. 2).

Targeting the Latent Reservoir

Given these immune evasion strategies, the question now becomes, can the immune response clear latently infected cells from the body? Without proper immune clearance, eradication of HIV-1 infection is likely impossible given the current antiretroviral therapies. Targeting the latent reservoir can occur at two points in the life cycle of HIV-1 infection.

The first would be targeting HIV-1 infection prior to productive integration in resting CD4⁺ T cells. This approach would require vaccination to induce a maximal immune response that limits the size of the latent reservoir early in viral infection. Recently, some lines of evidence suggest this may be possible. Vaccination strategies that elicit an effector memory phenotype in a monkey model of SIV infection prior to challenge were shown to be partially protective and were hypothesized to have reduced or eliminated the seeding of the latent reservoir in protected animals (Hansen et al. 2011). Targeting of non-productively infected CD4⁺ T cells could limit the initial seeding of the latent reservoir, as hypothesized recently in the human model of HIV-1 control (Buckheit et al. 2013b). Thus, a boosted immune response early during infection could lead to a reduced latent reservoir burden early in viral infection.

Secondly, latent HIV-1 infection could be targeted during the chronic phase of infection, thus leading to eradication. Recent efforts to eradicate HIV-1 infection have focused on a “shock and kill” methodology. Briefly, this method dictates the reactivation of HIV-1 latently infected cells using specific small molecule activators while continuing antiretroviral therapy to prevent new round of HIV-1 infection. Reactivation would lead to the production of viral proteins, and the elimination of these infected cells by either viral cytopathic effects or immune-mediated clearance and the presence of antiretroviral drugs will prevent new rounds of viral infection. Recently, clearance of reactivated cells was shown using an *in vitro* primary cell model, after maximal pre-stimulation of effector CD8⁺ T cells. CD8⁺ T cells from

chronically infected individual express higher levels of exhaustion/inhibitory markers, and *in vivo* many of the reactivated viruses may contain immune escape variants. This proof of concept study suggests that immune therapy to boost the CD8⁺ T cell response in chronically infected individuals may be required to clear reactive latently infected cells (Shan et al. 2012). Interestingly, a rare group of patients known as elite controllers have been shown to maintain undetectable viral loads without antiretroviral therapy for years despite the presence of low frequencies of latently infected CD4⁺ T cells (Buckheit et al. 2013a). This suggests that in some cases, the immune system is capable of eliminating reactivated latently infected CD4⁺ T cells, thereby preventing virologic breakthrough.

Conclusions

Overall, HIV-1 latency represents a unique epiphenomenon due to the stable integration of HIV-1 in CD4⁺ T cells. Classically, immune memory allows the maintenance of potent immune responses to a variety of antigens that have been previously encountered by the host. Whether by evolutionary design or by happenstance, HIV-1 has co-opted these processes to persist within the host, even in the face of strong immune pressure and the advent of suppressive antiretroviral therapy, representing a sizable barrier to HIV-1 eradication. The archiving of escape containing isolates within the latent reservoir and the transcriptional quiescent of long-lived memory CD4⁺ T cells represent the two main functional barrier to the immune clearance of HIV-1 infection, although efforts are underway to perturb this balance and potentially achieve eradication.

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Immunopathogenesis of HIV Coinfections

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Definition

HIV-infected patients, particularly those with advanced immunodeficiency, are frequently coinfecting with other common and opportunistic infections. Here, we discuss the immunopathogenesis of six common infections that cause significant morbidity in HIV-infected patients globally. These include coinfection with cytomegalovirus (CMV), *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, hepatitis B virus (HBV), hepatitis C virus (HCV), and *Plasmodium falciparum*. We summarize the epidemiology and global burden of each coinfection; highlight the immune response, pathogenesis, and natural history; and discuss the immune interplay between HIV and the co-pathogen and also the effect of cART in the setting of HIV

coinfections, including the pathogenesis of immune restoration disease (IRD).

Introduction

While AIDS-related deaths have declined globally with the availability of combination antiretroviral therapy (cART), coinfections remain a significant global burden, particularly in HIV-infected individuals who initiate cART late. Coinfections can have a significant impact on the natural history of HIV, and HIV can have a significant effect on the natural history of the specific coinfection, often characterized by unusual clinical presentations and/or increased severity.

Coinfections can directly enhance HIV viral replication through a number of mechanisms including upregulation of chemokines and cytokines, systemic or local immune activation that increases the number of target cells including activated CD4⁺ T cells, and enhancement of HIV transcription through transactivation of the HIV long terminal repeat (LTR) (Lisco et al. 2009). Conversely, HIV can have a direct impact on the innate and adaptive immune response to the specific coinfection leading to a change in natural history and altered response to treatment.

Immune restoration disease (IRD) or immune reconstitution inflammatory syndrome (IRIS) is an additional complication seen in the setting of HIV coinfections. Following cART initiation, restoration of immunity to the specific coinfection can be aberrant, and in some situations, the manifestations of the coinfection can worsen, further complicating the clinical presentation. This is associated with significant morbidity and mortality.

In this chapter, we will discuss the immunopathogenesis of six common and important infections that cause significant morbidity in HIV-infected patients globally. These include coinfection with cytomegalovirus (CMV), *Mycobacterium tuberculosis* (Mtb), *Cryptococcus neoformans*, hepatitis B virus (HBV), hepatitis C virus (HCV), and malaria. Specifically, we review the natural history of each coinfection in the setting of HIV, the specific immune defects

induced by HIV, the effects of cART on the immune response to the coinfection, the pathogenesis of IRD, and how the coinfection has a specific impact on immunopathogenesis of HIV and long-term persistence of virus on cART.

Cytomegalovirus (CMV)

Epidemiology and Global Burden of Disease

CMV is a common pathogen that infects more than half of the human population (nearly 100% in developing countries) and can range from mild or subclinical disease in immunocompetent individuals to severe disseminated disease in immunodeficient patients and severe neonatal congenital disease. CMV is the most common viral opportunistic infection in patients with AIDS.

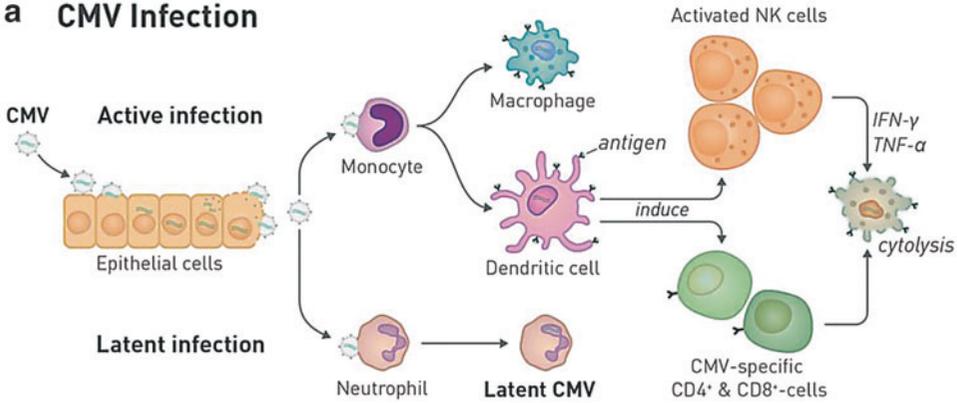
Natural History of CMV Infection

As with many other viruses, CMV can actively replicate and also remain latent. In most immunocompetent individuals, latent CMV causes no adverse effects; however, in the setting of immunosuppression such as advanced HIV infection, latent CMV can reactivate and cause significant end-organ disease such as retinitis, colitis, or pneumonitis. CMV can persist in a variety of cells and tissues and induces CMV-specific CD8⁺ T cells, even in the absence of active replication. With aging, there is a profound expansion of CMV-specific CD8⁺ T cells.

Immune Response to CMV and Pathogenesis

Both innate and adaptive immunities are important in the host control of CMV. Primary CMV infection in immunocompetent patients usually involves CMV replication in the mucosal epithelium, after which CMV disseminates to monocytic cells where it establishes latency. Restricted gene expression limits recognition by immune effector cells. The differentiation of monocytes to macrophages can produce productive CMV infection. CMV particles can be processed by antigen-presenting cells which then stimulate antigen-specific T cells and natural killer (NK) cells, both of which may directly lyse virus-infected cells by cytolysis or block replication

a CMV Infection



b

	CMV	+HIV	+ART
Immune response	CMV-specific CD4 ⁺ & CD8 ⁺ T-cells	↑	↓↓
	CMV-specific IFN-γ release	↑	↓↓
Clinical response	CMV reactivation	rare	↑↑↑
	Disseminated CMV disease	rare	↑↑↑
	End-organ damage	rare	↑↑↑
	Chronic immune activation	↑ (elderly)	↑↑

Immunopathogenesis of HIV Coinfections, Fig. 1 Pathogenesis of CMV infection and impact of HIV and cART. (a) CMV primarily replicates in the mucosal epithelium and then establishes latent infection in mononuclear cells. The differentiation of monocytes to macrophages can produce productive infection. DCs

present CMV antigens and can induce CMV-specific T cells and NK cells, which can both directly lyse virus-infected cells by cytolysis and block replication through secretion of IFN-gamma or TNF-alpha. (b) Impact of HIV and cART on the immune response and clinical manifestations of CMV

through secretion of interferon (IFN)-gamma or tumor necrosis factor (TNF)-alpha (Fig. 1a; Crough and Khanna 2009). The recruitment and activation of immune cells further increase local IFN-gamma concentrations. CMV-specific CD8⁺ T cells are critical for clearance of CMV-infected cells, although IFN-gamma secreting CD4⁺ T cells are also important for recovery from primary CMV infection.

To escape the host response, CMV encodes for a multitude of inhibitors of major histocompatibility complex (MHC) presentation, thereby reducing peptide presentation and avoiding T-cell recognition. CMV also encodes for inhibitors of IFN downstream signaling (Crough and Khanna 2009). The reactivation of CMV from latency is thought to be mediated through TNF-alpha (Crough and Khanna 2009).

Impact of HIV on CMV

Defects in cell-mediated immunity (CMI) seen in HIV infection result in an increased severity of CMV end-organ disease and increased risk for CMV reactivation and replication (Fig. 1b). Asymptomatic excretion of CMV in urine can often be detected in HIV-infected patients with low CD4+ T-cell counts, and transient CMV viremia may be noted (Crough and Khanna 2009). In vitro, there is enhancement of productive CMV infection by coinfection with HIV and of HIV replication by coinfection with CMV. Increasing CMV viremia during HIV disease progression has been described.

Impact of CMV on HIV

Evidence of past infection with CMV is almost universally present in sexually active adults, particularly in men who have sex with men (MSM). Even in the absence of active replication, latent CMV infection has been implicated as a contributing factor to chronic immune activation (► [Chronic Immune Activation in HIV](#)) in HIV-infected patients. In some studies, CMV-specific CD8+ T cells account for over half the total CD8+ T cells. High CMV-specific immune responses are strongly associated with the level of atherosclerosis in HIV-infected, CMV-seropositive individuals and accelerated immunosenescence as well as reduced number of total CD4+ T cells, particularly naïve CD4+ T cells.

Impact of cART on CMV, Including IRD

The availability of cART has dramatically reduced the incidence of CMV disease in HIV-infected patients and CMV viremia. However, HIV-infected patients on cART maintain strong CMV-specific CD8+ T cells (Naeger et al. 2010; Fig. 1b). A randomized placebo-controlled study suggested that daily valganciclovir of 900 mg – an antiviral agent active against CMV – reduced CD8 T-cell activation and CMV viral load (Hunt et al. 2011).

There have been numerous reports of CMV-related IRD, particularly involving ocular CMV disease – immune recovery vitritis and immune recovery uveitis. However, formal studies of CMV-IRD immunopathogenesis are lacking. A lower CD4+ T-cell count is a risk factor for CMV-IRD.

***Mycobacterium tuberculosis* Coinfection**

Epidemiology and Global Burden of Disease

Approximately 14 million people worldwide have HIV and *Mycobacterium tuberculosis* coinfection, and TB is the most common opportunistic infection in individuals with HIV infection, accounting for about 26% of AIDS-related deaths (Opportunistic Infections of Global Significance).

Natural History of *M. tuberculosis* Infection

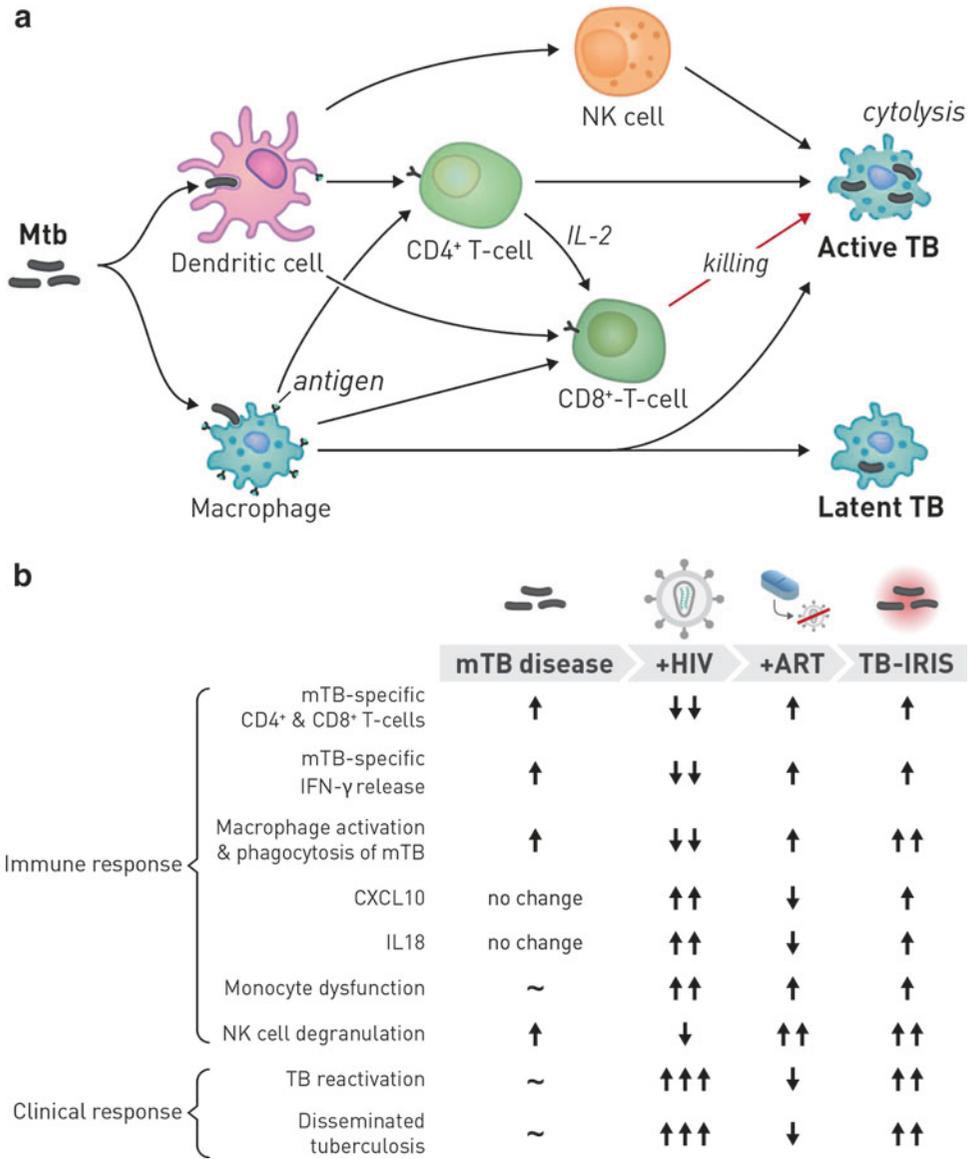
The majority of infected individuals achieve long-term control of *M. tuberculosis* (*Mtb*) infection resulting in latent tuberculosis infection (LTBI), which is associated with a 5–10% lifetime risk of reactivation, triggered usually by immunosuppression. In HIV-infected individuals however, this risk of reactivation increases by about 20-fold, and the risk of acquiring primary *M. tuberculosis* infection increases by 2.2–5.5-fold.

Immune Response to *M. tuberculosis* and Pathogenesis

M. tuberculosis is an obligate intracellular pathogen and is primarily controlled by CMI. Acquired through inhalation, alveolar macrophages and dendritic cells are the first cells that respond to *Mtb* (Fig. 2a). Immune control of *Mtb* infection is mediated by the concerted effects of multiple cell types, including CD4+ and CD8+ T cells, CD1-restricted T cells, B cells, macrophages, neutrophils, fibroblasts, and multinucleated giant cells that all contribute to granuloma formation to contain the infection. The inflammatory process that kills or “walls off” the mycobacterial infection involves chemokines and cytokines that promote a response from type 1 T helper (Th1) cells and monocytes, production of granulysin and other cytotoxic molecules by CD8+ T cells, and macrophage products such as nitric oxide synthase-2 and TNF-alpha.

Impact of HIV on *M. tuberculosis* Infection

CD4+ T-cell depletion seen in HIV infection results in the loss of cellular immune response to *Mtb*, due to a decline in memory CD4+ T cells and antigen-specific CD4+ T cells and a relative



Immunopathogenesis of HIV Coinfections, Fig. 2 Pathogenesis of *Mtb* infection and impact of HIV and cART. (a) Phagocytosis of *Mtb* by DCs leads to recruitment of MTB-specific CD4+ T cells which in turn activates NK cells and MTB-specific CD8+ T cells. Activated CD8+ T cells mediate killing of macrophages infected with *Mtb*. IFN-gamma enhances phagocytosis

and antigen presentation of free mycobacteria. *Mtb* can also establish latent infection that can persist indefinitely and can reactivate at any time despite the presence of MTB-specific CD4+ and CD8+ T cells. (b) Impact of HIV and cART on the immune response and clinical manifestations of *Mtb*

increase in IFN-gamma + CD8+ T cells and dysfunctional monocytes (discussed in Chang et al. 2013). This is associated with an increased rate of reactivation disease and disseminated disease (Fig. 2b). Suppression of cellular immune

responses by regulatory T cells (Treg) and impairment of TNF-alpha-mediated apoptotic responses also play a role. HIV infection is also associated with depletion of CD4+ T cells in granulomas, and both lymph nodes and other tissues infected by

Mtb demonstrate large numbers of neutrophils and necrosis.

Impact of *M. tuberculosis* on HIV

When mononuclear phagocyte cell lines are infected by *Mtb* or interact with *Mtb*-derived molecules, a number of signal transduction cascades are triggered, some of which result in the activation of transcription of the HIV LTR. Indeed, different phylogenetic strains of *Mtb* induce different degrees of HIV replication and may account for the diverse host immune response seen in HIV-TB coinfection.

Impact of cART Including ART-Associated TB and TB-IRIS

The improvement of CMI seen in response to cART reduces the rate of primary TB and reactivation of LTBI by at least 65%, irrespective of the CD4+ T-cell count at which cART is commenced (Suthar et al. 2012). However, the incidence of TB disease increases during the first 3 months of cART before subsequently declining, predominantly in patients with CD4+ T-cell counts of <50/μl. It appears that the *Mtb* infection is unmasked by restoration of immune responses against *Mtb*. In some patients, this presents as typical TB (ART-associated TB) whereas in others it presents as an IRIS with inflammation that is exaggerated or atypical (unmasking TB-IRIS). ART-associated TB is characterized by increased Th1 responses to the RD1 antigens of *M. tuberculosis* with increased production of IFN-gamma, the IFN-gamma-inducible chemokines CXCL10 and CXCL9, and interleukin (IL)-18 (discussed in Chang et al. (2013)).

Commencement of cART in HIV-infected patients who have recently received treatment for TB may also result in an IRIS in 20–25% of patients, termed paradoxical TB-IRIS in which there is worsening of TB clinical disease characterized by a pronounced and/or atypical inflammatory response. TB-IRIS occurs most commonly in patients with high pathogen load such as those with disseminated TB or drug-resistant TB and those with severe CD4+ T-cell deficiency (discussed in Chang et al. (2014)).

Th1 responses against *Mtb* antigens are also prominent in paradoxical TB-IRIS, characterized

by increased IFN-gamma + T-cell responses against tuberculin, ESAT-6 and CFP-10. However, there is also evidence that abnormalities of innate immune responses contribute to the immunopathogenesis of paradoxical TB-IRIS, including lower production of CCL2 (Oliver et al. 2010) and increased NK cell degranulation capacity (Pean et al. 2012) before cART is commenced, and increased production of CXCL10 (Oliver et al. 2010) and pro-inflammatory cytokines (Tadokera et al. 2011) after cART is commenced (Fig. 2b).

Barber et al. have proposed a model of TB-IRIS in which CD4+ T-cell depletion results in the failure of macrophage activation and accumulation of mycobacteria-laden macrophages. These cells become activated after cART is commenced due to an increase in IFN-gamma-producing CD4+ T cells, leading to the excessive production of IL-6 and TNF-alpha (Barber et al. 2012). When HIV infection is suppressed by cART, the recovery of adaptive and innate immune responses may occur in an uncoordinated manner with increased production of IL-18 and CXCL10 driving Th1 responses and increased production of IL-6 and TNF-alpha driving inflammation (Chang et al. 2013; Fig. 2b).

Cryptococcus Coinfection

Epidemiology and Global Burden of Disease

Globally, nearly a million cases of cryptococcal meningitis (CM) are estimated to occur each year, resulting in an estimated 624,700 deaths within 3 months of infection, with more than 80% of deaths occurring in sub-Saharan Africa (Park et al. 2009).

Natural History of *Cryptococcus* Infection

Cryptococcosis is most often due to reactivation of latent infection and can have a variety of clinical manifestations – in particular meningoencephalitis or cerebral cryptococcoma. Immunosuppression and particularly HIV infection are major risk factors for reactivation and disease.

Immune Response to *Cryptococcus* and Pathogenesis

Host defense against cryptococcosis has largely been thought to be mediated by CMI, though evidence for a role for innate and humoral immunity also exists. Protective CMI is based primarily on cryptococcal-specific Th1-type CD4+ T cells which produce IL-12, IL-2, IFN-gamma, and TNF-alpha (Hole and Wormley 2012) with IFN-gamma playing a particularly critical role. NK cells and monocytes have also been found to preferentially track into the CNS in the setting of CM.

Cryptococcus invasion of the CNS is the key to its pathogenesis and can occur via transcellular, paracellular, or vomocytosis in a host macrophage (Fig. 3a). The polysaccharide capsule is continually shed and interferes with macrophage phagocytosis, depletes complement, and impairs leukocytosis. The production of degradative enzymes enables cryptococci to degrade membranes, thereby compromising intra- and intercellular integrity leading to dissemination into the brain.

Impact of HIV on CM

The prominent CD4+ T-cell depletion seen in HIV infection significantly impairs the adaptive immune response to *Cryptococcus* spp. (Fig. 3b). Effective killing of *Cryptococcus* by alveolar macrophages is also impeded by HIV infection. HIV proteins can alter blood-brain barrier (BBB) integrity. The HIV viral protein, tat, can disrupt tight junctions and cross the BBB, contributing to BBB degradation, and may act as a chemoattractant for monocytes (Strazza et al. 2011). Gp120 binds to CXCR4 or CCR5, and the changes in brain microvascular endothelial cell cause tight junction dysfunction and an increase in BBB permeability (Strazza et al. 2011). Nef has been associated with increased secretion of CCL2 (Strazza et al. 2011). Further, expression of CCR5 (the predominant HIV coreceptor) found in the brain increases BBB permeability.

HIV-infected monocytes cross the BBB more readily and can repopulate CSF-resident macrophages. Thus, the presence of HIV in the CNS likely facilitates entry of a wide range of leukocytes into the perivascular space and

Cryptococcus spp. into the brain compartment, together leading to ongoing inflammation and BBB breakdown.

Impact of *Cryptococcus* spp. on HIV

The CNS is a known anatomical reservoir of HIV. The impact of cryptococcal CNS invasion and cryptococemia on HIV transmission and HIV replication and reservoir has not been formally investigated.

Impact of cART on CM, Including IRD

The preventative effect of cART on reactivation of *Cryptococcus* infection in HIV-infected patients has not been investigated, but *Cryptococcus* is rarely observed in HIV-infected patients with a CD4+ T-cell count of more than 100 cells/ml.

A proportion of HIV-infected patients with acute CM who commenced cART very early during antifungal treatment may develop cryptococcosis-associated immune reconstitution inflammatory syndrome (C-IRIS). Predictors of C-IRIS include a lower baseline CD4+ T-cell count, poor CD4+ T-cell recovery post-cART initiation, reduced CSF inflammation, a persistently positive culture of cryptococci from CSF at time of cART initiation, and lower IFN-gamma responses to cryptococcal mannoprotein (CMP) (discussed in Chang et al. 2014; Fig. 3b). Cytokine profiles of CCL2, CCL3, and CXCL10 measured in the CSF may assist in assessing risk for C-IRIS.

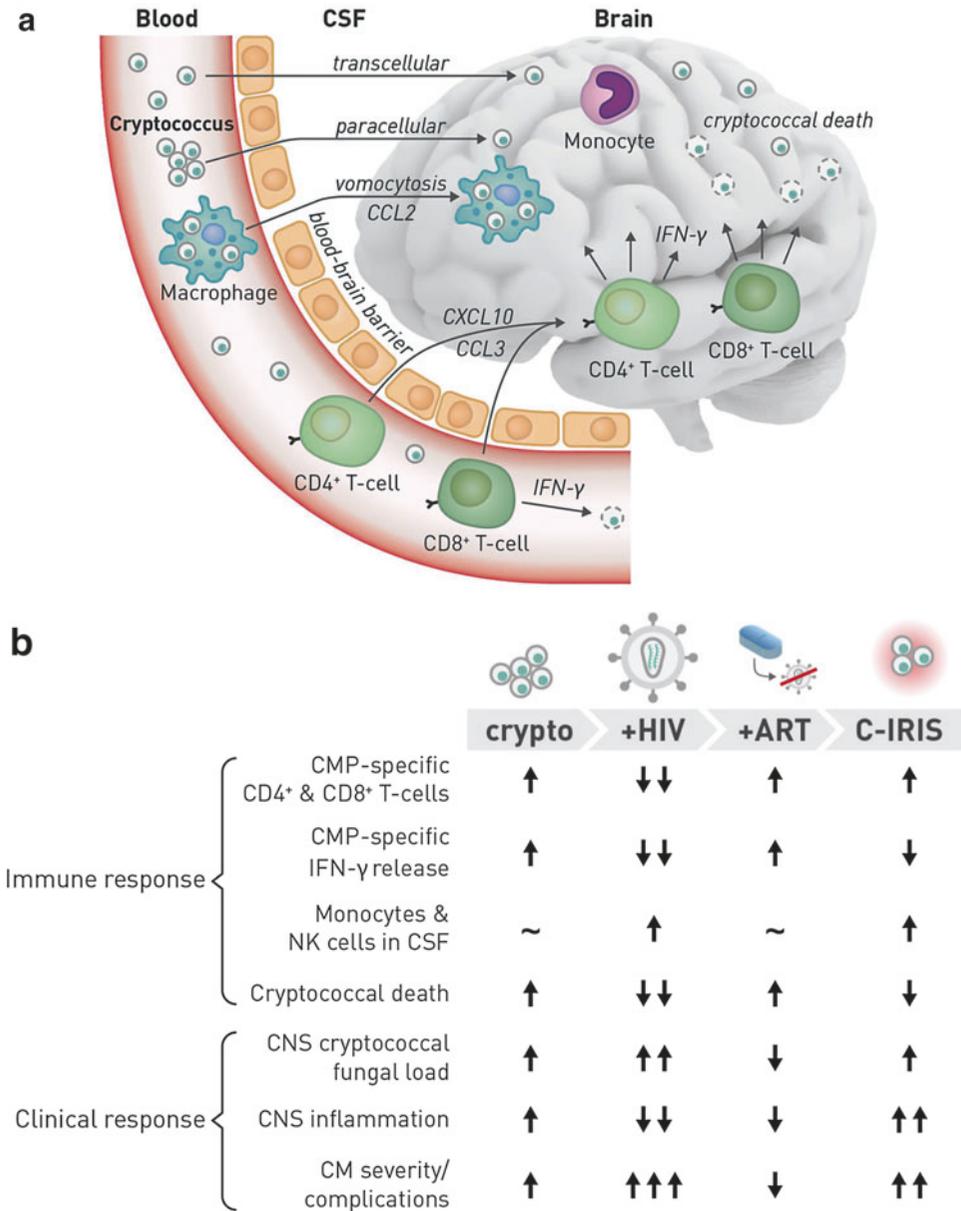
Hepatitis B Virus (HBV) Coinfection

Epidemiology and Global Burden of Disease

Despite the widespread availability of an effective vaccine, there are currently 300 million people with chronic HBV infection. Approximately 10–25% of HIV-infected patients are coinfecting with HBV. Liver-related mortality is a common cause of non-AIDS-related death in HIV-infected individuals on cART.

Natural History of HBV

Following infection with HBV, the initial hepatitis may or may not be symptomatic. Successful



Immunopathogenesis of HIV Coinfections, Fig. 3 Pathogenesis of cryptococcal meningitis and impact of HIV and cART. (a) *Cryptococcus* spp. is able to cross the BBB by paracellular, transcellular, or vomocytosis, using macrophages as a Trojan horse.

Antigen-specific CD4⁺ T cells release IFN-gamma which is critical for host protection. (b) Impact of HIV and cART on the immune response and clinical manifestations of CMV

clearance and resolution of infection is reduced in young infants and in the setting of immunosuppression. Chronic HBV infection is characterized by fluctuations in both hepatic enzymes and HBV viral load, which may eventually lead to liver

fibrosis and hepatocellular carcinoma in a subset of patients. Immune clearance is associated with development of antibodies to the HBV protein e antigen (HBeAg) and occasionally to the HBV protein s antigen (HBsAg). Hepatic

flares – more than twofold rise in alanine transferase (ALT) – often precedes development of these antibodies, or what is commonly referred to as “seroconversion.”

Immune Response to HBV and Pathogenesis

In patients with chronic HBV infection (CHB), HBV-specific CD4⁺ and CD8⁺ T-cell responses are significantly diminished; there are generalized CD4⁺ T-cell and dendritic cell (DC) hypo-responsiveness and dysregulation, altered ratios of total T cells to Treg cells, and impaired NK cell response to TLR9 stimulation (discussed in Chang et al. 2013). A strong positive correlation was recently demonstrated between activated T cells, monocytes, and NK cells and the IFN-stimulated genes (ISG), CXCL10, and abnormal liver function tests in CHB (Bayard et al. 2012). CXCL10 is produced by hepatocytes within the HBV-infected liver and has been associated with intrahepatic immune activity and liver damage (Fig. 4a). CHB is also associated with hypo-responsiveness of the TLR2 and TLR4 pathways. The importance of NK cells in HBV control may be limited to the intrahepatic compartment.

Impact of HIV on HBV

Patients with HIV-HBV coinfection have higher HBV DNA, lower rates of seroconversion, and faster progression to liver disease compared to HBV-mono-infected patients. This is accompanied by impaired HBV-specific T-cell response and increased intrahepatic apoptosis with an increase in liver-infiltrating IL-10-producing CD8⁺ T cells.

HIV significantly depletes CD4⁺ T cells in the gastrointestinal (GI) tract leading to increased microbial translocation (► [Microbial Translocation](#)), measured by elevated levels of lipopolysaccharide (LPS), part of the cell wall from gram-negative bacteria (Fig. 4b). HIV-HBV-coinfecting patients have significantly increased levels of circulating LPS and soluble (s)CD14 and inflammatory chemokines such as CXCL10 compared to HBV-mono-infected patients (Crane et al. 2014). LPS can directly stimulate Kupffer cells and hepatic stellate cells, contributing to liver fibrogenesis. This

pro-inflammatory environment with production of CXCL10 promotes chronic immune activation by recruitment of activated T cells, monocytes, and NK cells into the liver.

Impact of HBV on HIV

HIV has limited impact on the natural history of HIV infection. However, several nucleoside reverse transcriptase inhibitors (NRTI) that inhibit HIV reverse transcriptase (RT) also inhibit the HBV RT. Although HBV is a DNA virus, its replication occurs through an RNA intermediate which requires RT activity by the HBV polymerase. Therefore, HIV and HBV are often treated together using NRTI such as tenofovir and lamivudine that act against HIV and HBV, called HBV-active cART. Whether the liver is a potential anatomical reservoir for latent HIV is uncertain.

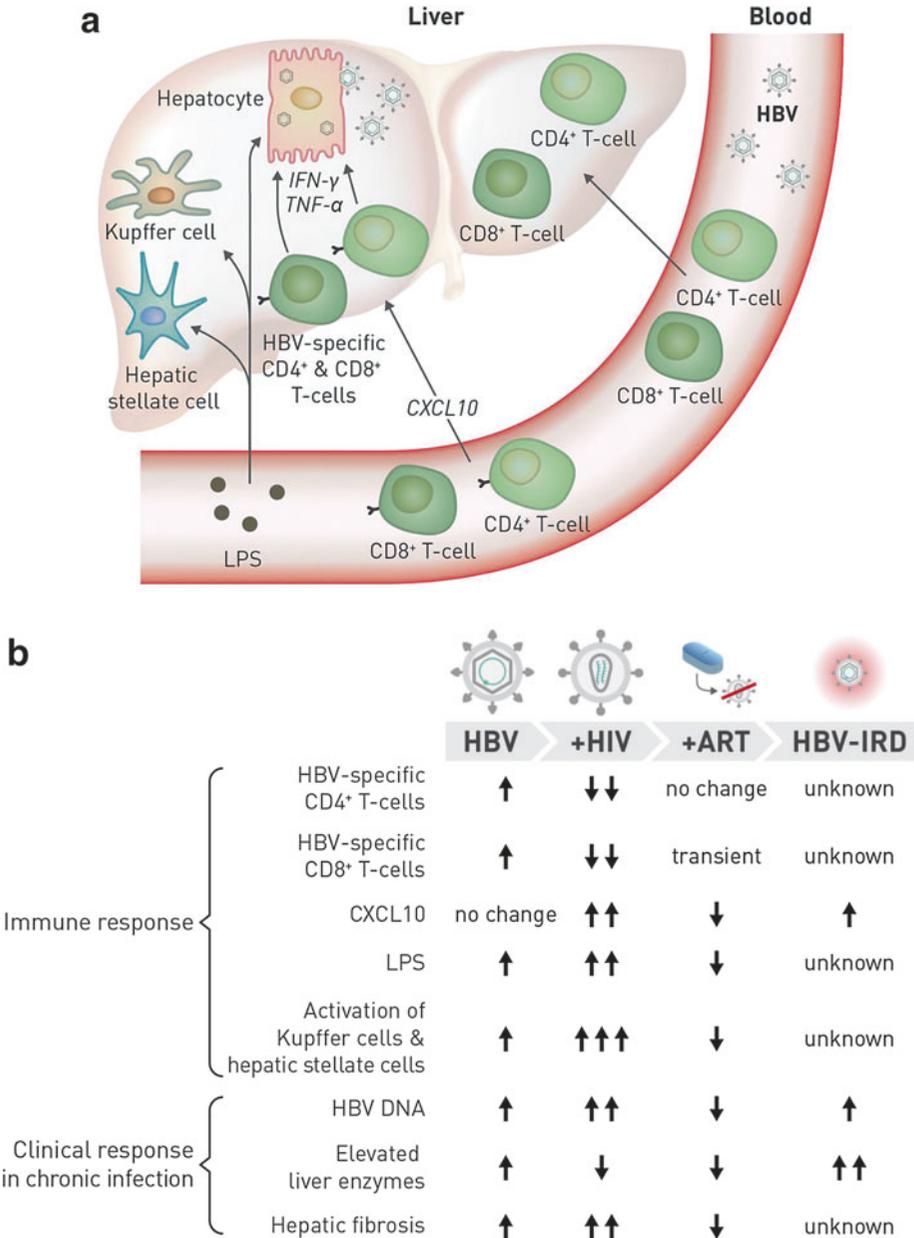
Impact of cART on CHB, Including IRD

HBV-active cART has substantially reduced liver-related mortality and improved rates of HBeAg and HBsAg seroconversion. HBV-active cART has been shown to lead to transient recovery of HBV-specific and mitogen-responsive CD8⁺ T cells. As with other coinfections, HBV-IRD has been described and can manifest as worsening liver function (or hepatic flare) which is often asymptomatic, but occasional deaths have been reported in this setting. Patients with low CD4⁺ T-cell counts and high HBV DNA prior to initiation of HBV-active cART are at highest risk of HBV-related IRD. The elevated CXCL10 seen in HIV-HBV coinfection does not decline despite initiation of HBV-active cART, and this may drive ongoing recruitment of inflammatory T cells into the liver contributing to hepatic destruction (Crane et al. 2009; Fig. 4b).

Hepatitis C Virus Coinfection

Epidemiology and Global Burden of Disease

Approximately 30% of HIV-infected individuals in high-income countries are coinfecting with hepatitis C virus (HCV) – likely acquired via the parenteral route (► [Comorbidity: Hepatitis C](#)).



Immunopathogenesis of HIV Coinfections, Fig. 4 Pathogenesis of chronic hepatitis B infection and impact of HIV and cART. (a) HBV primarily infects hepatocytes and is non-cytopathic. HBV antigens are presented by classical antigen-presenting cells as well as hepatocytes and induce both HBV-specific CD4⁺ and CD8⁺ T cells. HBV-infected hepatocytes can be cleared by HBV-specific T cells with the production of IFN-gamma and TNF-alpha or by direct cytotoxicity. In chronic HBV infection, there is a low frequency of exhausted HBV-specific T cells which are unable to

effectively clear infected hepatocytes. In chronic HBV infection, both HBV-specific and non-HBV-specific T cells are recruited to the liver by chemokines such as CXCL10, leading to hepatotoxicity and chronic liver inflammation. Finally, lipopolysaccharide (LPS) can contribute to HBV pathogenesis by direct activation of hepatocytes, Kupffer cells, and hepatic stellate cells (HSC). Stimulation of HSC by LPS increases production of pro-inflammatory cytokines leading to fibrosis. (b) Impact of HIV and cART on the immune response and clinical manifestations of chronic HBV

Natural History of HCV

Acute HCV infection is usually asymptomatic, but 75% of cases progress to chronic persistent infection. Approximately 5–10% of patients with chronic HCV will develop liver cirrhosis and/or hepatocellular carcinoma (HCC) per decade of infection. Treatments for HCV are rapidly improving, and cure is now possible for many patients.

Immune Response to HCV and Pathogenesis

Acute HCV Mono-infection

An early innate immune response is followed by an adaptive immune response to HCV. This leads to expression of ISGs, NK cell activation, and presentation of HCV antigens by immature dendritic cells to initiate adaptive immune responses, involving both CD4+ and CD8+ T cells (reviewed in Dustin et al. (2014)). HCV-specific CD4+ and CD8+ T cells are critical. An early, vigorous, and sustained CD4+ T-cell proliferative response against multiple HCV proteins and development of neutralizing antibody responses have been associated with an increased likelihood of immune clearance.

Chronic HCV Mono-infection

Hepatocellular injury and death occur as a result of host immune response rather than virus-mediated damage (Dustin et al. 2014). The severity of hepatic necro-inflammation is a key predictor of the rate of fibrosis and hence progression to cirrhosis and late-stage complications. Increased CD4+ T-cell activation in the parenchyma is associated with more severe hepatitis, and increased HCV-specific CD8+ T-cell in liver-derived T cells is associated with worsened hepatic necro-inflammatory activity (discussed in Chang et al. 2013). The leukocyte subsets that infiltrate the portal tracts and lobules differ – CD4+ and CD8+ T cells and B cells accumulate in the former, while CD8+ T cells predominate in the latter. Microvesicular fat accumulation (steatosis) is common. The recruitment of inflammatory cells into the liver is facilitated by expression of the IFN-inducible chemokines CXCL10 and CCL3, CCL4, and CCL5 that are ligands for CXCR3 and CCR5, respectively. These chemokines have been

shown to be increased in HCV-infected liver – in particular, circulating plasma CXCL10 levels have been shown to correlate with intrahepatic mRNA expression in chronic HCV infection (Askarieh et al. 2010).

Hepatic Fibrosis

Activated Kupffer cells produce pro-inflammatory and pro-fibrogenic cytokines, such as TNF-alpha and transforming growth factor (TGF)-beta (reviewed in Boltjes et al. (2014)). These activated hepatic stellate cells mediate liver fibrosis. Insulin resistance has also been implicated in promoting hepatic fibrosis.

Impact of HIV on HCV

Acute HCV

HIV-HCV coinfection leads to an increased HCV viral load, a lower rate of HCV clearance, a higher and faster risk of progression to chronic hepatitis and hepatic fibrosis, and hence higher rates of cirrhosis, liver failure, and hepatocellular carcinoma. HIV-HCV coinfection is associated with reduced HCV-specific T cells, thereby reducing the rates of spontaneous clearance. Higher rates of HCV persistence after primary infection have been shown in patients with lower CD4+ T-cell counts.

Chronic HCV

Studies of individuals with existing chronic HCV, before and after HIV seroconversion, have shown that HCV viral loads increase following HIV infection. Patients with lower CD4+ T-cell counts have higher HCV viremia, lower HCV-specific antibody responses, and a decline in functional HCV-specific CD8+ T-cell responses in the peripheral blood, while the HCV-specific CD8+ T-cell responses in the liver are maintained. A direct interaction between HIV and HCV may be relevant given that envelope protein of HIV (gp120) has been shown to increase HCV replication *in vitro*.

Hepatic Fibrosis

Using *in vitro* models, HIV has been shown to potentially have a direct role in liver disease

progression. HIV infection of both hepatic stellate cell (HSC) lines and primary cells has been demonstrated in vitro and has been associated with elevated levels of markers of HSC activation such as alpha smooth muscle actin and the monocyte chemoattractant CCL2. HIV may also have an indirect role via depletion of CD4+ T cells in the GI tract leading to elevated circulating LPS which can also directly activate HSC and Kupffer cells and therefore can potentially promote fibrosis.

Impact of HCV on HIV

Hepatitis C infection also results in continuous immune activation and thus may accelerate progression to AIDS. The role of the liver as an anatomical reservoir for HIV is unclear.

Impact of cART, Including IRD

Hepatic flares are common in HIV-HCV-coinfected patients initiating cART, but these are usually mild and self-limited, although they can rarely result in hepatic decompensation. These episodes have been associated with an increased Th1 response and higher levels of anti-HCV antibodies (Cameron et al. 2011).

Malaria Coinfection

Epidemiology and Global Burden of Disease

Malaria is caused by parasites of the genus *Plasmodium* spp., of which *P. vivax* is more geographically widespread, but *P. falciparum* is most deadly with more than 1 million deaths each year and thus will be the focus of the discussion here. There is considerable geographical overlap with areas of high HIV prevalence.

Natural History of Malaria

Severe anemia, cerebral malaria, metabolic acidosis, and acute respiratory distress syndrome are the major causes of death. Malaria infections begin with the injection of parasite sporozoites by infected mosquitoes, which then invade hepatocytes and undergo massive proliferation to form merozoites which are released into the blood to

invade erythrocytes. These infected red blood cells (iRBCs) express malaria antigens, some of which (such as pfEMP1) cause attachment of the iRBC to endothelial surfaces. Sequestration of iRBC in capillaries may disrupt blood flow, causing tissue hypoxia and lactic acidosis, as well as induce inflammatory responses, resulting in end-organ damage.

In malaria endemic areas, humans usually acquire protective natural immunity to severe clinical disease after one or two episodes of infection, whereas immunity to mild disease accumulates very slowly. Pregnancy-associated malaria results in morbidity to both the mother and baby.

Immune Response to Malaria and Pathogenesis

Immunity to malaria is primarily mediated by humoral immunity, and protection is mediated by acquisition of antibodies to malaria-associated neo-antigens expressed on the surface of the infected erythrocyte. Preerythrocytic antibodies may prevent sporozoite invasion of hepatocytes. The sporozoites can drain to the lymph nodes where they are able to prime T and B cells (Riley and Stewart 2013). IFN-gamma-producing CD4+ and CD8+ T cells are able to inhibit parasite development into merozoites, and merozoite-specific antibodies can agglutinate and opsonize the parasite and can inhibit the invasion of red blood cells through receptor blockade (Riley and Stewart 2013). Antibodies to variant surface antigens (VSA) expressed on iRBCs may also opsonize and agglutinate iRBCs and prevent their sequestration in the microvasculature (Riley and Stewart 2013).

Parasites and iRBCs activate DCs through pattern recognition receptors and are phagocytosed, and their antigens are presented to T cells (Riley and Stewart 2013). Th1 cells promote B-cell differentiation and antibody secretion and also secrete IFN-gamma which activates macrophages which in turn phagocytose and kill opsonized merozoites and iRBCs (Riley and Stewart 2013) by also secreting pro-inflammatory cytokines in response. Elevated plasma pro-inflammatory cytokines such as TNF-alpha and IL-6 are seen

in severe malarial disease and cerebral malaria in patients infected with *P. falciparum*. In pregnancy, this pro-inflammatory state is not only seen in the blood but also in placenta.

Impact of HIV on Malaria

HIV-infected patients have increased incidence and severity of malaria and repeated malaria infections (Chang et al. 2013). Cohort studies suggest HIV infection was associated with increased peripheral parasitemia and increased placental parasite densities (Hochman and Kim 2009).

In vitro, HIV-infected human macrophages exhibit lower opsonic phagocytosis activity, and HIV-infected pregnant women develop fewer antibodies to VSAs (Hochman and Kim 2009). HIV-related inflammation upregulates adhesion molecules on endothelial cells which may compound the adherence and sequestration seen in malaria (Hochman and Kim 2009).

Impact of *P. falciparum* on HIV

HIV-infected patients with malaria have a more rapid decline in CD4 T cells and have an increased, albeit transient viremia, at the time of increased parasitemia (Hochman and Kim 2009). Binding of recombinant *P. falciparum* adhesin to chondroitin sulfate A on human placental cells is associated with increased HIV replication (Gonzalez et al. 2012). Placental HIV viral RNA is increased, and CCR5 receptor expression is upregulated in women with placental malaria which may increase mother-to-child HIV transmission (Gonzalez et al. 2012).

Impact of cART Including IRD

There are few studies on the efficacy of cART in preventing severe malaria in children or adults. There are no reported cases of malaria-associated IRD possibly because protective immune responses are humoral rather than cellular.

Conclusions

Coinfections remain a major threat to HIV-infected patients globally, particularly in

resource-limited settings where access to cART is limited and diagnostic methods and treatment for coinfections are scarce. Coinfections in HIV not only increase patient morbidity and mortality but can impact directly on HIV viral replication, microbial translocation, and overall systemic immune activation. A deeper understanding of the interactions between HIV, host deficiency, and exogenous pathogens is necessary to develop immunotherapy, novel molecules, and vaccine strategies to adequately manage and/or prevent coinfections in HIV-infected patients.

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Inflammasome and HIV

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Definition

Inflammation is meant to be a transient and non-specific process dedicated to exert a physical and chemical barrier against an incoming injurious stimulus. This process is ignited in the challenged tissues by injured or infected cells which release some alarmins prone to rapidly warn and attract resident immune cells at the challenged site. Some of these cells will then capture the threat and recognize some danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) via their interaction with specific pattern recognition receptors (PRRs). Some of these PRRs are able to associate with other cellular factors and the caspase-1 to form specific multiprotein complexes, called inflammasomes. Formation of these platforms allows a rapid amplification of the inflammatory cascade upon caspase-1 activation, thereby leading to the maturation and release of the active IL-1 β pro-inflammatory cytokine. Recently, a plausible explanation for the massive CD4⁺ T-cell depletion observed during HIV infection relied on the activation of specific inflammasomes in abortively infected CD4⁺ T cells leading to an inflammation-associated form of cell death called pyroptosis. Although still in its early stages, this hypothesis raises hope for potential therapeutic approaches in the field of HIV pathogenesis.

HIV and Chronic Inflammation: Proof of Statement

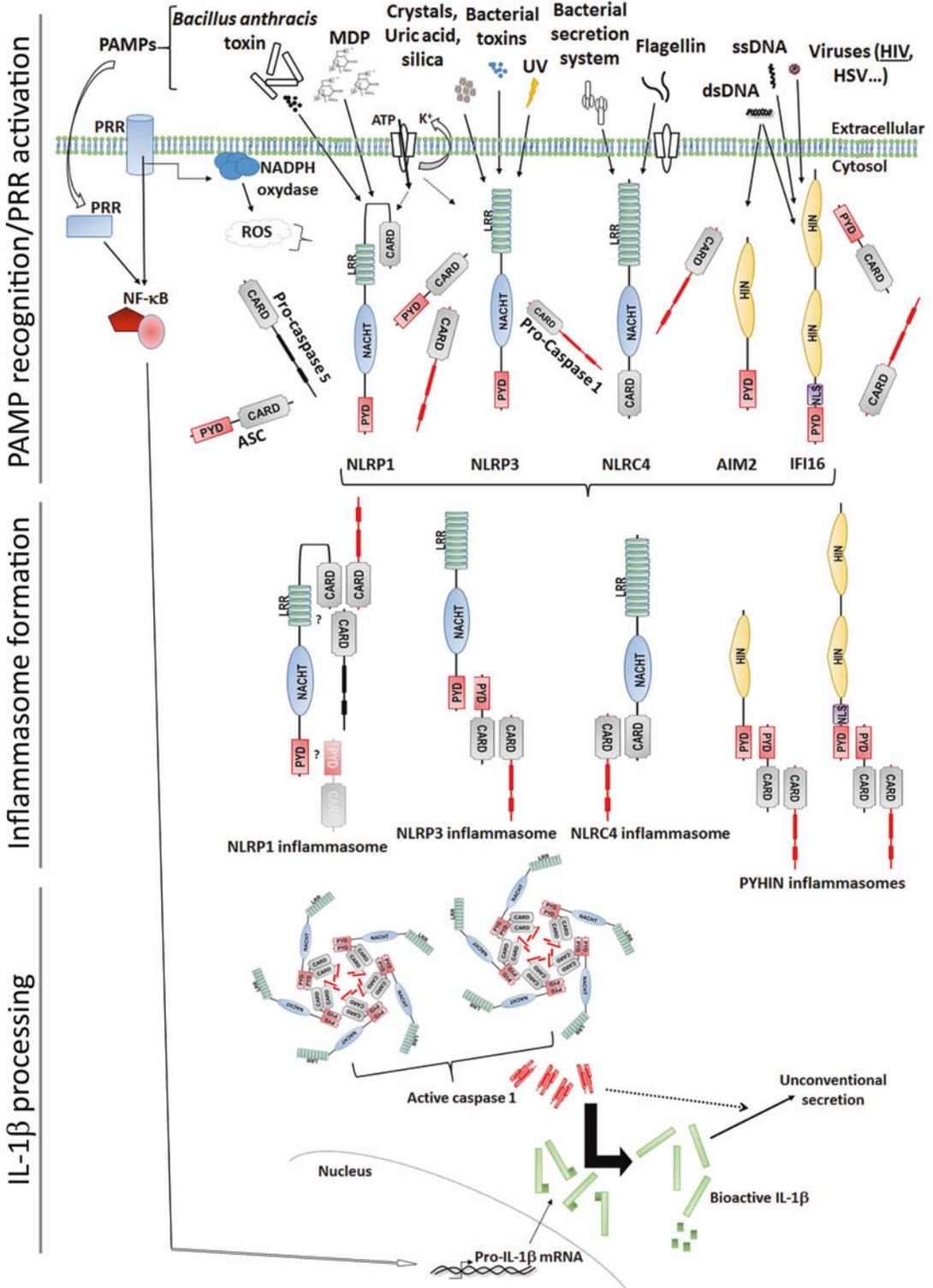
While the prevalence of acquired immunodeficiency syndrome (AIDS) has engaged in a decreasing curve, mainly due to combinations of

antiretroviral therapy (ART) in the last three decades, the HIV-mediated pathogenesis could be now referred to as a chronic disease associated with non-AIDS-related illness and morbidity. Indeed, non-AIDS disorders appear to be higher than expected in HIV-infected adults under ART with a particularly alarming situation regarding cardiovascular diseases, kidney disease, osteoporosis, cancer, and some neurological diseases. There is now a growing line of evidence that most of these complications come from a systemic immune activation linked to an uncontrolled chronic inflammation (“► [Chronic immune activation in HIV](#)”). In fact, the link between HIV-mediated pathogenesis and persistent inflammation was already strongly evidenced in initial reports showing an increase of inflammatory markers in sera from untreated HIV-infected patients at all stages of the disease. Nevertheless, this trend was also described for infected patients under ART regimen suggesting that, although viral replication was controlled and immune functions were almost fully restored, there was still an evident uncontrolled and sustained inflammatory state with a consequent shift toward non-AIDS-related disorders (Deeks et al. 2013). Although traditional risk factors (obesity, alcohol, smoking, etc.) and antiretroviral drug toxicity (insulin resistance, diabetes, hyperlipidemia, etc.) could contribute to such advert effects, they do not entirely recapitulate and explain the symptoms preceding non-AIDS morbidity. There are many other different events and factors thought to contribute to the persistent inflammatory state among which a chronic activation of some T-cell subsets and cells of the monocyte/macrophage lineage; breakdown and leakage of the gut mucosa leading to chronic translocation and exposure to gut microbial products; prominent coinfections by other pathogens like cytomegalovirus (CMV), Epstein-Barr virus (EBV), or herpesviruses (HSVs); and altered functions of T regulatory cells (Treg) and immune regulatory cytokines might be the most plausible reasons. However, while most of these conditions should be largely attenuated in appropriately treated patients, the inflammatory state still persists. There is an intense research activity aiming at further elucidating the reasons for the remaining

CD4⁺ T-cell loss and HIV-associated chronic inflammation occurring in ART-treated patients, and recent studies suggest that a residual HIV replication in tissues could persistently fuel abortive infection of resident CD4⁺ T cells, thus leading to viral DNA accumulation which could be sensed by a specific PRR contributing to inflammasome activation and pyroptotic cell death.

Inflammasome Assembly

The inflammasome is a high molecular weight multiprotein complex (~700 kDa) whose assembly ultimately leads to the clustering and activation of caspase-1, involved in the processing of the pro-inflammatory cytokines IL-1 β and IL-18 and their release via an unconventional secretion pathway (Schroder and Tschopp 2010). Caspase-1 belongs to the family of cysteine proteases and reported to be key mediators of apoptotic and inflammatory processes. They are usually synthesized as inactive proenzymes or zymogens which contain three domains subsequently processed into large and small subunits that associate to form the active enzyme. While most caspases share a common structure, they could be divided based on their known roles in apoptosis (caspase-3, caspase-6, caspase-7, caspase-8, and caspase-9 in mammals) or in inflammation (caspase-1, caspase-4, caspase-5, and caspase-12 in humans). For instance, inflammatory caspases were firmly established as critical mediators of innate immune responses rather than being proapoptotic factors, although they could also promote a non-homeostatic and lytic mode of cell death called pyroptosis in parallel to the release of the pro-inflammatory cytokines IL-1 β and IL-18. Caspase-1 (also named ICE for IL-1 β -converting enzyme) is the prototypic member of this family of inflammatory caspases (including human caspase-4/11 and caspase-5) which all contain an N-terminal caspase recruitment domain (CARD) (Fig. 1). However, the reason why these caspases are more prone to regulate inflammatory processes is still poorly understood, but a possible explanation would rely on the secretion of caspase-1, also described for caspase-5, upon



Inflammasome and HIV, Fig. 1 (continued)

inflammasome activation. Indeed, this secretion is not firmly established for the other proapoptotic caspases, and it is therefore tempting to speculate that a parallel secretion of caspase-1 could favor the survival of activated cells. In agreement with this, a recent study reported that secretion of an oligomeric NLRP3 inflammasome stimulated further activation of caspase-1 extracellularly, therefore amplifying the inflammatory response.

Interestingly, different types of inflammasome have been identified to date, mainly based on the sensor involved in its assembly (Fig. 1). At least four canonical and one noncanonical inflammasomes have been reported until now:

1. The founding inflammasome coined **NALP1 inflammasome** was reported to contain NLRP1 (Nod-like receptor protein 1 and also named NALP1 for NACHT-, LRR-, and PYD-containing protein), the adaptor protein ASC (apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain), caspase-1, and caspase-5 (Martinon et al. 2002) and was mainly involved in the production of IL-1 β upon TLR-mediated recognition of bacterial and more recently parasitic infections.
2. The **NLRP3 inflammasome** composed, in addition to NLRP3, of the caspase recruitment domain (CARD)-containing protein CARDINAL, ASC, and caspase-1 was shown to regulate inflammation in a reactive oxygen species (ROS)-dependent manner upon detection of chemical irritants and host-derived danger signals (ATP, hyaluronic and uric acids, asbestos, silica, etc.) or bacterial toxins and RNA (Schroder et al. 2010; Tschopp and Schroder 2010). Interestingly, this inflammasome was recently reported to be activated upon ionic flux perturbations possibly created by viroporins like the M2 viral protein from influenza virus, the SH protein from RSV, or the protein 2B from encephalomyocarditis virus (Triantafilou and Triantafilou 2014).
3. The **NLRC4 inflammasome** (previously named IPAF inflammasome) characterized by the ability of NLRC4 to interact directly with caspase-1, therefore rendering ASC presence dispensable for minimal activity, was reported to be activated upon infections with gram-negative bacteria possessing type III or IV secretion systems such as *Legionella pneumophila*, *Salmonella Typhimurium*, *Pseudomonas aeruginosa*, or *Shigella flexneri* (Schroder and Tschopp 2010).
4. Some DNA sensors were recently involved in inflammasome assembly and activation upon viral DNA recognition. Among these, a protein from the HIN-200 family called absent in melanoma-2 (AIM-2) was reported to trigger **AIM-2 inflammasome** formation upon recognition of bacterial, viral, and even host DNA in cases of autoimmune disorders (Rathinam et al. 2012). Interestingly, IFI16, another member of the same family of proteins, was recently

Inflammasome and HIV, Fig. 1 Activation of inflammasomes controls IL-1 β processing and release. This figure represents a schematic view of inflammasome activation and IL-1 β processing and release in three steps: (1) PAMPs recognition/PRR activation: various PAMPs and DAMPs induce a first signal upon recognition by specific PRR leading to NF- κ B activation and pro-IL-1 β expression. Concomitantly, a second signal, which may involve potassium efflux, membrane damage, and generation of reactive oxygen species (ROS), is generated through either NLRP1 (responding to Bacillus anthracis lethal factor or muramyl dipeptide (MDP)), NLRP3 (responding to crystalline compounds, bacterial toxins, or UV), NLRC4 (responding to bacterial secretion system subunits or flagellin), AIM2 (recognizing cytosolic dsDNA from bacterial, viral, and mammalian origin), or IFI16 (recognizing cytosolic or nuclear dsDNA and ssDNA from viruses like HIV-1 and HSV).

(2) Inflammasome Formation: once activated, these cellular sensors form multiprotein complexes with ASC (except NLRC4) and procaspase 1, leading to inflammasome assembly. Each complex is formed through homotypic interactions and allows proximity-induced dimerization and activation of caspase-1. (3) IL-1 β processing: the precursor form of IL-1 β (pro-IL-1 β), expressed upon PRR-mediated NF- κ B activation, is then cleaved by caspase-1 and released from cells via an unconventional secretion pathway. Abbreviations used: *CARD* caspase activation and recruitment domain, *NLS* nuclear localization signal, *NACHT* NAIP, CIITA, HET-E, and TP1 domain, *LRR* leucine-rich repeats, *PYD* pyrin domain, *HIN* hemopoietic IFN-inducible nuclear proteins, *PRR* pattern recognition receptor, *ATP* adenosine triphosphate, *NADPH* nicotinamide adenine dinucleotide phosphate oxidase

proposed to contribute also to inflammasome activation upon abortive HIV infection of CD4⁺ T cells (Monroe et al. 2014), and this will be discussed below. This recent discovery leads to the hypothesis that other members from the same family, like PYHIN1 (also named IFIX) or myeloid cell nuclear differentiation antigen (MNDNA), could contribute to inflammasome activation, although the nature and source of recognized DNA require further investigation. Therefore, a new kind of inflammasome could emerge, namely, the **PYHIN inflammasome**, also reinforced by the fact that the majority of these proteins possess a pyrin domain at their N-terminus, hence inducible by type I and type II interferons, highly suggestive of a role in antiviral immunity. Although their mechanism of action is now intensively scrutinized, it seems that an evolutionary close family of proteins, namely, pyrin domain-only proteins (POP1, POP2, and POP3), could antagonize PYHIN inflammasomes function by competing with ASC recruitment. Another important aspect will be to decipher the subcellular localization of DNA recognition and subsequent spatio-temporal regulation of PYHIN inflammasomes as HIN-200 proteins harbor one or more nuclear localization signal(s) (NLS) and were indeed, in the case of IFI16, visualized in the nucleus upon herpes simplex virus (HSV) infection.

5. Recently, a noncanonical inflammasome was identified in mice and characterized by the contribution of caspase-11 in pyroptosis and in caspase-1-dependent IL-1 β and IL-18 secretion upon gram-negative bacteria infection (Lamkanfi and Dixit 2014). However, little is known in humans for which caspase-4 and caspase-5 are thought to be the orthologs of murine caspase-11, based on sequence identity. Although this noncanonical inflammasome seems to intervene in specific settings of infection, the contribution in other inflammasomes of caspase-11 in mice, and caspase-4/5 in humans, will need to be revisited in light of the discovery that *caspase-1* knockout mice were also impaired in caspase-11 expression.

In a mechanistic view, the assembly of inflammasomes is quite simple, although the amount, subcellular localization, and heterogeneity of the factors involved suggest this process to be tightly regulated. Briefly, the sensors are the central proteins in the inflammasome complex which is induced upon recognition of their specific ligand. Most of these sensors, except NLRC4 which harbors only an N-terminal CARD domain, possess an N-terminal pyrin domain (PYD) allowing interaction with the PYD domain of ASC (Fig. 1). ASC is also composed of a CARD domain which makes it an essential component of the inflammasome upon binding with the CARD domain of procaspase-1, and also procaspase-5 for NLRP1 inflammasome, therefore creating a bridge between the sensor and the protease. Then, ASC-mediated recruitment favors the proximity-dependent caspase-1 autocleavage leading to the generation of multimers containing stably active caspase-1 molecules, a structure reminiscent of apoptotic protease-activating factor 1 (APAF-1)-mediated apoptosome assembly required for caspase-9 activation. The activated caspase-1 present in inflammasomes cleaves the cytosolic -1 β (pro-IL-1 β) and pro-interleukin-18 (pro-IL-18) to convert them to active cytokines which will be released by an unconventional secretion pathway (Keller et al. 2008). While pro-IL-18 might be constitutively expressed in some myeloid cells, pro-IL-1 β expression requires a priming wave initiated upon ligand recognition by a PRR and converging toward NF- κ B activation (signal 1). Therefore, inflammasome activation could be viewed as a synergistic structure able to tune the amplification of pro-inflammatory processes with the ability to potentiate the activation and secretion of highly pyrogenic cytokines.

Inflammasome and Innate Immunity: IGNITION!

Inflammation arises from the activation and migration of various immune cells, mainly initiated by a complex cascade of events upon detection and recognition of a potential threatening

entity. Briefly, the process of inflammation starts with signals released by injured or infected cells at the challenged site which, consequently and rapidly, warn and attract resident neighboring immune cells, like macrophages, monocytes, or dendritic cells. These newly attracted cells would, therefore, take over and “ignite” the innate immune response by inducing expression and release of pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6, among others) upon ingestion and recognition of the incoming threat. The pro-inflammatory components will fuel the innate immune response while chemokines will further attract specific immune cells to help in containing, degrading, and clearing the harmful elements. However, an uncontrolled and prolonged inflammation could have deleterious consequences and therefore needs to be turned off upon healing or resolution of infection. Indeed, if inflammation is not properly regulated at both the onset and after completion, then a chronic inflammatory state could occur as described for some autoimmune diseases like multiple sclerosis (MS) or inflammatory bowel diseases (IBDs).

In the case of microbial infections, specific molecular signatures of the incoming pathogen (nucleic acid, proteins, or glycolipids) could be sensed by specific PRRs, particularly in cells from a myeloid lineage which constitutively express a broad range of these receptors (“Pathogen Associated Molecular Patterns” and “Pathogen Recognition Receptors (general)”). In most circumstances, PAMPs will be sensed extracellularly by PRRs from the toll-like receptor (TLR) or C-type lectin receptor (CLR) families or intracellularly by proteins belonging to the RIG-I-like receptor (RLR) or the Nod-like receptor (NLR) families, therefore covering a wide range of possible recognized ligands or patterns. Stimulation of these pathways will converge toward the activation of the master transcriptional regulator NF- κ B and the subsequent transcription of pro-IL-1 β (signal 1), in parallel with the assembly of the engaged sensor with specific cellular components like ASC and the procaspase-1, therefore forming inflammasomes (Martinon et al. 2002; Pettrilli et al. 2005) (Fig. 1). Inflammasomes constitute, therefore, an effector arm of the innate

immune response by selectively regulating the availability of active pro-inflammatory cytokines.

An important remaining question is the characterization of the molecular mechanisms driving inflammasome assembly and activation. Briefly, some drugs or chemical messengers, like nigericin or ATP respectively, are potent activators of inflammasomes by modulating ion efflux, in particular calcium (Ca²⁺) and potassium (K⁺), through ion channel receptors (like the P2X purinoreceptor 7 P2X₇) therefore leading to a lowered intracellular potassium concentration. This hypotonic stress condition, coupled to the clustering of P2X₇ receptor with the hemichannel receptor Pannexin-I, sets the condition up for inflammasome assembly and activation. Nevertheless, all inflammasomes are not sensitive to ion efflux, like the NLRC4 inflammasome, and activation of another more common pathway was then thought to take place. Interestingly, most of the inflammasomes agonists induce the generation of reactive oxygen species (ROS), one of the most evolutionarily conserved responses upon infection or stress. This kind of free radicals could be produced, upon rapid uptake of oxygen, by the activation of a membrane-bound enzymatic complex composed of different nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits. Outstandingly, the involvement of ROS in inflammasome activation was supported by reports showing that ROS production blockade by pharmacological compounds or knockdown of NADPH oxidase expression was potently inhibiting NLRP3 inflammasome. Furthermore, the thioredoxin-interacting protein (TXNIP or VDPU1), a negative regulator of the antioxidant factor thioredoxin, was reported to link oxidative stress with inflammasome activation. Therefore, a tight regulation of the redox system appears to influence inflammasome activity, and it is tempting to hypothesize that an increased quantity of ROS might continuously feed a preexisting inflammatory environment.

All these speculations might make sense in the case of HIV infection when looking at the different biological parameters obtained from infected patients. Indeed, it was known for a long time that HIV-infected patients were presenting all the

features of high oxidative stress with elevated levels of ROS at the onset of infection, and further during all stages of the disease, in marked contrast with a significant decrease of antioxidant levels, like vitamin A, C, and E, glutathione, and a pronounced reduction of thioredoxin in tissues. This imbalance between oxidants and antioxidants species might, therefore, perpetuate an inflammatory environment. But what is the origin of such dysregulation and why ART treatments could apparently not halt these damages are key questions to be addressed in order to improve future therapeutic strategies.

Inflammasome and Acute HIV Infection

The influence of ROS-mediated inflammasome activation could be central in setting up the pathological symptoms as it was shown to greatly contribute to both immune activation and inflammation. However, there are very few reports on HIV and inflammasome in the literature which might not reflect the interest in such topic but rather the fact that the possible conditions and settings necessary to unveil any link between both could be particularly challenging. Indeed, most of the reports on a possible role of inflammasome upon HIV-1 infection were essentially done in myeloid cells or were aimed at deciphering genetic polymorphisms in inflammasome-related genes correlating with susceptibility to HIV-1 infection. Interestingly, some myeloid subsets express constitutive pro-IL18 and are prone to express pro-IL-1 β with suboptimal doses of PAMPs like LPS, making these cells particularly relevant in inflammasome-mediated active IL-1 β and IL-18 secretion. Hence, myeloid cells constitutively express most inflammasome components which could be further greatly enhanced upon PRR-mediated recognition of PAMPs or DAMPs. A recent study, from Pontillo and colleagues, demonstrated that IL-4-differentiated monocyte-derived dendritic cells (MDDCs) pulsed with HIV-1 were highly upregulating *NLRP1*, *NLRP3*, *NLRC4*, *CASP1*, and *IL-1beta* mRNA expression. Interestingly, MDDC from HIV+ patients, derived in the same conditions, showed increased basal levels of

inflammasome-related gene expression, only slightly upregulated when challenged with HIV-1, suggesting that an established chronic inflammatory state in these patients would greatly perturb an optimal response (Pontillo et al. 2013). The same authors reported, also, that HIV-1 was able to induce a NLRP3 inflammasome response accompanied by a significant IL-1 β secretion in MDDC from healthy individuals, indicating a possible functional role of this inflammasome during acute HIV-1 infection (Pontillo et al. 2012b). Furthermore, it was also noticed that HIV-1 susceptibility was associated with single-nucleotide polymorphisms (SNPs) in the *NLRP3* and the *IL1-beta* genes, therefore further supporting a potential role of inflammasome in HIV-1 infection (Pontillo et al. 2012a). Interestingly, a recent study investigating HIV neuropathogenesis revealed a significant association between lentivirus-induced brain disease and NLRP3 inflammasome activation in microglia and likely contributing to neuronal loss in cerebral cortex and neurological deficits as evidenced in vivo in a model of feline immunodeficiency virus (FIV) infection (Walsh et al. 2014).

In a model of human monocyte-derived macrophages (MDMs), a study analyzed the ability of HIV-1 to induce inflammasome activation and showed that HIV-1 was able to promote IL-1 β secretion by inducing the first signal for NLRP3 inflammasome activation as soon as 6 h post-challenge and evidenced in combination with typical inflammasome stimuli, like ATP, Nigericin, or silica. Although, the mechanism of such activation was not characterized, the same level of inflammasome activation was observed when cells were challenged with VSV-G-pseudotyped envelope-deficient (Δ Env) HIV-1 viruses, therefore conclusively showing that HIV-1 contribution to inflammasome activation was envelope independent. Two other very recent reports claimed that the early steps of HIV-1 infection could effectively induce inflammasome activation in human myeloid primary cells or cell lines. Indeed, both studies demonstrated that TLR8-mediated recognition of infection was required for pro-IL-1 β transcription, caspase-1 activation, and secretion of active IL-1 β . While NLRP3

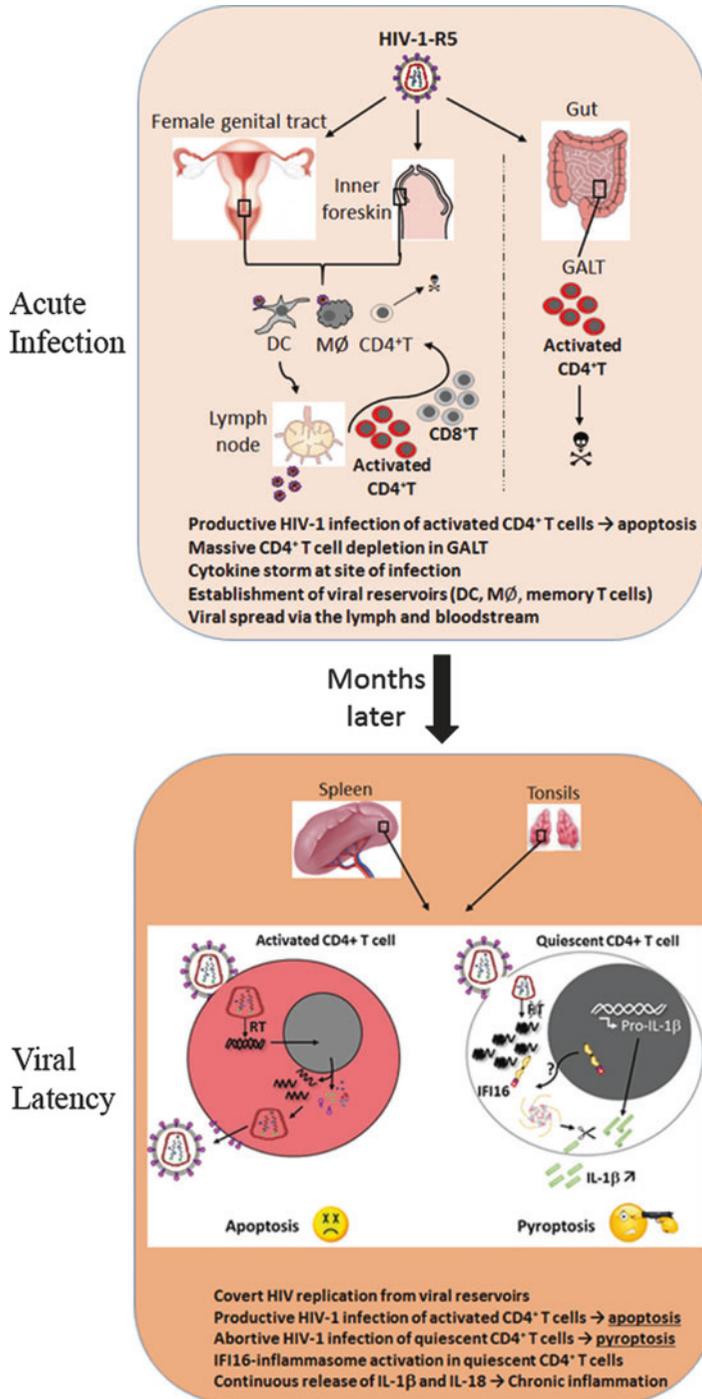
inflammasome was seemingly involved in both reports, some important discrepancies appeared, however, between them. The first study using freshly isolated human primary monocytes noticed no impact of viral fusion inhibitors (Maraviroc and T20) on HIV-1-mediated inflammasome activation, while dynasore, a cell-permeable dynamin inhibitor, or methyl- β -cyclodextrin (M β CD), a cholesterol trapping cyclic oligosaccharide, were potently blocking the virus-mediated effects, suggesting that viral endocytosis was required to induce inflammasome activation but without the need for productive HIV-1 infection (Chattergoon et al. 2014). In contrast, the other report, mainly done in the myelomonocytic leukemia THP-1 cell line, argued that HIV-1 entry, reverse transcription, and integration, but not the maturation step, were necessary to induce inflammasome activation, in parallel with the contribution of the lysosomal protease cathepsin B and virus-mediated intracellular ROS increase (Guo et al. 2014). In light of the current literature, sensing of HIV-1 during the early steps of infection leading to inflammasome activation might be an important aspect to consider in the establishment of inflammatory process, but further investigation will be definitely required to unveil the cellular mechanisms involved and, overall, to assess if uncontrolled inflammasome activation during acute infection could effectively contribute to the chronic inflammation usually observed in HIV-infected patients.

Inflammasome and Later Stages of HIV Infection

The real interest in inflammasome role upon HIV infection started with a study aimed at analyzing the virus-mediated killing of CD4⁺ T cells in human lymphoid tissues (Doitsh et al. 2010). In fact, it was well known that HIV-1 infection could promote apoptosis of CD4⁺ T cells, mainly in an envelope-dependent manner, but the massive cellular depletion occurring in some mucosa and secondary lymphoid organs could not be explained by the low rate of productive HIV infection of CD4⁺ T cells (<5%) (“► HIV

Associated Immune Exhaustion” and “► Lymphocyte Apoptosis”). The concept of “bystander” CD4⁺ T-cell death was born with the notion that a virus-mediated distal effect on neighboring cells could be the cause of such depletion. Based on previous knowledge showing that resting CD4⁺ T cells are particularly refractory to productive infection, Doitsh and colleagues revisited the HIV-mediated bystander CD4⁺ T-cell death, but using a model of human lymphoid aggregated cultures (HLACs) made from freshly dissected human tonsillar tissues or spleen. With this model, they could reproduce the expected massive CD4⁺ T-cell depletion, upon 12 days of infection with a X4-tropic HIV-1 strain, while CD8⁺ T cells levels remained almost unchanged. Surprisingly, they uncovered that CD4⁺ T-cell death was not blocked by reverse transcriptase inhibitors but could be avoided with cultures pretreated with entry or fusion inhibitors, highly suggestive of a cell death mechanism involving nonproductive infection of CD4⁺ T cells. Indeed, they demonstrated that abortive viral DNA synthesis occurring in nonpermissive, quiescent CD4⁺ tonsil T cells was a critical parameter leading to intracellular viral DNA intermediates accumulation which could be detected by specific cellular sensors (“Viral DNA Sensors”), while surprisingly not affecting the cellular DNA damage response, and promoting caspase-1- and caspase-3-mediated pro-inflammatory and pro-apoptotic innate immune responses, respectively.

Of note as well is the fact that their study was done with X4-tropic HIV-1 which, although only appearing in less than 50% of infected patients, is known to be associated with more pronounced depletion of CD4⁺ T cells compared to R5-tropic viruses. Whether the latter could also reproduce such effect was under the scope, but the authors proposed that apoptosis linked to HIV-1 productive infection could predominate during acute infection, for which a massive depletion of CCR5⁺ memory CD4⁺ T cells in gut-associated lymphoid tissue (GALT) was described, while pyroptosis induced in tonsil and spleen tissues may reflect later stages of HIV-1-induced disease (Fig. 2). Interestingly, some studies showed that the probability of X4-tropic virus emergence was



Inflammasome and HIV, Fig. 2 Mechanisms of CD4⁺ T-cell depletion during acute and asymptomatic phases of HIV-1 infection. *Upper scheme:* HIV-1-R5 acute infection in the cervix of the female genital tract or in the inner foreskin targets different mucosal immune cell subsets. HIV-1 productively infects resident CD4⁺ T cells leading

to their apoptosis, while some mucosal-resident dendritic cells (DCs) and macrophages (MØ) can intercept incoming virions in order to initiate immune response. HIV-1 captured by DC can remain infectious during days and reach proximal lymphoid tissues upon DC migration. DC-mediated immune responses allow generation of

higher in HIV-infected patients under ART than in those nontreated which could support the notion of an imbalanced chronic inflammation due to pyroptosis in the later stage of the disease (“► [Pyroptosis and HIV Replication](#)”).

Another interesting point, which remains to be answered, relies on the role of other cellular enzymes shown to recognize viral DNA intermediates and negatively regulate HIV-1 replication, like the three prime repair exonuclease 1 (TREX1), also named DNase III. Trex1 is a 3′–5′ exonuclease abundantly expressed and reported to prevent autoimmune disease by processing aberrant replication single-stranded DNA (ssDNA) intermediates (Yang et al. 2007). Interestingly, TREX1 was also shown to metabolize reverse-transcribed (RT) endogenous retroviral DNA, therefore attenuating both the DNA damage and the interferon stimulatory DNA pathways (Stetson et al. 2008). The discovery linking TREX1 with RT products prompted scientists to investigate the role of TREX1 during retroviral infection, and a study reported that TREX1 was, indeed, degrading HIV DNA intermediates generated during HIV-1 infection and, thereby, preventing recognition from cellular DNA sensors (Yan et al. 2010). Downregulation of TREX1 expression in CD4⁺ T cells, as well as in MDM, was shown to promote HIV DNA accumulation leading to an antiviral response, supposedly upon recognition of HIV DNA by specific DNA sensors. Although the antiviral response was mainly linked to type I interferon production in an IRF-3-dependent manner, it would be interesting to investigate if TREX1, or other DNases,

could exert a negative feedback control on inflammasome activation and decipher any potential lack or overwhelmed TREX1 activity upon HIV abortive infection of quiescent CD4⁺ T cells leading to pyroptosis (“► [Pyroptosis and HIV Replication](#)”).

Nevertheless, the proposed model of pyroptotic-mediated CD4⁺ T-cell depletion would perfectly fit with a long-term escalating and uncontrolled inflammatory process linked to chronic infection and immune activation, but the cellular sensor involved was still missing. It is only very recently that the same group of research uncovered IFI16 as a critical sensor of intracellular HIV-derived DNA accumulation upon abortive infection (“PRRs in HIV Recognition” and Monroe et al. 2014). Indeed, the authors developed an unbiased proteomic approach involving an affinity chromatography using a biotinylated 500-bp HIV-1 Nef DNA fragment hypothesized to represent a Nef-derived reverse-transcribed DNA likely present during abortive HIV infection. The HIV DNA was therefore incubated with tonsillar CD4⁺ T cells lysates followed by streptavidin immunoprecipitation prior to mass spectrometry analysis of the cellular proteins co-immunoprecipitated with HIV-Nef DNA. With this methodology, the authors identified numerous proteins for which IFI16 and IFIX were among the top 6 hits. Upon shRNA-mediated gene silencing experiments, they consistently observed that cell death was not occurring only when tonsillar cells were transduced with shIFI16-expressing vectors, while still observable in shSTING-, shFIX-, shAIM2-, and shDNA-

Inflammasome and HIV, Fig. 2 (continued) HIV-specific CD8⁺ and CD4⁺ T cells which can migrate to the mucosal site of infection. Lymph nodes become a site of intense HIV-1 replication due to interactions between HIV-containing DC and CD4⁺ T cells thus establishing viral reservoirs and facilitating infection propagation via the lymph and bloodstream. The same events occur in gut-associated lymphoid tissues (GALTs) upon HIV-1-R5 infection but with the hallmark of a massive CD4⁺ T-cell depletion. *Lower scheme:* In a model of spleen-derived or tonsils-derived cell culture, CD4⁺ T cells are either productively infected or resistant to HIV-1 replication. In permissive activated CD4⁺ T cells

(*left*), reverse transcription of the viral genome is efficient without recognition from cellular DNA sensors, leading to integration of viral DNA into the host cell genome and production of new virions. Productive HIV-1 infection results in caspase-3-dependent apoptosis. In quiescent CD4⁺ T cells, HIV-1 infection is restricted resulting in abortive reverse transcription and accumulation of viral DNA intermediates. These RT products are detected by IFI16 in the cytoplasm which forms inflammasomes able to activate caspase-1, involved in pro-IL-1 β processing, and release of the bioactive cytokine. Abortive HIV-1 infection results in caspase-1-dependent pyroptosis corresponding to a “cell suicide” mechanism

PK1-treated samples. While this study allowed the identification of IFI16 as a HIV DNA sensor involved in cell death of nonpermissive quiescent CD4⁺ T cells, it remains to determine the mechanism by which IFI16 is exerting its effects. Indeed, this DNA sensor was already reported to detect and respond to other viral infections, like CMV, HSV, or EBV, and drive inflammasome assembly with procaspase-1 and ASC. Furthermore, IFI16 is predominantly nuclear due to the presence of a NLS seemingly acetylated, and in the case of HSV infection, functional IFI16 inflammasomes were effectively identified in the nucleus. However, IFI16 was shown to rapidly translocate into the cytoplasm upon transfection of HIV-derived stem-rich ssDNA and also upon 4 h of HIV infection of human MDM (Jakobsen et al. 2013). The mechanisms involved in IFI16 cytoplasmic translocation upon HIV infection and how IFI16 recognizes viral DNA intermediates will need to be identified in the future.

There is, therefore, a convergence toward a possible detection of HIV-derived DNA intermediates by IFI16 inflammasome which would ultimately lead to a caspase-1-dependent pyrogenic form of cell death (Fig. 2). This was further confirmed by the group of Warner Greene in a recent report aimed at tackling HIV-1-mediated pyroptosis of tonsillar and spleen CD4⁺ T cells in their HLAC model by using pharmacological compounds (Doitsh et al. 2014). Importantly, in this study, they reproduced the pyroptotic phenotype with a R5-tropic virus, whose occurrence was also confirmed in a model of Lamina Propria Aggregate Culture (LPAC) by others (Steele et al. 2014). Interestingly, in the LPAC model, the researchers observed that microbial exposure (*Escherichia coli*) led to an increased productive infection, thereby shifting HIV-1-R5-induced caspase-1-mediated pyroptosis of lymphoid CD4⁺ T cells toward a predominant caspase-3-dependent apoptosis of infected cells. Anyway, these reports suggest that pyroptotic cell death is likely occurring in tissues in vivo, although the relevance might be questionable due to the very small proportion of CCR5⁺ cells present in some tissues. Nevertheless, Doitsh and colleagues identified in the HLAC a subset of CD4⁺/CCR5⁺

lymphoid cells, also expressing CXCR4, presenting constitutively high levels of pro-IL-1 β and becoming the predominant IL-1 β -producing cells upon HIV-R5 challenge (Doitsh et al. 2014). Outstandingly, they finally confirmed the caspase-1-dependent pyroptosis of tonsillar or splenic lymphoid CD4⁺ T cells in the HLAC model by demonstrating that VX-765, a clinically available caspase-1 inhibitor from Vertex Pharmaceuticals Incorporated, potently blocked IL-1 β release and greatly rescued cell death occurring upon HIV-1 infection. Importantly, HIV-1 infection was not restored to productive infection upon treatment with the caspase-1 inhibitor, which could have been one major concern in a therapeutic perspective. Targeting caspase-1 activity might become, therefore, a promising therapeutic strategy in combination with ART to dampen chronic inflammation while concomitantly inhibiting viral replication.

Conclusion

The hallmark of HIV-mediated immunopathology lies in an apparent chronic immune activation which correlates with a timely established chronic inflammatory state. This permanent immune dysregulation not only could affect the proper homeostasis and activity of immune cells but is also seemingly contributing to collateral damages. In the era of ART regimen, an apparent shift toward non-AIDS-related health problems is currently observed, with a particular emphasis on symptoms linked to chronic inflammation and senescence. There is now a growing interest in inflammasome role during HIV infection, particularly when considering the residual inflammatory state and appearance of immunosenescence observed during viral latency and further, even in ART-treated patients. The preliminary results reported recently show great promise and suggest that therapeutic strategies aimed at dampening inflammasome activity could represent a new and valuable add to antiretroviral therapy. Hence, treatments aimed at targeting the host rather than the virus itself would circumvent any potential emergence of viral resistance. Although the link between HIV and this damaging

inflammatory response leading to self-destruction of CD4⁺ T cells will warrant further investigation, the recent discoveries raise hope for new therapeutic avenues against AIDS.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [Lymphocyte Apoptosis](#)
- ▶ [Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense](#)

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Inflammatory Cytokines

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Definition

Cytokines are soluble mediators of immune responses. Their secretion is a method that cells use to communicate and interact. Cells give and receive these instructional signals to perform and direct an immune response. Cytokines are produced by cells in response to a variety of stimuli, including Toll-like receptor agonists, pathogens, or other cytokines. These soluble factors bind to receptors on target cells and depending on the cytokine deliver a pro- or anti-inflammatory signal. In addition, a subset of cytokines termed chemokines deliver a chemotactic signal to cells expressing the appropriate receptor. In HIV infection, inflammatory cytokines, such as TNF- α , are elevated during the initial immune response of an acute infection, and although the intensity subsides, this inflammatory cytokine response persists throughout chronic infection even after successful antiretroviral treatment.

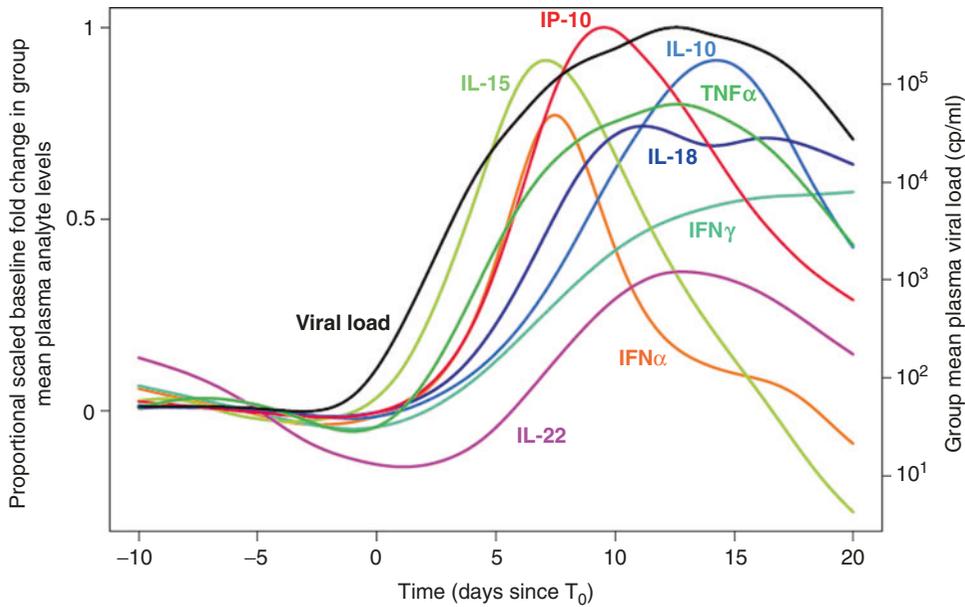
Introduction

Inflammation is a general term describing the movement of activated immune cells to a site of infection or trauma. This is mediated by a broad array of inflammatory soluble proteins which include but are not limited to cytokines, interferons, interleukins, and chemokines. They promote the innate and adaptive immune responses against infection and potentiate downstream control of pathogens. Within days of HIV infection, a cascade of inflammation begins by the virus triggering an innate immune response through pattern recognition receptors on immune cell surfaces.

The adaptive response begins with presentation of viral antigens on infected cells to CD4⁺ and CD8⁺ T cells and binding of virus to free or B cell-bound antibodies. All of these actions prompt immune cells to send out soluble mediators to direct HIV-specific immune responses. While effective early immune responses may help control virus and reduce viral set point, a maladapted response may lead to loss of control, high viral load, and maintenance of a persistent inflammatory state. With the advent of modern technology, a broad array of cytokines are now able to be easily measured to monitor immune responses to HIV. Recent research has revealed an impressive cytokine storm in early HIV infection, which resolves but does not normalize after initial infection. This phase is followed by persistent viral infection, with immune responses unable to control or eliminate the virus, and the inflammatory cytokine response fuels systemic pathogenesis during chronic infection.

Cytokines in Acute HIV Infection

HIV infection can be symptomatic or asymptomatic, and studies have suggested that patients with symptomatic acute HIV infection present with higher viral load and progress to immune deficiency and AIDS more rapidly than those with asymptomatic infection. Due to the difficulty in identifying subjects with HIV at the earliest stages of infection, the interaction between cytokine levels in early HIV and subsequent viral pathogenesis is only recently beginning to be understood. Study of paid plasma donors, who are required to be afebrile at the time of plasma donation, revealed the trajectory of cytokines through acute HIV infection up to seroconversion (Fig. 1). HIV induces successive waves of systemically detectable cytokines, beginning with inflammatory mediators like IP-10 (CXCL10), IFN- α , and TNF- α , followed by both pro- and anti-inflammatory cytokines such as IFN- γ and IL-10, respectively (Stacey et al. 2009). The cytokine storm seen in acute HIV infection is much greater in breadth and magnitude than what is appreciated in other acute infections such as



Inflammatory Cytokines, Fig. 1 The proportional fold change relative to baseline in plasma levels of selected analytes and viral load in 35 plasma donors with acute HIV infection is shown. Time is plotted in days relative

to T₀, the day of first detectable plasma HIV (Copyright © American Society for Microbiology (Journal of Virology, Vol. 83, 2009, p. 3719–33, ► <https://doi.org/10.1128/JVI.01844-08>))

hepatitis B or C viruses. One question that arises in study of HIV is whether cytokine levels during acute infection predict subsequent disease progression. Early HIV is characterized by massive CD4⁺ T cell depletion in gut-associated lymphoid tissues, and systemic cytokine responses might affect the activation and propensity to infection for CD4⁺ T cells. A study of 30 cytokines in acute HIV-infected African women revealed a subset of 5 cytokines that could predict subsequent viral load during chronic, untreated infection. It was found that IL-12p40, IL-12p70, and IFN-γ were associated with lower viral load and IL-7 and IL-15 were associated with higher viral load (Roberts et al. 2010). Clearly, the degree of inflammation induced during acute HIV infection can affect the subsequent clinical course.

Although HIV is a blood-borne pathogen and can be acquired by routes such as injection drug use or blood transfusion, most transmission occurs via mucosal surfaces through sexual activity. It is increasingly appreciated that mucosal cytokine responses play an important role in

HIV transmission. Study of mucosal cytokines is complicated by difficulties in sample collection, though advances have been made in recent years to standardize collection of samples using techniques such as vaginal wicks. Microbicides are designed to inhibit HIV replication or transmission locally within the genital tract, and early trials of microbicides failed in spite of in vitro anti-HIV activity. It has since been shown that potential microbicides such as nonoxynol-9 can increase genital tract inflammation, paradoxically increasing HIV transmission risk. A recent trial designed to down-modulate inflammation in the genital tract of rhesus macaques showed promise in decreasing transmission of SIV (Li et al. 2009). In humans genital tract inflammatory cytokines at the time of acute HIV infection have been shown to be associated with higher systemic viral load and more rapid CD4⁺ T cell depletion. While much remains to be understood about how mucosal cytokines interact with HIV, the available evidence points to a detrimental role of mucosal inflammatory cytokines in predisposing to HIV acquisition.

Interaction Between HIV and Cytokines During Chronic Infection

During early HIV infection, cytokines first appear during the viral ramp-up, and there is engagement of the innate immune response, which begins to drive the expansion of the antiviral T cell population to limit HIV viral replication. Cytokines including IL-2, IL-4, and IFN- α are not only required to support both humoral and cellular arms of the immune response but also drive activation of HIV-infected cells and replication of virus (Poli and Fauci 1992). However, not all cytokines affect viral replication in the same way. *In vitro* studies using cytokine cocultured with HIV-infected cells show differential effects on viral replication; some cytokines may aid in viral control while others continue to drive HIV replication (Keating et al. 2012). For example, HIV antigen-stimulated T cells produce the inflammatory cytokine TNF- α , which in turn pushes infected cells to increase viral replication. Inflammatory cytokines, including IL-2, IL-6, and IL-18, can trigger latently infected cells to turn on viral production, potentially useful in attempts to flush the virus out of latently infected cells to eliminate HIV from the body. On the other hand, the chemokines MIP-3 β (CCL19) and 6CKINE (CCL21) turn on resting CD4⁺ T cells to be more permissive to infection, allowing expansion of the virus reservoir (Klatt et al. 2013).

After the acute phase of the immune response, when viral replication slows down, the viral level may be controlled by an antiviral immune response mediated by the presence of antibodies and by the induction of cellular immune mechanisms to target and kill HIV-infected cells. The viral load reaches a set point and will remain at this steady state until the last stage of chronic infection. During this time, polyfunctional T cells expressing multiple cytokines have been shown to be more effective in controlling HIV replication (Perreau et al. 2013). However, activation-induced senescence in chronic infection leads to low-function T cell profiles, with decreased cytokine output leading to loss of HIV control and a greater susceptibility to opportunistic infections and microbial translocation. With continued immune activation either by

HIV or other pathogen stimulation, loss of T cell responses and increases in systemic immune activation lead to increased production of inflammatory cytokines.

While immune responses during early infection may be required for HIV control to achieve a lower viral set point, non-sterilizing immunity and uncontrolled viral replication can create a chronic inflammatory state. Cytokine and chemokine production, migration of immune cells through sites of infection, and loss of effective immune responses due to over-activation lead to immunological aging (Deeks 2011). These are all consequences of chronic activation and play a part in the development of HIV-associated comorbidities. In addition, viral or fungal coinfections or movement of commensal microbes into the bloodstream, otherwise known as microbial translocation, may occur because of weakened immune responses in the mucosa and may contribute to a persistent systemic inflammatory state. Inflammatory cytokines are elevated during chronic infection. Elevated levels of TNF- α are seen throughout the course of HIV infection, and levels are significantly higher in uncontrolled HIV replication compared to low viral levels due to successful control (Keating et al. 2011). Even in the individuals who control viremia such as elite controllers or those taking antiretroviral therapy, TNF- α concentrations are significantly higher than in HIV-negative subjects. This shows that even low-level viral replication causes persistent inflammation that is detectable in the plasma. Another inflammatory cytokine, IL-6, has been investigated in chronic HIV infection. There are no significant differences in inflammatory markers during HIV control, but significantly elevated concentrations are found in late-stage AIDS and are associated with comorbidities such as cardiovascular disease, kidney dysfunction, liver fibrosis, and an overall increased risk of death (Kuller et al. 2008).

Inflammatory Cytokines and HIV-Associated Morbidity

With immune senescence and reduced T cell function, where are the inflammatory cytokines

coming from? It is known that monocytes or macrophages express HIV coreceptors and are susceptible to HIV infection, and they are likely important sources of inflammatory cytokines in HIV disease. They are easily triggered by antigenic stimulation through Toll-like receptors, they move in response to chemokines released at infection sites, and they target sites of infection by binding to cell adhesion molecules expressed in the vascular endothelium. Macrophages produce both IL-6 and TNF- α in addition to a number of chemokines that direct immune trafficking; they are responsible for systemic surveillance and regularly move throughout blood and tissue searching for pathogens. Movement of immune cells to sites of infection and continued production of inflammatory cytokines may result in breakdown of tissue integrity, activation of cells to produce collagen, and development of fibrosis.

Adipocytes and adipose-resident immune cells are considered another major source of inflammatory cytokines. The primary function of adipocytes is metabolic regulation and energy storage, and this is sensitive to triggers that engage adipocytes to increase fat storage and produce inflammatory mediators. Cytokines act in triggering adipose cell growth, disrupting insulin-glucose homeostasis and causing insulin resistance. Adipocytes produce MIP-1 α and MCP-1, inducing CCR5-expressing inflammatory (M1) macrophage infiltration and IL-8 secretion, promoting neutrophil infiltration into adipose tissues. While it is not known whether adipocytes possess receptors for HIV, adipocytes from HIV⁺ individuals are responsive to viral antigens and express higher TNF- α and IL-18 mRNA after stimulation compared to uninfected individuals (Koethe et al. 2013). Other adipose hormones, including leptin and adiponectin, are triggered upon inflammation and play a role in the development and regulation of obesity. Leptin, a hormone structurally similar to IL-6, activates macrophages to increase TNF- α , IL-6, and IL-12 production; it promotes antigen-specific Th1 IFN- γ secretion and suppresses Th2 IL-4 production.

Uncontrolled viremia drives accelerated inflammation and induces a number of HIV-related comorbidities including cancers, cardiovascular

disease, liver fibrosis, HIV-associated nephropathy, and neurocognitive dysfunction. Even during immune-mediated viral control or antiretroviral treatment with full viral suppression, there are elevations in inflammatory cytokines. These have been measured in large HIV-treatment cohorts and found to have correlations with increased incidence of cancers, cardiovascular disease, and HIV-associated neurocognitive disorders. Mechanisms of morbidity are not well understood, but infiltration of immune cells has been linked to localized sites of pathogenesis. Cytokine, viral antigen, or microbial antigen activation of the vascular endothelium elevates expression of cell adhesion molecules, enabling targeting of immune cells to sites of infection. In response to chemokine signals, activated monocytes bind to the vascular endothelium and, in the case of HIV-associated neurocognitive disorder, cross the blood-brain barrier. Continued production of inflammatory cytokines disrupts regulatory function in the brain and results in neuronal damage and neurocognitive dysfunction. Similar mechanisms of immune cell infiltration, disruption of cellular homeostasis, and development of fibrosis are found in other comorbidities. Increases in the inflammatory cytokines TNF- α , IL-6, and IL-1 can be measured in the CSF and plasma and used as biomarkers of systemic comorbidities. More research needs to be conducted to understand the common factors that may be at play in each HIV-associated comorbidity.

Potential Therapeutic Roles of Inflammatory Cytokines in HIV

The hallmark of HIV disease is the progressive loss of CD4⁺ T cells, leading to AIDS. With the advent of highly active antiretroviral therapy (HAART), infected individuals are living longer, with improved quality of life. However, the immune systems of HAART-controlled patients are never fully restored, and to some degree, there is continual immune activation. Thus the common γ -chain cytokines (IL-2, L-7, IL-15, IL-21), which are critically important to T cell development, homeostasis, and function, have been proposed as potential adjuvants to HIV therapy. These cytokines and

their functions promote immunity to chronic infections and have been tried or proposed as therapies for HIV, with the intent of full restoration of the T cell compartment.

IL-2 is known to induce expansion of T cells, and as CD4⁺ T cells are the primary target of HIV infection, it was considered a strong candidate for use as an immunotherapeutic agent. There have been phase I and II studies reaching as far back as the 1980s and 1990s (Keating et al. 2012) and more recent phase III trials utilizing IL-2 alone or in combination with HAART to achieve full restoration of the T cell compartment. Although targeted increases in CD4⁺ T cells were achieved in clinical trials, there was no clinical benefit to the increase with respect to morbidity or disease progression. This was attributed in part to increases in regulatory T cells disproportionately to effector T cells. Additional potential uses of IL-2 therapy were investigated with the intention of purging the viral reservoir and deferring or allowing interruptions in HAART to offset the high cost and side effects of lifetime antiretroviral treatment. However, there were no observed clinical benefits with IL-2 therapy, and sometimes there was increased disease progression, resulting in higher morbidity and mortality rates (Keating et al. 2012). Thus IL-2 therapy has largely been abandoned as an immune-based therapeutic intervention in HIV.

The failure of IL-2 therapy brought to light the fact that merely increasing the size of the T cell compartment through immune therapy would not be sufficient to yield clinical benefits, even when combined with HAART. Alternative cytokine therapies were proposed; IL-7, a homeostatic cytokine that promotes differentiation and proliferation of memory CD4⁺ and CD8⁺ T cells, and IL-15, a cytokine that augments proliferation of CD8⁺ memory T cells, were both considered for use as adjuvants to HAART (Carcelain and Autran 2013; Toe et al. 2013). Both IL-7 and IL-15 have been included in multiple preclinical studies (and clinical trials for IL-7) and have shown some promise as potential adjuvants to HAART. Unfortunately, IL-15 was shown to inhibit CD4⁺ T cell recovery and impair viral control (Toe et al. 2013). These studies have raised questions about the viability of IL-15 as

an HAART adjuvant therapy. However, IL-7 remains a viable prospect, and further clinical studies, especially in the acute phase, are needed to fully vet the potential for IL-7 therapy. Other uses for IL-7 have focused on curative strategies to eradicate the latent viral reservoir. It is known that activating resting CD4⁺ T cells will induce viral replication in latently infected cells. If this is done in the presence of antiretroviral drugs that successfully prevent new infections, then it is thought that the reservoir of latently infected cells could be purged. There is evidence that IL-7 can induce latent cells to begin producing virus again, thus making these cells targets for antiretroviral drugs. There are ongoing trials investigating the ability of IL-7 to reactivate the viral reservoir; the results of these studies are pending (Carcelain and Autran 2013). Thus, it remains to be determined if IL-7 can provide the adjuvant to HAART that was hoped of the other common γ -chain cytokines.

The inflammatory cytokine IFN- α has many effects on the host immune system. It is a critical part of antiviral defense and is primarily produced by antigen-presenting cells upon encountering a wide range of foreign antigens. The evidence for dysfunction in the IFN system in HIV has been well documented since the beginning of the epidemic. This led to the investigation of its use as a therapeutic. From these studies IFN- α was first approved as a treatment for AIDS-associated Kaposi sarcoma in 1988 (Keating et al. 2012). As more treatments have been developed for HIV, the use of IFN- α as a frontline treatment for HIV has disappeared. However, pegylated IFN- α is the current standard of care for hepatitis C virus (HCV) infection, though likely to be supplanted soon by new classes of drugs. Recently, the safety and tolerability of IFN- α monotherapy was investigated in antiretroviral-naïve HIV-infected patients (Keating et al. 2012).

Interaction of Cytokines with Intrinsic HIV Restriction Factors

As the potential of IFN- α therapy is investigated, it is important to have a firm understanding of the

type 1 IFN genes that are upregulated in response to treatment. In HIV infection one important group of IFN-stimulated genes is a group that codes for proteins with potent antiviral activities. These are collectively referred to as retroviral restriction factors and impede the replication of many viruses, including HIV, in a variety of ways. There have been three major restriction factors described: apolipoprotein B mRNA editing complex catalytic subunit (APOBEC-3), tripartite motif-containing protein 5 (TRIM5), and tetherin (Neil and Bieniasz 2009). The first host gene to be associated with HIV restriction was the APOBEC-3 family, where three proteins have been identified as HIV restriction factors, APOBEC-3A/F/G. When APOBEC-3 proteins are expressed in infected cells, they are incorporated into viral particles during replication. Subsequently, when those particles infect other target cells, APOBEC-3 proteins catalyze the deamination of deoxycytosine, causing production of viral RNA with high levels of G to A mutations resulting in nonfunctional viruses. Interestingly, they are also highly upregulated by the common γ -chain cytokines IL-2, IL-7, and IL-15 (Neil and Bieniasz 2009; Stopak et al. 2007). The other two major restriction factors, TRIM5 and tetherin, function to inhibit HIV through completely different mechanisms, and both are highly upregulated by IFN- α . When expressed in target cells, TRIM5 is able to recognize viral particles, bind to them, and inactivate them prior to reverse transcription. While human TRIM5 has very weak activity versus HIV, the rhesus macaque TRIM5A α has potent inhibitory activity against SIV-1. Genetic manipulation and engineering of human cells to produce rhesus TRIM5 α in conjunction with IFN- α therapy would potentially induce novel antiviral activity. Another restriction factor induced by IFN- α , tetherin, maintains a potent inhibitory activity against HIV-1. Tetherin induces the formation of protein-based tethers that anchor budding virus particles to the surface of the infected cell, preventing further dissemination of the virus to uninfected cells. These “tethered particles” are susceptible to internalization and lysosomal degradation.

Currently, it is not clear how IFN- α therapy could be harnessed to enhance the function of

restriction factors. HIV encodes multiple proteins to antagonize the activity of APOBEC-3 proteins and tetherin, which poses a barrier to the effectiveness of immunotherapy that increases expression of these restriction factors. Clearly, there is a need for a better understanding of how these factors elicit inhibitory activity and interact with downstream adaptive immunity against HIV. A recent study has indicated that the amount of restriction factor induced is critical to realizing any potential clinical benefits (Abdel-Mohsen et al. 2014). In addition, there are over 30 restriction factors that have been associated with direct inhibition of HIV-1 in vitro that are expressed in human peripheral blood. When measured in whole peripheral blood mononuclear cells or isolated CD4⁺ T cells, many of these restriction factors are induced to supraphysiologic levels following in vivo IFN- α therapy (Abdel-Mohsen et al. 2014). Interestingly, both tetherin and A3G have significantly increased expression in CD4⁺ T cells following IFN- α therapy (Pillai et al. 2012). These studies highlight the fact that suppressive effects of restriction factors are critical to exogenous IFN- α treatment. Given the well-documented negative effects of endogenous IFN- α , it is provocative that exogenous IFN- α treatment is able to significantly increase restriction factor expression without increasing CD4⁺ T cell activation (Abdel-Mohsen et al. 2014; Keating et al. 2012). Thus the future of IFN- α as an HIV therapy is yet to be determined, and its potential points to the growing need for both in vitro and in vivo investigations into cytokine treatment to modify restriction factor expression.

Conclusions

As the ability to measure multiple cytokines simultaneously has improved, it has become clear that HIV causes a profound disruption of the cytokine network from the earliest points of HIV infection, shortly after first detection of systemic virus. This dysregulation of cytokines can be partially but not completely reversed via suppression of viral replication through pharmacologic or immunologic control. While HIV likely

exploits proinflammatory cytokines to promote viral replication, there are multiple potential avenues to use these factors to help control HIV replication or ameliorate the damage inflicted by the virus on CD4⁺ T cells. In summary, our knowledge of the depth and breadth of cytokine perturbation is increasing exponentially, and with this new knowledge comes the potential of harnessing the immune system to limit viral replication and collateral damage to the host immune system.

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Inhibition of HIV-1 Spread: Cell-Free Versus Cell-Cell

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Definition

Heterosexual transmission is currently the major mode of HIV transmission worldwide. In addition to the CD4⁺ T cells which are the

principal target, several other potential HIV target cell types including antigen-presenting cells (APCs) (dendritic cells, macrophages) residing at mucosal sites can be also infected. The capacity of these APCs to replicate cell-free virus is low but they transfer HIV-1 to CD4 T lymphocytes efficiently. The various modes of infection are responsible for rapid and efficient replication of HIV-1 and its dissemination through the organism. Preventing cell-to-cell transmission of HIV-1 is crucial for inhibiting HIV-1 propagation, and either antiretroviral drugs or anti-HIV-1-specific antibodies (Abs) can be used.

Introduction

The annual number of new HIV infection has been in decline since 2005 and the major mode of HIV transmission is now through sexual contact. Mucosal sites (genital tracts or anal mucosa) contain various types of immune cells, antigen-presenting cells (APCs) including dendritic cells (DCs) and macrophages, and various types of T cells (principally activated or memory CD4 T lymphocytes), all initially targeted by HIV-1 (Shattock and Moore 2003; Su and Moog 2014). However, the capacity of APCs to replicate HIV is far lower than that of CD4 T cells and infected APCs are rarely detected *in vivo*. Consequently, the precise chronology of infection and replication capacity is still a matter of debate.

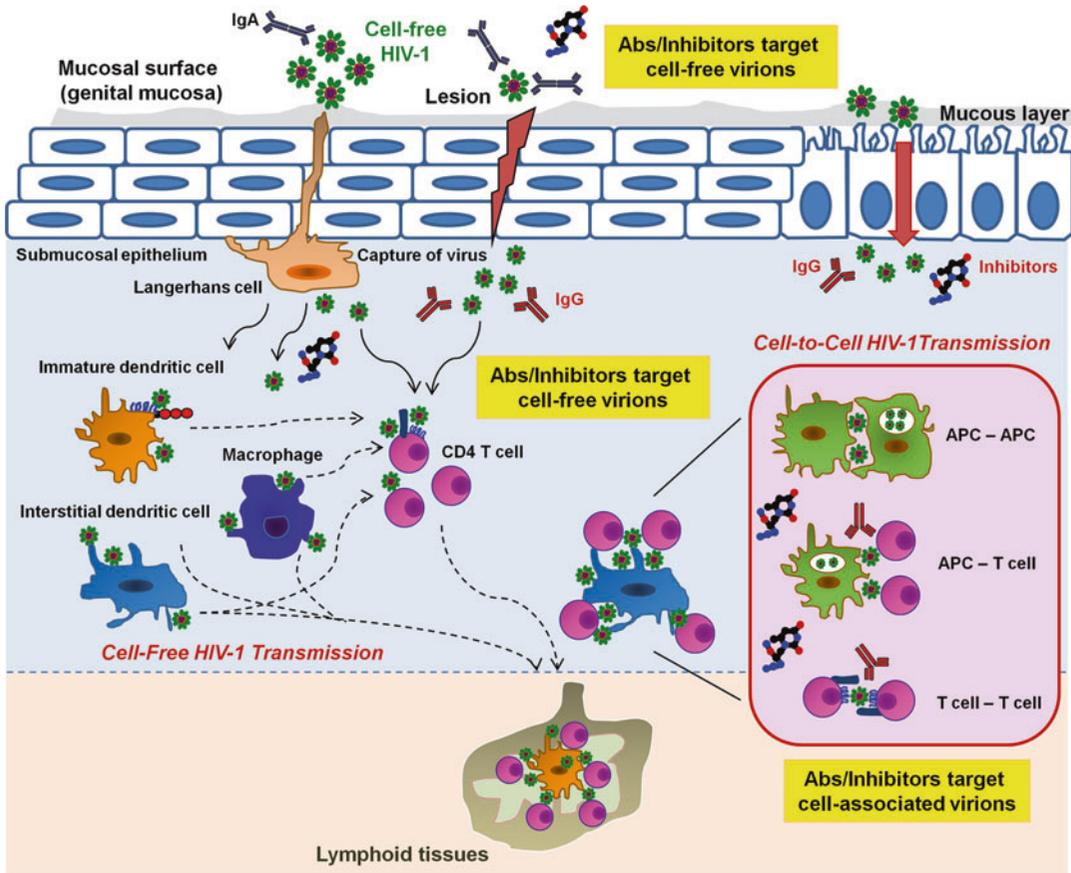
The mechanism of the migration of viral particles through the epithelial barrier remains unclear. Various modes of infection have been proposed, and they include transfer through epithelial cells, transport of HIV via DCs present at mucosal surfaces, and direct infection of resident CD4 T cells (Cunningham et al. 2013; Su and Moog 2014) (Fig. 1). Thereafter, HIV migrates to lymphoid tissues. There are two mechanisms by which HIV-1 infects immune cells: infection by cell-free virus and cell-to-cell transmission. It has been suggested that this second mode of HIV infection makes a large contribution to HIV propagation and dissemination through the body. Spread of HIV-1 infection through direct cell-to-cell transmission has been shown to be

100–1,000-fold more efficient than infection by cell-free virions (Su and Moog 2014).

The mucosal immune/humoral response involves essentially IgG and secretory IgA. IgG and IgA able to neutralize free viral particles would be expected to inhibit the infection of mucosal HIV target cells before the establishment of systemic infection. Such Abs, by preventing the infection of the first target cells of the virus, may therefore constitute the first line of defense at the portal of virus entry (Fig. 1). Also, once cells are infected, subsequent virus dissemination through cell-to-cell contact should also be inhibited. Here, the different modes of HIV-1 spread, through cell-free or cell-to-cell transmission, are described; then the inhibitory activities of Abs and antiretroviral inhibitors for both types of transmission are reviewed and their contribution to protection against HIV-1 is considered.

The Mechanisms of Cell-Free and Cell-to-Cell Transmission

The relative significance of cell-free and cell-associated virus to HIV-1 infection is still a matter of debate, but numerous studies suggest that direct cell-to-cell transmission of HIV makes a large contribution to its rapid dissemination throughout the body. This mode of transmission has substantial consequences for the design of treatments or vaccine strategies. Indeed, cell-associated HIV may be less sensitive to various therapeutic approaches, and particularly Abs, than cell-free virus. Moreover, the cellular environment of the mucosa may contribute to determining the mode of infection. In inflamed or injured tissues, the recruitment of activated T cells would tend to favor direct infection of these target cells. Several *in vitro* assays have been developed to study the infection of cells by free viral particles and by cell-to-cell transmission. Infection with free virus is dependent on the expression of the HIV-1 receptors and co-receptors and a “conventional” fusion of HIV-1 to the target cells. By contrast, there are various mechanisms associated with cell-to-cell infection (commonly called “transfer”) depending on the cells involved and



Inhibition of HIV-1 Spread: Cell-Free Versus Cell-Cell, Fig. 1 The mucosal port of HIV-1 entry. HIV-1 infection can occur via genital mucosal tissues, which comprise a multicellular layer of stratified squamous epithelial cells. Both cell-free and cell-associated infectious HIV-1 virions can infect host cells. Cell-free HIV-1 can gain access to Langerhans cells which can transport the virus into the subepithelium. Mucosal lesions may also provide an accessible pathway for HIV-1. In the subepithelium, immature DCs and macrophages which are enriched in HIV-1 attachment factors like C-type lectins and CD4⁺ T cells are infected either directly by cell-free virions or by cell-

associated virions. HIV-1-infected immature DCs then migrate to lymphoid tissues that are enriched in CD4⁺ T cells, facilitating HIV-1 dissemination through the body. These infected immature DCs can differentiate into mature DCs during migration. Mature DCs can present HIV-1 antigens to CD4 T cells in the lymphoid tissues and initiate antiviral adaptive immune responses. Cell-free virus can be bound and neutralized by mucosal IgA (IgA) at mucosal surfaces. Antiviral inhibitors and adaptive immune responses, such as secreted IgG, are important for preventing HIV-1 infection and dissemination in vivo

their phenotypes. In the environment of the mucosa, the transmission of cell-free virus is plausible, as is the transfer of cell-associated virus between various APCs and neighboring lymphocytes (Fig. 1).

The interactions between APCs and T cells were among the first to be studied. This cell-cell interaction was shown to enhance viral spread substantially, and several distinct mechanisms are involved (formation of virological synapses,

endosomes, and increased binding of virus to alternative receptors) (Sattentau 2010; Su and Moog 2014). Two mechanisms of HIV-1 transfer from DCs to T lymphocytes have been described: *trans*-infection and *cis*-infection (Coleman et al. 2013). The efficient and rapid passage of virus has been described as *trans*-infection. Several publications report that immature DCs capture HIV and efficiently transfer it to neighboring CD4 target cells (Sattentau 2010). This direct transfer can be

rapid and is highly dependent on the type of donor cells (DCs, macrophage, or T cells) and particularly on the receptors expressed and the function of the donor cells; it involves the formation of virological synapses between two cells, secretion of exosomes, or direct binding to alternative receptors (Su and Moog 2014). The *trans*-infection of CD4 T lymphocytes was demonstrated to be transient as HIV-1 transfer decayed within the first 24 h (Turville et al. 2004).

The second mode of transfer is *cis*-infection. This long-term transfer occurs after conventional HIV-1 fusion and *de novo* virus production in donor cell (Turville et al. 2004); it involves close cell-to-cell contacts following synthesis of new viral particles and possibly the formation of virological synapses.

During chronic infection, most HIV-1 replication is in CD4⁺ T lymphocytes from lymphoid tissue. Therefore, cell-to-cell transmission between T cells is likely to be the most common mode of HIV-1 spread in this stage. Indeed, virological synapses formed between T cells have been extensively described (Dale et al. 2013). Altogether, these various modes of cell-to-cell transmission of HIV may favor HIV escape recognition by or interaction with Abs/inhibitors and allow it to hijack the immune system. Inhibition of cell-to-cell HIV transfer is therefore central to the fight against this infection.

Role of Antibodies and Antiretroviral Inhibitors in Preventing HIV-1 Transmission

The protective role of neutralizing antibodies (NAbs) against HIV has been demonstrated. HIV-specific Abs (mainly IgG) can neutralize free viral particles and inhibit infection of various HIV mucosal target cells *in vitro* (Su and Moog 2014; Holl et al. 2006). However, their role in inhibition of HIV transfer is more controversial. Here, recent observations which may be informative about which Abs and antiretroviral inhibitors could contribute to protection against HIV-1 transmission through cell-free or cell-associated pathways are discussed.

Mechanisms of Inhibition of HIV Transmission via Cell-Free Mechanisms

Neutralization Activity

Abs of various subtypes can inhibit infection of HIV target cells through various mechanisms that are or are not dependent on additional effectors cells or mechanisms. The most studied HIV inhibitory activity of Abs is neutralization. Neutralization is the inhibition of HIV replication by Abs in the absence of other additional components, such as Fc receptors (FcR) or complement. Five neutralizing monoclonal antibodies (NAbs) have been analyzed extensively over more than 10 years (IgG1 b12, 2F5, 4E10, 447-52D, and 2G12), and new broadly neutralizing antibodies (bNAbs) have recently been discovered (e.g., PGT monoclonal Abs 121–123 and 125–128, VRC01, VRC03, and PG9, PG16, etc.) (Su and Moog 2014; Kwong et al. 2013). These NAbs are able to inhibit a broad spectrum of HIV-1 strains (cell-free infection) *in vitro*, as assessed in conventional neutralization assays with peripheral blood mononuclear cells (PBMCs) or HIV-1-permissive cell lines (TZM-bl) (Heyndrickx et al. 2012). They target crucial conserved epitopes of HIV (CD4bs, gp41), thereby impeding attachment and fusion processes. However, NAbs isolated from infected patients are rare and complex and result from a long maturation process (Kwong et al. 2013). bNAbs could not be induced by vaccination; however partial protection (31% efficacy) was observed in the RV144 phase III Thailand trial through the induction of non-neutralizing antibodies (NNAbs) and a moderate T-cell response (Haynes et al. 2012).

Fc Receptor-Mediated Inhibition

In addition to neutralizing HIV-1, NAbs can also inhibit HIV-1 infection through additional mechanisms. The FcR-dependent mechanism of inhibition has been observed for various HIV target cells expressing these receptors, such as DCs, Langerhans cells, and macrophages (Su and Moog 2014). The involvement of interactions with FcR in HIV inhibition is confirmed with the cell line TZM-bl expressing various Fc γ Rs. This Fc-mediated inhibition increased by 10–1,000-

fold the inhibitory activity of NAbs against the infection of FcR-bearing cells. Some HIV-1-specific Abs lacking neutralizing activities display Fc-mediated inhibitory activities (Su and Moog 2014; Holl et al. 2006). Such Abs, able to inhibit HIV-1 replication by the Fc γ R-dependent mechanism only, are referred to as non-neutralizing inhibitory Abs (NNIAbs) (Holl et al. 2006). Binding to Fc γ RI can augment the conventional entry-inhibiting neutralizing activity of Abs recognizing multiple epitopes on gp120 and gp41 of HIV-1 (Perez et al. 2013). Neutralization titers for NAbs 4E10 and 2F5 were increased as much as 5,000-fold in the presence of Fc γ RI expression on the surface of TZM-bl (Perez et al. 2013). For APCs bearing Fc γ R, binding of immune complexes formed between Abs and HIV leads to destruction of the virus by phagocytosis and its degradation by specific lysosomes. High affinity between IgG and HIV-1 may therefore be relevant for both neutralizing activity and FcR-mediated transcytosis of the virus-IgG complex. These findings illustrate the potential role of Fc γ R-mediated innate and adaptive immune functions in the mechanism of HIV protection. In addition, FcR can be also involved in Abs-dependent enhancement of HIV-1 binding to, and infection of, certain cell types.

ADCC Activity

ADCC (antibody-dependent cellular cytotoxicity) is also related to FcR-dependent mechanisms (Forthal et al. 2013; Su and Moog 2014). It mainly involves FcRIII (CD16) and consists of recognizing an infected target cell by Abs. The additional binding of the Fc domain of Abs to the FcR of an effector cell (mainly NK cells) induces the lysis of the infected cell following NK cell degranulation.

ADCVI Activity

ADCVI (antibody-dependent cell-mediated virus inhibition) results from an interaction between an infected target cell, an HIV-specific Ab, and an effector cell expressing one or several Fc γ Rs. ADCVI encompasses multiple effector function activities related to lytic (e.g., ADCC) and non-cytolytic (e.g., production of β -chemokines) mechanisms dependent on Fc γ Rs that may

interfere with HIV infection and replication (Forthal et al. 2013; Su and Moog 2014; Holl et al. 2006).

Overall, the inhibitory activity of NAbs against virus infection of these target cells is due to distinct mechanisms of inhibition: classical neutralization of the virus infectivity involving the Fab part of the antibody and FcR-dependent mechanisms of inhibition, such as phagocytosis, ADCC, or ADCVI. Therefore, cell-free virus can be inhibited by various inhibitory mechanisms expressed by Abs to prevent infection of diverse HIV target cells.

Mechanisms of Inhibition of HIV-1 Spread via Cell-to-Cell Transmission

Inhibition of cell-associated HIV-1 infection (also called cell-to-cell transfer) is even more complex. Numerous mechanisms lead to cell-to-cell transfer and *cis/trans*-infection, and they differ according to the donor and target cells. Several groups have studied the inhibition of HIV-1 transfer by Abs, but a multitude of diverse models of HIV-1 transfer have been used (Fig. 2). Inhibitory activity may differ between donor and target cells, viral strains, and technique for assessing transfer; consequently, results are divergent and controversial, with some indicating that HIV-1 transfer is resistant to NAbs, whereas others demonstrate an efficient inhibition of HIV-1 replication by entry inhibitors or NAbs (reviewed in Fig. 2) (Schiffner et al. 2013; Su and Moog 2014). The chapter below presents some mechanistic explanations for inhibition of cell-to-cell transfer by HIV-1-specific Abs or inhibitors.

Inhibition of Cell-to-Cell Transfer Is Cell Type Dependent

Co-cultures of various HIV-1 donor cells with specific target cells in cell-to-cell transfer assays allow efficient HIV-1 transmission mainly via the formation of virological synapses. T cell-to-T cell and APC-to-T cell transfer experiments have been used to analyze the activity of specific anti-HIV-1 Abs and inhibitors against HIV-1 transmission. Most work on T cell-to-T cell HIV transmission describes the close contact between CD4 T lymphocytes leading to HIV transfer in *trans*

HIV transfer	Donor cells	Target cells	Virus types	Methods of detection	Inhibition of cell-to-cell transfer by Abs/inhibitors No/weak inhibition of cell-to-cell transfer by Abs/inhibitors	Comparison of inhibition cell-free v.s. cell-to-cell transfer	References
T-to-T cell	Jurkat	CD4 ⁺ /CD8 ⁺ T cells	HIV Gag-gFP (NL4-3)	Cytometry	sCD4, Leu3a, b12	C.F. > transfer	Chen 2007 J Virol.
	Jurkat	CD4 ⁺ T cells, MT4	HIV Gag-gFP ΔCT HIV-NL4-3 Gag-gFP HIV-NL-GI (JREFL)	3D confocal imaging 3D confocal fluo-imaging	2F5, T20, AMD3100, patient Nabas AMD3100, Cytochalasin D HIV-1 neutralizing serum 2	C.F. (cell-free) serum 2: 87% for cell-free 38% for transfer	Hubner 2009 Science
	MOLT2/CCR5	CD4 ⁺ T cells	NL4-3, HIV-1 _{89.6}	Cytometry	b12, 2F5, 4E10, Leu3a, C34	Not Done (N.D.)	Massanella 2009 AIDS
	JH _{89.6} , PBMC	AS301.R5	HIV-1 _{89.6} , HIV-1 _{89.6}	qPCR, LSCM	2G12, b12, 2F5, Q4120, T20, TAK779, PRO2000	C.F. = transfer	Martin 2010 J Virol.
	Jurkat	CD4 ⁺ T cells, MT4	HIV Gag-gFP, HIV Gag-Cherry pNL4-3	Electron microscope BlaM assay	AMD3100, polyclonal patient sera (1:50 dilution)	patient sera: 100% for cell-free 50% for transfer	Dale 2011 Cell H. M.
	293T, MOLT	CD4 ⁺ T cells	NL4-3, HIV-1 _{89.6} , pseudovirus-Luc pNL4-3-eGFP	Cytometry, FRET Fluo-imaging	sCD4, Leu3a, b12	C.F. > transfer (19 patient sera with NL4-3)	Sanchez-Palomino 2011 Vaccine
	Jurkat	CD4 ⁺ T cells	HIV-1 Gag-gFP/ΔCT (NL4-3) NL-GI (Env cloned)	Cytometry	Leu3a, b12, 4E10, (2G12, T20, AMD3100)	C.F. > transfer (except Leu3a, 2G12, AMD3100)	Durham 2012 J Virol.
	HEK293, HeLa	Jurkat, MT4, Jurkat, Raji SupT1, CD4 ⁺ T cells	NL4-3 (VSV-G), Gag-GFP/RFP HIV-inGLUC	Cytometry	17b, patient serum 1 & 2 2G12,	C.F. > transfer (2F5-2G12-4E10)	Zhong 2013 PLoS One
	Jurkat	CD4 ⁺ T cells	NL4-3, NL4D8 strains	Luciferase assay	2F5, 4E10	C.F. > transfer	Malbec 2013 J Exp Med
TZM target cell	PBMC (CD8 ⁺)	TZM-bl	JR-FL, ADA, ZA110, 015, 016, SF162, NL4-3, Env-pseudovirus	Cytometry, β-gal assay	Broadly neutralizing antibodies	C.F. > transfer	Abela 2012 PLoS Path.
	Mature MoDC (monocyte-derived DC)	TZM-bl	HIV-1 clones Lai, NL4-3, Lai/Bal-Env, 89.6, Gag-eGFP	Virus attachment assay Luciferase assay Ab-cell binding	anti-gp41 (2F5, 4E10), T20 active anti-gp120 (2G12, b12, VRC01), lose potency anti-gp41 (2F5, 4E10) active anti-gp120 (2G12, b12, VRC01, PG16) less susceptible	C.F. = transfer (anti-gp120) C.F. = transfer (anti-gp41) C.F. > transfer (anti-gp120) C.F. = transfer (anti-gp41 & b12)	Sagar 2012 J Infect Dis.
APC-to-T cell	Skin-derived DC	CD4 ⁺ T cells	Primary HIV-1 _{89.6} , HIV-1 _{89.6} , HIV-1 _{89.6} , HIV-1 ₁₀₁ , HIV-1 ₁₀₁ , HIV-1 ₁₀₁	ELISA	b12, 2G12, 4.8D, 2F5	N.D.	Frankel 1998 J Virol.
	Mature blood DC	PBMC	Primary HIV-1 _{89.6} , HIV-1 ₁₀₁ , HIV-1 ₁₀₁ , HIV-1 ₁₀₁	Immunocytochemistry ELISA	T20, CD4-IgG3, PRO 140, RANTES	N.D.	Ketas 2003 J Virol.
	Immature MoDC	ASR5, MT2	HIV-1 _{89.6} , HIV-1 ₁₀₁ , HIV-1 ₁₀₁ , HIV-1 ₁₀₁	ELISA, Cytometry	2F5, b12	N.D.	Ganesh 2004 J Virol.
	Myeloid DC (myDC)	CD4 ⁺ T cells	ADA-Luc, HIV-1-Ypr-GFP	Confocal imaging	b12,	N.D.	van Montfort 2007 J Imm.
	Raji-DC-SIGN	CD4 ⁺ T cells	Cloned dual-tropic HIV-1	Capture, Binding	AMD3100, TAK779, sCD4	N.D.	Cavrois 2007 PLoS Path.
	Immature MoDC	Resting PBLS	81A, NL4-3	ELISA, Confocal	sCD4, sCD4-hlgG	N.D.	Yu HU 2008 PLoS Path.
	Immature MoDC	CD34-LC	HIV-1-GFP	Cytometry	2G12, 4.8d, V3-21, 2F5, 4E10 (all used 20 μg/ml)	N.D.	van Montfort 2008 J Virol.
	Mature MoDC	Hos-CD4 cells	HIV-Luc/GFP-Ypr (HXB2)	Luciferase assay	less inhibition for X4-tropic virus	N.D.	Groot 2008 Blood
	Myeloid DC	Jurkat LTR-GFP	Cloned HIV-1-LAI (HXB2)	Immunofluorescence	1388, Q4120, TAK-779, 2D7, T20 AZT	N.D.	Su 2012 Blood
	Raji-DC-SIGN, myDC	CD4 ⁺ T cells	JR-CSF, LAI	Capture, Cytometry	2G12, b12, VRC0103, 2F5, 4E10, AZT, T20	C.F. = transfer, except b12	Duncan 2013 AIDS
	Immature MoDC	CD4 ⁺ T cells	HIV-1 _{89.6}	LSCM, ELISA, Cytometry	4B3, 246-D, 5F3, 1570-D ART: AZT, NVP, RAL	C.F. = transfer (MOI equivalent)	Su 2014 J Virol.
	Macrophages (MDM)	CD4 ⁺ T cells	Primary HIV-1 _{89.6} , HIV-1 ₁₀₁ , HIV-1 ₁₀₁	Cytometry	VRC01, AZT	C.F. = transfer	
	Macrophages (MDM)	CD4 ⁺ T cells	HIV-1-NL4-3-LucR with Env Bal, YU-2	Luciferase assay			
	Plasmacytoid DC	CD4 ⁺ T cells	Primary HIV-1 _{89.6} , T1F HIV-1 _{89.6}	Cytometry			

Inhibition of HIV-1 Spread: Cell-Free Versus Cell-Cell, Fig. 2 Inhibition of cell-to-cell HIV-1 transfer by HIV-1 specific Abs/inhibitors

via the formation of virological synapses to be resistant to inhibitory Abs and entry inhibitors (Fig. 2); the Jurkat cell line was used as the donor cells in these assays. Other studies, based on other cell lines or primary lymphocytes in HIV-1 transfer assays, found that, on the contrary, Abs were able to inhibit HIV-1 transfer (Fig. 2). Indeed, the characteristics of the cell-to-cell contact appear to depend on the donor/target cells used. Zhong et al. recently showed that HeLa and Jurkat T cells produced and released 10- and 40-fold less cell-free HIV than HEK293, respectively (Fig. 2). However, they showed that co-cultures of Jurkat donor cells with HeLa, MT4, and Jurkat target cells resulted in HIV spread being about 100-fold more efficient, associated with greater resistance of HIV-1 transmission to NAbs. In contrast, co-cultures of HEK293 donors and MT4 target cells, which are highly permissive to cell-free HIV-1, were most sensitive to all NAbs tested. These findings suggest that the strength of the synapse established and the efficiency of HIV-1 transfer influence the inhibitory activity of Abs.

Abela et al. showed that bNAbs VRC01 did not block cell-mediated HIV-1 transmission from HIV-1-infected PBMC donor cells to TZM-bl target cells and proposed that cell-to-cell transmission enables HIV-1 to evade inhibition by these Abs (Fig. 2). Sagar's team reported a similar phenomenon (Fig. 2): using TZM-bl as target cells, they found that NAbs directed against the transmembrane domain membrane proximal external region gp41 were much more effective than CD4bs-directed Abs at restricting cell-associated HIV-1 transmission (Fig. 2). This preferential inhibition by anti-gp41 Abs may be linked to the use of TZM-bl as target cells. Indeed, these cells overexpress CD4/CCR5 HIV-1 receptor/co-receptor entry molecules which may artificially augment the first attachment step of HIV-1; consequently, the inhibitory activity of anti-gp120 Abs will be weaker than that of anti-gp41 Abs.

Although the cell lines used as effectors and target cells are generally accepted as appropriate models for analysis of HIV-1 transfer, they are poorly representative of HIV-1 transfer because they may not reflect the cross talk that takes place between primary immune cells *in vivo*.

Immunological synapses formed between cell lines differ from those involving primary cells (Sattentau 2010). For example, the close interaction (cross talk) observed between primary DC/T cells involves adhesion molecules such as ICAM-1 and LFA-1 (Su et al. 2014; Rodriguez-Plata et al. 2013), independent of HIV-1 envelope proteins (Rodriguez-Plata et al. 2013); this is absent from TZM-bl cell lines.

The immunological synapses formed between primary DCs and T cells may be the most pertinent because DCs can be considered to be APCs closely interacting with autologous lymphocytes for the initiation and modulation of the immune response. Interestingly, with these two primary cell types, almost every NAb tested showed efficient inhibitory activity (Fig. 2). By dissecting the early steps of HIV-1 transfer from DCs to autologous primary CD4 T lymphocytes, Su et al. showed that NAbs, added to HIV-1-exposed DCs at the same time as CD4 T lymphocytes, were able efficiently to inhibit the transfer of HIV-1 *in trans* and *in cis* from DCs to CD4 T lymphocytes (Fig. 2). Similar inhibitory activity by Abs/inhibitors has also been observed by others (Fig. 2) (Su and Moog 2014), suggesting that HIV-1 transfer from DCs to T lymphocytes is not resistant to Abs/inhibitors.

Virus Types in the Cell-to-Cell HIV-1 Transfer Assay
Most work with cell-to-cell transfer assays has used laboratory-adapted HIV-1 strains and particularly the molecular clone NL4-3 or recombinant Env-pseudovirus particles encoding a green fluorescent protein (GFP) or luciferase protein (Luc) (Fig. 2). Primary HIV isolates, such as subtype B HIV-1_{BaL}, have also been tested by several laboratories (Fig. 2). Although primary viral isolates are more biologically relevant than pseudovirus particles, the most pertinent viruses for studies of the inhibition of HIV-1 transfer would be HIV-1 transmitted/founder (T/F) viruses. Indeed, they are of particular interest as they display the specific features supporting their selection, propagation, and dissemination. However such viruses have not yet been studied in analyses of the inhibition of HIV-1 transfer, and clearly this is required.

Inhibition of HIV-1 Binding, Fusion, or Replication and Readout for Cell-to-Cell HIV-1 Transfer

Various steps of the inhibition of HIV-1 transfer have been studied. Indeed, cell-associated HIV-1 infection of target cells depends on the following steps: CD4 binding, co-receptor binding, membrane fusion, and viral replication. The results, or readout, will depend on the virus used (pseudovirus particles expressing or not expressing GFP/Luc or fully replication-competent virus). Van Montfort et al. analyzed HIV-1 binding by confocal microscopy and showed that transfer of virus to T cells can be enhanced if the virus is coated with Abs and taken up via the Fc receptor on DCs (Fig. 2). They showed that 2F5-neutralized virus captured by immature DCs was able to bind and subsequently be transferred to CD4 T lymphocytes following the formation of virological synapses, whereas NAb b12 had no such effect. Blocking DC-SIGN completely abolished the uptake of 2F5-neutralized virus, indicating that DC-SIGN mediated the capture of the 2F5/HIV-1 immune complex by immature DCs. However in this study the late steps of virus fusion and replication of the virus transferred was not defined. On the contrary, by analyzing these late steps of HIV-1 replication, inhibition of HIV-1 transfer by Abs/inhibitors was observed. Cavrois et al. showed that truncated recombinant soluble CD4 (sCD4) inhibits the uptake of surface-bound HIV-1 virions using virion-based fusion assays (Fig. 2). This inhibition of *trans*-infection by surface-accessible HIV was confirmed by Yu et al. (Fig. 2). Frankel et al. showed that NAbs b12, 2F5, and 2G12 were able to inhibit HIV replication in the supernatants of co-cultured DC/T cells (Fig. 2).

Massanella et al. reconciled the diverse effects of Abs on HIV-1 transfer (Fig. 2). They showed that, although anti-gp41 NAbs 4E10 and 2F5 were not able to block the formation of virological synapses, HIV-1 spread between T cells was fully sensitive to all NAbs tested; this suggests that HIV is transmitted between T cells by a neutralization-sensitive mechanism involving HIV budding from infected cells and capture by target cells. Indeed, DCs may sequester intact infectious HIV-1 particles into compartments destined for lysosomal degradation. Cavrois et al. showed

that the virus internalized into these compartments does not substantially contribute to productive transfer of infection but rather leads to viral degradation (Fig. 2). This model of HIV-1 binding and transfer was also described recently for macrophages infected with HIV-1 (Fig. 2). Thus, the internalized virus did not need to be inhibited by NAbs as it was destined for degradation. However, they found that HIV-1 is transmitted by a sensitive viral fusion step. Fluorescence microscopy and electron tomography studies showed that the viruses were retained on the external plasma membrane and consequently remained accessible to neutralizing monoclonal Abs and to various HIV-1 fusion inhibitors (Fig. 2). This illustrates how better understanding of the mechanisms of HIV-1 binding to and entry into immunologically privileged endocytic compartments will help reveal inhibition-relevant characteristics that will contribute to the analysis of cell-to-cell HIV-1 transmission.

Nevertheless, although they analyzed virus replication, some studies found that Abs and antiviral drugs fail to inhibit T-cell-associated HIV-1 transmission (Fig. 2). Ganesh et al. measured early HIV replication by p24 ELISA and flow cytometry and found that the percentage of HIV-1 transfer from DCs to T cells was not substantially affected by NAbs b12 and 2F5 (Fig. 2). This lack of inhibition may be due to virus produced by the donor cells. Indeed, Holl et al. showed that DCs become highly competent for HIV replication in the context of co-culture with CD4 T cells (Holl et al. 2010). If donor DCs were infected before addition of NAbs, they will escape from Ab inhibition and a first round of HIV replication will occur. It is therefore essential to know if transfer studies are performed with noninfected HIV-1-loaded DCs, or with HIV-1-infected DCs/macrophages, or with HIV-1-infected PBMC, or with productively infected T cells in the context of T cell-to-T cell HIV-1 transmission (Fig. 2). If donor cells are infected before addition of Abs, this will unavoidably affect the apparent inhibitory activity of NAbs especially if early HIV-1 replication is analyzed.

Increased transmission of CXCR4-tropic virus associated with NAbs was reported by van

Montfort et al. (Fig. 2). Again, this may be explained by modifications of DC features in co-cultures; indeed, maturation of DCs has been observed in the presence of CD4 T lymphocytes (Fig. 2) (Su and Moog 2014). Such DC maturation may favor transmission and replication of X4 viruses using DC donor cells.

Overall, the modification of phenotype and the effective replication of HIV in co-cultured donor DCs raise numerous issues for the analysis of inhibition of HIV transmission by Abs. Further studies are needed to clarify these phenomena to facilitate the analysis of HIV replication in transfer experiments from DCs.

FcR-Mediated Inhibition of Cell-Associated Infection

As donor and target cells produce virus, it is important to determine which of these cell types produce the newly synthesized virus. This is particularly important for DCs and macrophages as these cells express FcRs. Abs can bind FcRs and inhibit HIV-1 transfer via FcR-mediated inhibitory activity. Some NNABs, like 246-D, which do not affect HIV-1 transfer from infected DCs to CD4 T lymphocytes, have been found to reduce significantly the percentage of infected DCs in co-cultures (Su et al. 2012). For these Abs, a strong association was found between Fc γ R-specific binding capacity, inhibition of HIV-1 replication, and DC maturation. This suggests that the binding of these Abs to DCs induces the maturation of these cells, resulting in lower levels of R5 virus replication (Su et al. 2012). Indeed, the major effect of Fc γ Rs engagement in DCs is induction of maturation. Also, HIV infection and provirus formation are impaired in immature DCs in the presence of HIV-specific IgG and complement: the long-term transfer of HIV from DCs to CD4 T cells was impeded by HIV-specific IgG, whereas the addition of these Abs and/or complement to activated PBMC did not protect them from HIV infection (Wilflingseder et al. 2007). There may be unconventional mechanisms of HIV inhibition in DCs but not in PBMC, leading to the suggestion that the lower levels of infection mediated by HIV/IgG immune complexes may result from interactions between

virus-bound IgG and Fc γ RIIb expressed on DCs (Wilflingseder et al. 2007). Therefore, Fc-mediated inhibitory activity of Abs on the infection of DCs may also participate in decreased HIV-1 transfer to T cells.

Comparison of HIV-1 Inhibitory Activity of Abs Against Infection with Cell-Free Versus Cell-to-Cell Transfer

Comparisons of the inhibitory activities of Abs between cell-free and cell-associated virus is tricky because of the diverse inhibitory mechanisms involved. Su et al. showed that the inhibitory activity of NABs against HIV-1 transfer was similar to that observed in neutralization assays involving PBMCs or CD4 T lymphocytes (Fig. 2). Both cell-to-cell and cell-free viral spread are similarly sensitive to entry inhibition (Fig. 2). However, studies comparing inhibition of cell-free transmission and cell-associated transfer with T-cell lines mostly observed weaker inhibition of cell-associated transfer (Fig. 2). For example, Chen et al. demonstrated that patient sera that neutralize 78–99% of cell-free infection but have little or no effect on Jurkat cell line-to-T cell HIV-1 transfer (Fig. 2). At the same concentration, the same patient sera were capable of blocking only 38–50% of cell-associated HIV-1 transfer (Fig. 2). Zhong et al. using luciferase assays showed that NABs efficiently inhibit cell-free HIV-1 but were less effective against cell-associated virus and that cell-to-cell transmission can overcome multiple combinations of donor/target cell lines barriers (Fig. 2). In addition, two studies using TZM-bl cells as target cells also found that NABs directed against the CD4-binding site were very much less potent in HIV-1 transfer studies (Fig. 2). Overall, they found HIV-1 transfer involving cell lines to be quite resistant to Ab inhibition compared to cell-free infection. Conversely, the type of cell-free assay may also influence the outcome of the comparison. For PBMC neutralizing assay conditions, the cells are incubated with free virus particles and are then cultured for several days. During this period, the cells are in close contact with each other and cell-to-cell HIV-1 transfer may occur. Therefore, conventional neutralization assays

involving PBMC and readouts of HIV-1 replication after several rounds of HIV-1 replication may in fact assess the capacity of Abs to inhibit cell-to-cell transmission of HIV-1; consequently, comparisons between the inhibition of cell-free and of cell-associated virus need to be interpreted with caution.

Antiretroviral Inhibitor-Mediated Inhibition of Cell-Associated Infection

Cell-associated HIV-1 infection may increase the probability of viral escape from antiretroviral inhibition. Sigal et al. observed that the antiretroviral drugs tenofovir and efavirenz substantially decreased cell-free infection whereas infections involving cell-to-cell spread were markedly less sensitive without requiring drug-resistance mutations (Sigal et al. 2011). This result suggests that antiretroviral therapy may not interfere with the persistence of viral replication due to cell-associated infection in vivo. On the contrary, others found that antiretroviral drugs blocked T cell-to-T cell HIV-1 transmission with an efficacy equivalent to that for blocking cell-free infection (Permyer et al. 2012). These divergent results were recently resolved by Duncan et al. (Fig. 2): using equivalent multiplicities of infection for macrophage-produced virus and for T-cell infection by the cell-free and cell-to-cell routes, they observed that inhibition by antiretroviral drugs (raltegravir, nevirapine, and azidothymidine) was equally effective in both cell-free and cell-to-cell infections (Fig. 2). They demonstrated that the effectiveness of inhibition is not influenced by the mode of viral transmission but is dependent on the multiplicity of infection.

Spread of HIV-1 Infection In Vivo and Their Inhibition by Antibodies

HIV-1 transmission in vivo occurs by the transfer of blood, vaginal secretions, semen, or breast milk. The combination of both cell-free particles and HIV-1-infected immune cells present in these bodily fluids in vivo can influence early HIV transmission and also its inhibition. Almost all studies of cell-to-cell HIV-1 transfer have been

based on focused on in vitro models, and there have been few relevant studies in vivo.

The effects of Abs and antiretroviral drugs on HIV-1 transfer in vivo remain unclear. The protective role of HIV-1-specific Abs has been extensively studied in various experimental models of infection, including nonhuman primate models (NHP), with cell-free virus challenges (Klein et al. 2013; Su and Moog 2014), but never following infection with cell-associated virus. Cell-free virus infection at mucosal sites may involve various steps of HIV-1 transfer from cell-to-cell for efficient replication and dissemination (Fig. 1). The potential role of Fc γ R-mediated innate and adaptive immune functions in addition to neutralization has been repeatedly demonstrated in the mechanism of HIV protection (Su and Moog 2014). Dennis Burton's group has shown that neutralizing monoclonal IgG1 b12, devoid of Fc-Fc γ R functions, has decreased protective potential following vaginal challenge in NHPs (Hessell et al. 2007). NNIAbs were able to reduce the viral load in challenged macaques without conferring complete protection (Moog et al. 2014; Burton et al. 2011). The activation of human macrophages, through Fc γ R cross-linking, may contribute to the natural protection against HIV infection. In addition, it has been reported that NNAb activities correlated with the reduction of acute viremia in vaccinated macaques, demonstrating that NNABs elicited by immunization may play an important role in the control of the acute phase of infection; possibly, this could be exploited to fight HIV (Forthal et al. 2013). A correlation between the presence of a particular allele of Fc γ R and an increased risk of infection has been evidenced in a subgroup of volunteers with low-risk practices (Forthal et al. 2013). These results suggest that some specific HIV Abs may possibly have a deleterious effect in a subpopulation of patients with a particular Fc γ R genotype. These various observations clearly indicate that, in addition to neutralization, Fc γ Rs are important in the mechanism of protection in vivo. Such effective protection observed in vivo suggests that HIV-specific Abs inhibit infection by cell-free virus and cell-to-cell transfer, both mechanisms contributing to HIV-1 replication and dissemination in the body.

Conclusion

The last decade has witnessed enormous advances in our knowledge about HIV-1 transmission. Nevertheless, the protective role of HIV-1-specific Abs/inhibitors against cell-to-cell transfer of HIV both in vitro and in vivo, although extremely important, has not been fully elucidated. Neutralization of cell-free HIV-1 infection in vitro has been extensively studied, but deciphering the divergent findings for inhibition of cell-to-cell transfer of HIV-1 would be of great help in the development of anti-HIV strategies. The inhibitory activities of various agents are diverse and appear to depend on the donor and target cells, on the viruses used, and the way the results of transfer experiments are scored; nevertheless, numerous studies indicate that some NAbs can efficiently inhibit cell-to-cell HIV-1 transfer and replication. In addition, although experimental challenges involve cell-free viruses, there is likely to be cell-associated HIV-1 transfer following challenges at mucosal sites; NAbs that are effective in such assays may therefore be effective against cell-to-cell HIV-1 transmission in vivo. These are very encouraging observations for the development of effective vaccines. Further in vivo studies are needed to explore mechanistic aspects of inhibition of HIV-1 cell-to-cell transmission in more detail. In particular, work involving challenges with transmitted/founder viruses and infected cells, for example, may confirm the potential of NAbs and antiretroviral drugs for inhibition of HIV-1 transfer. Altogether, a better understanding of HIV-1 transmission via cell-free mechanisms and cell-associated mechanisms would lead to new therapeutic and next-generation vaccine approaches for inhibiting HIV-1 transmission (Su and Moog 2014; Kwong et al. 2013; Klein et al. 2013).

Cross-References

- ▶ Cellular Cofactors for HIV-1 Transcription
- ▶ HIV-1 Transmission Blocking Microbicides
- ▶ Mucosal Immunity to HIV-1

- ▶ Non-neutralizing Antibody Responses and Protection Against HIV-1
- ▶ Role of Antibodies in HIV Transmission

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Initial Antiretroviral Regimens

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Definition

The first antiretroviral drug regimen used to treat a previously treatment-naïve HIV-infected patient.

Introduction

Although there are many possible choices for initial antiretroviral therapy (ART) in an HIV-infected patient, only a small subset of the potential options are now recommended in treatment guidelines used in most resource-rich settings. Recommendations are based on a combination of efficacy, safety, tolerability, convenience, and in some cases cost. This review will focus primarily on regimens recommended and used in the United States, where cost has traditionally not been a criterion for guideline

selection. However, that may change as less expensive generic drugs become available.

US guidelines include those from the Department of Health and Human Services (DHHS), which are updated approximately once a year (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015), and those from the International Antiviral Society (IAS-USA), updated every 2 years (Gunthard et al. 2014). In the past, it was customary to classify individual drugs as “preferred” or “alternative.” Clinicians then used the guidelines to combine a “backbone” consisting of two nucleoside or nucleotide analog reverse transcriptase inhibitors (NRTIs) with a “third agent” (at that time a protease inhibitor [PI] or non-nucleoside reverse transcriptase inhibitor [NNRTI]).

This approach has been replaced by the categorization of specific regimens – as opposed to agents – as either “recommended” or “alternative.” The reason for this change is that not all recommended third agents (to which integrase strand transfer inhibitors [INSTIs] have been added) have been studied equally with the two recommended NRTI backbones, and when they have, efficacy may differ. However, this review will discuss agents by class, as well as discuss specific regimens.

The Nucleoside “Backbone”

It may seem surprising that today, almost 20 years after the introduction of highly active antiretroviral therapy (HAART) in the mid-1990s, all recommended initial ART regimens still include two NRTIs plus a third agent. Multiple “NRTI-sparing regimens,” two-drug regimens, and even single-drug regimens have been studied, but while it is assumed that there is nothing “magic” about three drugs, nothing else to date has performed well enough to replace this standard approach in US guidelines or practice.

Only two backbones are currently recommended: tenofovir disoproxil fumarate (TDF) with emtricitabine (FTC) and abacavir (ABC) with lamivudine (3TC). Both are coformulated as single tablets and also with third agents as single-tablet regimens (STRs).

Tenofovir/Emtricitabine (TDF/FTC)

TDF/FTC is a component of more recommended regimens than ABC/3TC, as it has been more extensively studied, and it was more effective than ABC/3TC when combined with either efavirenz (EFV) or ritonavir-boosted atazanavir (ATV/r) in patients with high baseline viral loads (Sax et al. 2011). It is generally safe and well tolerated but does have two important potential toxicities: nephrotoxicity and loss of bone mineral density.

The primary mechanism of TDF-associated nephrotoxicity is proximal tubulopathy, typically manifested by proteinuria, glycosuria, and/or urinary phosphate wasting. The presence of glucose in the urine in a nondiabetic, a high fractional excretion of phosphate (>20%), and overt Fanconi syndrome (with or without evidence of glomerular dysfunction) are fairly specific for tubulopathy. However, patients on TDF may develop a decline in the estimated glomerular filtration rate (eGFR) without laboratory evidence of tubulopathy. In such cases, while the decline in kidney function may be due to other factors (e.g., hypertension, diabetes, or other nephrotoxic agents), it may still be appropriate to discontinue TDF to avoid exacerbating progression of kidney disease. Alternatively, the dosing interval can be increased based on the eGFR according to labeled indications. Discontinuation is preferred over dose modification in patients with evidence of TDF-associated tubulopathy.

A decline in bone mineral density (BMD) is observed after the initiation of all currently available NRTI-based regimens. This decline occurs within the first 6–12 months of therapy and then stabilizes over time. The decline is more pronounced with TDF-containing regimens. It also appears to be at least partially reversible, since discontinuation of TDF is associated with an increase in BMD. This effect should be a consideration in patients with preexisting osteoporosis or osteopenia. In the United States, routine BMD testing is now recommended in HIV-infected postmenopausal women and men over 50 years old.

When patients fail therapy with a TDF/FTC-containing regimen, they typically first develop

the M184V mutation, conferring high-level resistance to 3TC and FTC but increasing susceptibility to TDF and zidovudine (ZDV). With ongoing viral replication, K65R, the signature TDF mutation, may emerge, resulting in a decrease in TDF and ABC susceptibility but further increasing ZDV susceptibility. From a resistance standpoint, ZDV would be a logical NRTI choice after failure of TDF/FTC with K65R and M184V, but this strategy is no longer recommended because of the toxicity and poor tolerability of ZDV, as well as the availability of other options.

TDF and FTC are available as a coformulated product and are also combined with other agents (efavirenz, rilpivirine, and elvitegravir/cobicistat) in three STRs.

Abacavir/Lamivudine (ABC/3TC)

The DHHS guidelines currently recommend ABC/3TC as an NRTI backbone in combination with the integrase strand transfer inhibitor (INSTI) dolutegravir. A coformulated single-tablet regimen combining these three drugs is available. It is recommended as an alternative backbone in combination with ritonavir- or cobicistat-boosted darunavir. The DHHS guidelines no longer list the combination of ABC/3TC with either EFV or boosted ATV as either recommended or alternative regimens because of the lower efficacy in patients with baseline viral loads above 100,000 c/mL compared with TDF/FTC (Sax et al. 2011). This difference in efficacy based on viral load was not observed with the combination of ABC/3TC with DTG, which is why it is a recommended regimen (Walmsley et al. 2013).

ABC can cause a serious hypersensitivity reaction (HSR) during the first weeks of therapy. Deaths have been reported with ABC rechallenge after resolution of HSR. Fortunately, the possibility of HSR can essentially be excluded by pre-testing for HLA-B*5701. ABC should not be prescribed to those who test positive, and the negative predictive value is 100%.

In some observational studies, ABC has been associated with an increased risk of myocardial infarction. While the mechanism of this potential association is unknown, and it has not been observed in clinical trials or in all observational

studies, guidelines recommend avoiding ABC in patients who are at high cardiac risk.

When patients fail therapy with an ABC/3TC-containing regimen, they typically first develop the M184V mutation, conferring high-level resistance to 3TC and FTC and a modest decline in susceptibility to ABC. M184V increases susceptibility to TDF and ZDV. With ongoing viral replication, L74V, the signature ABC mutation, may emerge, resulting in a further loss of ABC susceptibility. While TDF should retain activity in the presence of M184V and L74V, this sequencing strategy has never been shown to be effective clinically, perhaps because of the presence of K65R at low levels in patients failing ABC.

Both ABC and 3TC are available in generic form. The two drugs are coformulated and are also components of a single-tablet regimen in combination with dolutegravir.

Other NRTIs

No other NRTIs are currently recommended in current US guidelines. *Stavudine* (d4T) causes mitochondrial toxicity, including peripheral neuropathy, lipoatrophy, hepatic steatosis, and lactic acidosis. In addition to those toxicities, *didanosine* (ddI) also causes acute pancreatitis and has been associated with irreversible non-cirrhotic portal hypertension. Although still widely used worldwide, *zidovudine* (AZT, ZDV) causes gastrointestinal side effects, fatigue, macrocytic anemia, lipoatrophy, hepatic steatosis, and lactic acidosis.

Investigational NRTIs

Like TDF, *tenofovir alafenamide* (TAF) is a pro-drug of tenofovir (TNF). However, compared to TDF, TAF achieves higher intracellular TNF levels, including distribution into the lymphoid tissue, with lower plasma levels. There is early evidence that the lower plasma levels result in less loss of bone density and possibly less kidney toxicity and that the higher intracellular levels may increase activity against NRTI-resistant virus. It is expected that TAF will first be approved in coformulation with elvitegravir, cobicistat, and FTC. Later products will include coformulations with FTC, rilpivirine/FTC, and eventually darunavir/cobicistat/FTC.

Integrase Inhibitors

All but one of the DHHS-recommended regimens for initial ART contain INSTIs, which block the integration of reverse-transcribed viral DNA into the host DNA. As a class, INSTIs are highly effective, safe, and well tolerated. They also suppress viral load more rapidly than other antiretroviral classes, although the clinical significance of this characteristic is unknown.

Raltegravir (RAL)

RAL was the first approved INSTI. It was initially used primarily for treatment-experienced patients with drug resistance, but subsequent studies demonstrated outstanding efficacy and tolerability in treatment-naïve patients as well. RAL has few side effects. Creatine kinase elevation has been observed but is rarely clinically significant. There are few significant drug interactions. RAL is dosed twice daily, although a once-daily formulation is being studied. No coformulated products containing RAL have been approved. Failure on a RAL-based regimen can lead to INSTI mutations, which typically result in cross-resistance to elvitegravir. Dolutegravir may retain activity, except in the presence of the Q148 mutation in combination with at least two other INSTI mutations. RAL is a recommended INSTI in pregnant women.

Elvitegravir (EVG)

EVG is the only INSTI that requires pharmacologic “boosting.” Cobicistat (COBI) is generally used for that purpose, and while both drugs are available as stand-alone products, they are more commonly used as components of an STR that also includes TDF and FTC. This combination is a recommended regimen for initial therapy in DHHS and IAS-USA guidelines. Because it is a cytochrome P450 (CYP) 3A4 inhibitor, COBI interacts with many other drugs, with an interaction profile that is similar, but not identical, to that of the PI ritonavir. EVG/COBI is dosed once daily. It is generally well tolerated, although nausea can occur, typically resolving after the first few days of therapy (Clumeck et al. 2014; Wohl et al. 2014). Failure on an EVG-based regimen

can lead to INSTI mutations that typically cause cross-resistance to RAL. However, dolutegravir may retain activity except in the presence of the Q148 mutation in combination with at least two other INSTI mutations.

COBI inhibits tubular secretion of creatinine, resulting in a modest increase in serum creatinine and a decline in the estimated glomerular filtration rate (eGFR). This effect is seen within the first few weeks of therapy and then stabilizes. It is a laboratory effect only and does not represent true nephrotoxicity. Any progressive decline in eGFR cannot be attributed to COBI and should prompt an evaluation for other causes of kidney damage, including TDF toxicity.

EVG can also be given in combination with boosted PIs, although this is a strategy that would apply only to treatment-experienced patients.

Dolutegravir (DTG)

DTG is a potent, well-tolerated INSTI. The DHHS guidelines recommend DTG in combination with either ABC/3TC (with which it is coformulated as an STR) or with TDF/FTC. In treatment-naïve patients, it was superior to both EFV and DRV/r based on better tolerability (Walmsley et al. 2013; Clotet et al. 2014). In INSTI-naïve, treatment-experienced patients, it was superior to RAL. It has relatively few drug interactions, though somewhat more than RAL.

Like COBI, DTG inhibits tubular secretion of creatinine, with a resulting decline in eGFR that does not represent true nephrotoxicity.

No drug resistance has yet been reported in treatment-naïve patients taking DTG in an initial regimen, suggesting that it has a higher genetic barrier to resistance than either RAL or EVG. It remains to be seen whether its resistance barrier is as high as that of boosted PIs. DTG retains activity against some RAL- and EVG-resistant virus, especially when given at a higher dose (50 mg twice daily). The presence of a Q148 mutation in combination with at least two other INSTI mutations substantially decreases DTG susceptibility.

Investigational INSTIs

Cabotegravir is an unboosted INSTI that is being studied both in oral form and as a long-acting

injection that could be administered infrequently for treatment or prevention (preexposure prophylaxis) of HIV. Although it is an analog of DTG, resistance has been observed in patients failing cabotegravir.

GS-9883 is an unboosted, once-daily INSTI currently in phase II testing. It appears to have characteristics similar to those of DTG. If approved, it is expected to be coformulated with TAF/FTC.

Protease Inhibitors

PIs bind to HIV protease, preventing cleavage of polyproteins required for viral assembly. Although they have been mainstays of therapy since their introduction in the mid-1990s and are still widely used in treatment-experienced patients, they are less frequently used for initial therapy because of the availability of other options, most notably the INSTIs. PIs are almost always used in combination with either ritonavir (RTV) or COBI, which are used as pharmacoenhancers or “boosters” to increase serum levels, prolong half-life, and allow for lower doses and/or less frequent administration. In addition, patients without preexisting PI resistance do not appear to develop PI resistance on boosted PIs, which was not the case when PIs were used without boosting. For that reason, boosted PI-based regimens continue to be popular choices for initial therapy in patients with unreliable adherence.

Both RTV and COBI inhibit tubular secretion of creatinine, resulting in the early increase in eGFR, discussed above. The effect is somewhat more pronounced with COBI than with RTV. There are no other significant tolerability or toxicity differences between the two agents.

Both RTV and COBI are CYP3A4 inhibitors and therefore have many drug interactions.

Darunavir (DRV)

DRV boosted with RTV is now the only PI recommended in the DHHS guidelines for initial therapy, based on the demonstration of superiority over atazanavir in a large clinical trial (Lennox et al. 2014). DRV requires pharmacologic

boosting, traditionally with RTV. However, the coformulation of DRV with COBI is now approved and is currently categorized as an alternative PI by the DHHS guidelines. When used for initial therapy, ritonavir-boosted DRV (DRV/r) and COBI-boosted DRV (DRV/c) are given at doses of 800/100 mg and 800/150 mg, respectively. Treatment-experienced patients who have DRV resistance mutations should use only DRV/r at a dose of 600/100 mg twice daily.

DRV is well tolerated but can sometimes cause rash, which may or may not be self-limiting. It has more gastrointestinal side effects (e.g., loose stools) than RAL or DTG but fewer than ATV/r. DRV should be taken with food for maximal absorption.

DRV/r is a recommended PI in pregnant women.

Atazanavir (ATV)

ATV, boosted either with RTV or COBI, is now categorized as an alternative PI, based primarily on a large clinical trial showing inferiority to DRV/r and RAL (Lennox et al. 2014). This was based primarily on tolerability and toxicity differences. Specifically, ATV causes an increase in indirect bilirubin, which can sometimes lead to overt jaundice or scleral icterus. ATV was also associated with more gastrointestinal toxicity than RAL or DRV/r in that trial. Other known toxicities of ATV include nephrolithiasis, nephrotoxicity, and cholelithiasis. ATV requires gastric acid for absorption and should be used with caution and appropriate dose separation with antacids, H₂ blockers, and proton pump inhibitors. ATV should be taken with food.

ATV is usually dosed at 300 mg daily, boosted either with RTV (100 mg) or as a coformulated product with COBI (150 mg) (Gallant et al. 2013). However, unlike DRV, ATV does not achieve adequate serum levels when given at a dose of 400 mg daily without boosting, which is a consideration in patients where tolerability concerns or drug interactions prevent boosting. Boosting is required when ATV is given with TDF, which lowers ATV levels.

ATV/r is a recommended PI in pregnant women.

Other PIs

Lopinavir/ritonavir (LPV/r) was an important PI for many years and was the first to be coformulated with a booster. It is still the most widely used agent for second-line therapy in resource-limited settings. However, it is no longer recommended in the United States because of its poorer tolerability and greater toxicity and higher pill burden compared with DRV and ATV. The dose of RTV in LPV/r is 100 mg twice a day, compared to the 100 mg once-daily dose used with DRV and ATV. This may partly explain why it has more gastrointestinal side effects and metabolic toxicity, including dyslipidemia. LPV/r remains a recommended PI in pregnant women.

Fosamprenavir (FPV) is a well-tolerated PI that can be given either boosted with RTV (700/100 mg twice daily or 1400/100–200 mg once daily for PI-naïve patients) or unboosted 1400 mg twice daily. It is infrequently used because of the availability of better studied PIs with lower pill burdens (DRV and ATV). The use of unboosted FPV is not recommended as it can lead to cross-resistance with DRV. FPV can cause a rash, which may or may not require discontinuation.

Saquinavir (SQV) is boosted with RTV at a dose of 1000/100 mg twice daily. It is no longer recommended, mainly because compared to DRV and ATV, it has a higher pill burden (6 per day), a higher dose of RTV with greater metabolic toxicity, and the potential for QTc prolongation.

Nelfinavir (NFV) is the only PI that is given only in unboosted form. It is not recommended because of poorer efficacy and toxicity (diarrhea).

Indinavir (IDV) can be given either unboosted (800 mg every 8 h) or boosted with ritonavir (800/100 mg twice daily). It is no longer recommended because of toxicity, including insulin resistance, GI side effects, retinoid-like effects (alopecia, dry skin, mouth, and eyes), nephrolithiasis and nephrotoxicity, indirect hyperbilirubinemia, lipodystrophy, hyperlipidemia, and paronychia.

Tipranavir (TPV) is used only in treatment-experienced patients with PI resistance. Because

of its greater toxicity compared to DRV, it is typically reserved only for patients with virus that is susceptible to TPV but not to DRV.

Non-nucleoside Reverse Transcriptase Inhibitors

NNRTIs inhibit the activity of viral reverse transcriptase by binding directly to the enzyme. They have been mainstays of initial therapy for many years but are being used less frequently in the United States because of the availability of INSTIs. No NNRTI-based regimen is included in the list of recommended regimens in the April 2015 version of the DHHS guidelines.

Efavirenz (EFV)

EFV has been a gold standard agent for many years and is still a component of recommended regimens in most treatment guidelines used worldwide, with the exception of the DHHS guidelines. It is also available in a single-tablet regimen coformulated with TDF and FTC. It has an outstanding record for safety and efficacy but also has significant tolerability disadvantages. Specifically, it is associated with neuropsychiatric side effects such as dizziness, vivid dreams or nightmares, sleep disturbance, concentration difficulties, and less commonly depression and hallucinations. These effects are most pronounced during the first days to weeks of therapy and then improve or resolve. However, some side effects can be persistent, and one study found an increased risk of suicidality (suicidal ideation or attempted or completed suicide) in patients taking EFV. EFV can also cause a rash with early therapy, which may or may not require discontinuation.

EFV is a recommended NNRTI in pregnant women, but only after the first eight weeks of pregnancy because of the potential for neural tube defects.

Rilpivirine (RPV)

RPV is classified as an alternative agent in the DHHS guidelines, for use in patients with

baseline viral loads below 100,000 copies/mL and CD4 counts above 200 cells/mm³. It is available in a single-tablet regimen coformulated with TDF and FTC. Its primary advantage is outstanding tolerability. The disadvantages include poorer efficacy at high viral loads and low CD4 counts, the requirement for dosing with a meal, and the effect of proton pump inhibitors, H2 blockers, and antacids on absorption. In addition, resistance to RPV can lead to partial cross-resistance to all other NNRTIs, including etravirine, because of the signature E138K mutation. Like etravirine, RPV is active against EFV- and NVP-resistant virus with the K103N mutation. Clinical trials have demonstrated excellent efficacy and tolerability in patients who switched from other NNRTIs or PIs to RPV/TDF/FTC.

Nevirapine (NVP)

NVP is still widely used worldwide, but the initiation of NVP-based regimens is no longer recommended in the United States, primarily because of the potential for serious early toxicity: hepatotoxicity, including hepatic necrosis, and skin toxicity, including Stevens-Johnson syndrome and toxic epidermal necrolysis. These toxicities are more common in women and in patients with higher baseline CD4 counts. In addition, NVP has not been as well studied as EFV in combination with the two recommended NRTI backbones: TDF/FTC and ABC/3TC. NVP is also more likely than EFV to select for mutations that cause cross-resistance to etravirine, most notably Y181C.

Other NNRTIs

Etravirine (ETR) is an effective and well-tolerated agent that may have a higher barrier to resistance than other NNRTIs. However, it has not been well studied for initial therapy and is approved only for use in treatment-experienced patients, generally those with resistance to “first-generation” NNRTIs (NVP and EFV).

Delavirdine (DLV) is rarely used because of inconvenience (three times daily dosing) and relative lack of efficacy data compared to recommended NNRTIs.

Investigational NNRTIs

Doravirine (DOR) is an investigational NNRTI that appears to have activity against virus with common NNRTI mutations, including K103N and Y181C.

Entry Inhibitors

There are two approved entry inhibitors: *maraviroc* (MVC), a CCR5 antagonist, and *enfuvirtide* (ENF, T20). MVC can only be given to patients with exclusively R5-tropic virus, determined by tropism testing. ENF requires twice-daily subcutaneous injections and causes painful injection site reactions. Neither is recommended for initial therapy in treatment-naïve patients.

Choosing the Initial Regimen

The choice of the initial regimen should be made after consideration of a variety of factors, including comorbidities, concomitant medications, food requirements, possibility of pregnancy, and likelihood of adherence.

The most recent version of the DHHS guidelines lists five “recommended” regimens (Table 1), all but one of which consist of two NRTIs plus an INSTI. However, they point out that “alternative” regimens may be preferred in some patients. The following is a discussion of the choice of initial regimen for specific patient types and clinical scenarios:

Desire for a Single-Tablet Regimen (STR)

Two STRs are classified as “recommended” regimens in the DHHS guidelines: EVG/COBI/FTC/TDF and DTG/ABC/3TC. Either is an excellent choice for a large proportion of patients. The former has the advantage of including TDF/FTC as the NRTI backbone, which has generally been preferred in other regimens. However, there are potential renal and bone toxicities associated with TDF and drug interactions with COBI. DTG/ABC/3TC appears to have a higher barrier to resistance than EVG/COBI/FTC/TDF.

Initial Antiretroviral Regimens, Table 1 Advantages and disadvantages of antiretroviral drugs and regimens for initial therapy

Recommended initial regimens all consist of two NRTIs (the “backbone”) plus one “third agent”			
Drug/regimen	Forms and brand names	Advantages	Disadvantages
Nucleoside analog reverse transcriptase inhibitor “backbones” (to be combined with third agent)			
Tenofovir/emtricitabine (TDF/FTC)	<i>Truvada</i> (and part of <i>Atripla</i> , <i>Complera</i> , and <i>Stribild</i>)	<ul style="list-style-type: none"> • The recommended NRTI backbone in combination with most third agents • Three single-tablet regimens (STRs) available: <i>Atripla</i>, <i>Complera</i>, and <i>Stribild</i> • Both drugs are active against hepatitis B • A preferred NRTI backbone in pregnancy 	<ul style="list-style-type: none"> • Can cause kidney toxicity (monitor creatinine and urinalysis) • Causes more loss of bone density than other drugs
Abacavir/lamivudine (ABC/3TC)	<i>Epzicom</i> , <i>Kivexa</i> (and part of <i>Trizivir</i> and <i>Triumeq</i>)	<ul style="list-style-type: none"> • No kidney toxicity • Less effect on bone density than TDF • Available in an STR with DTG • A preferred NRTI backbone in pregnancy 	<ul style="list-style-type: none"> • Must pretest with HLA-B*5701 to avoid ABC hypersensitivity reaction • Less effective than TDF/FTC when combined with EFV or ATV/r in patients with viral loads >100,000 c/mL • May increase risk of myocardial infarction (controversial): avoid or use with caution in patients with multiple cardiac risk factors
Didanosine (ddI)	<i>Videx</i> , <i>Videx EC</i> , or generic	None	Not recommended
Stavudine (d4T)	<i>Zerit</i> , <i>Zerit XR</i> , or generic	None	Not recommended
Zidovudine (ZDV, AZT)	<i>Retrovir</i> or generic (and part of <i>Combivir</i> , <i>Trizivir</i>)	A preferred NRTI backbone in pregnancy (ZDV/3TC)	Not recommended
Third agents			
Non-nucleoside reverse transcriptase inhibitors			
Efavirenz (EFV)	<i>Sustiva</i> (and part of <i>Atripla</i>)	<ul style="list-style-type: none"> • Extensively studied • STR available (<i>Atripla</i>) • Remains in the blood for a long time: “forgiving” of missed doses • See TDF advantages (for <i>Atripla</i>) • Preferred NNRTI in pregnancy after the first 8 weeks 	<ul style="list-style-type: none"> • Can cause neurologic or psychiatric side effects, especially in the first few weeks; long-term effects also occur • Potential for rash • Resistance is common if treatment fails • May cause birth defects if given to pregnant women during the first trimester <ul style="list-style-type: none"> • Less CD4 increase than with other third agents (PIs, INSTIs) <ul style="list-style-type: none"> • See TDF disadvantages (for <i>Atripla</i>)
Rilpivirine (RPV)	<i>Edurant</i> (and part of <i>Complera</i> , <i>Eviplera</i>)	<ul style="list-style-type: none"> • STR available (<i>Complera</i>) • Better tolerated than <i>Sustiva</i> and <i>Atripla</i> (less rash, neurologic side effects, and effect on lipids) • See TDF advantages (for <i>Complera</i>) 	<ul style="list-style-type: none"> • Must be taken with a meal • Gastric acid is required: decrease absorption with proton pump inhibitors, H2 blockers, antacids • Resistance is common with treatment failure, including cross-resistance to ER • Recommended only if viral load <100,000 c/mL and CD4 > 200 cells/mm³ • See TDF disadvantages (for <i>Complera</i>)

(continued)

Initial Antiretroviral Regimens, Table 1 (continued)

Recommended initial regimens all consist of two NRTIs (the “backbone”) plus one “third agent”			
Drug/regimen	Forms and brand names	Advantages	Disadvantages
Delavirdine (DLV)	<i>Rescriptor</i>	None	Not recommended
Etravirine (ETR)	<i>Intencele</i>	<ul style="list-style-type: none"> • Well tolerated • May be less prone to resistance than other NNRTIs • Can be dissolved in water 	Not recommended for initial therapy because of lack of data
Nevirapine (NVP)	<i>Viramune</i> , <i>Viramune XR</i> , or generic	<ul style="list-style-type: none"> • Well tolerated and safe after the first few weeks/months • Safest NNRTI in pregnancy • Generic form available 	<ul style="list-style-type: none"> • Not recommended for initial therapy (US guidelines) • Can cause severe liver toxicity or skin rash during the first few weeks, especially in women with pretreatment CD4 counts >250 cells/mm³ or men with counts >400 cells/mm³ • Resistance is common if treatment fails; more likely to cause ETR cross-resistance than EFV
Protease inhibitors			
Atazanavir/ritonavir (ATV/r), atazanavir/cobicistat (ATV/COBI), or atazanavir (ATV)	<i>Reyataz</i> / <i>Norvir</i> , <i>Evotaz</i> , or <i>Reyataz</i>	<ul style="list-style-type: none"> • The best PI if boosting is not possible • Resistance is unlikely with failure (when boosted) • A preferred PI in pregnancy (ATV/r) 	<ul style="list-style-type: none"> • Can cause jaundice • Should be taken with food • Should not be taken with proton pump inhibitors • More GI side effects than DRV • Can cause nephrolithiasis, cholelithiasis, nephrotoxicity
Darunavir/ritonavir (DRV/r) or darunavir/cobicistat (DRV/COBI)	<i>Prezista</i> / <i>Norvir</i> or <i>Prezcobix</i>	<ul style="list-style-type: none"> • The only recommended PI (DRV/r) in the DHHS guidelines • The best tolerated PI • Resistance is unlikely with failure • A preferred PI in pregnancy (DRV/r) 	<ul style="list-style-type: none"> • Should be taken with food • Can cause rash • More GI toxicity than RAL
Lopinavir/ritonavir (LPV/r)	<i>Kaletra</i> , <i>Aluvia</i>	Resistance is unlikely with failure	<ul style="list-style-type: none"> • No longer a recommended PI for initial therapy in US guidelines • More GI side effects than ATV/r or DRV/r • More lipid effects than ATV or DRV
Fosamprenavir (FPV)	<i>Lexiva</i>	Can be given unboosted	<ul style="list-style-type: none"> • No longer recommended for initial therapy • Greater pill burden than recommended PIs, with no COBI coformulation • Potential for DRV cross-resistance (when given unboosted)
Indinavir (IDV)	<i>Crixivan</i>	None	Not recommended
Nelfinavir (NFV)	<i>Viracept</i>	None	Not recommended
Ritonavir (RTV)	<i>Norvir</i>	Used only as a low-dose booster for other PIs	Should not be used at full dose to treat HIV infection
Saquinavir (SQV)	<i>Invirase</i>	None	Not recommended
Integrase inhibitors			
Raltegravir (RAL)	<i>Isentress</i>	<ul style="list-style-type: none"> • Few side effects • Few drug interactions • Preferred INSTI in pregnancy 	<ul style="list-style-type: none"> • Twice-daily dosing • No STR available • Lower barrier to resistance than DTG

(continued)

Initial Antiretroviral Regimens, Table 1 (continued)

Recommended initial regimens all consist of two NRTIs (the “backbone”) plus one “third agent”

Drug/regimen	Forms and brand names	Advantages	Disadvantages
Elvitegravir (EVG)	<i>Vitekta</i> (and part of <i>Stribild</i>)	<ul style="list-style-type: none"> • Part of an STR • Tolerability advantages over EFV or PIs • See TDF advantages (for <i>Stribild</i>) 	<ul style="list-style-type: none"> • Lower barrier to resistance than DTG • <i>Stribild</i>: <ul style="list-style-type: none"> – See TDF disadvantages – COBI drug interactions similar to RTV – COBI inhibits creatinine secretion, resulting in decreased eGFR without true nephrotoxicity <ul style="list-style-type: none"> – Indicated only for patients with creatinine clearance >70 mL/min; should be discontinued if creatinine clearance falls to <50 mL/min
Dolutegravir (DTG)	<i>Tivicay</i> (and part of <i>Triumeq</i>)	<ul style="list-style-type: none"> • Few side effects • Few drug interactions • Higher barrier to resistance than RAL or EVG • <i>Triumeq</i>: <ul style="list-style-type: none"> – See ABC advantages – The only non-TDF-containing STR 	<ul style="list-style-type: none"> • Inhibits creatinine secretion, resulting in decreased eGFR without true nephrotoxicity • See ABC disadvantages (for <i>Triumeq</i>)

However, it requires pretesting for HLA-B*5701, and the potential toxicities of ABC must be considered. Both COBI and DTG inhibit tubular excretion of creatinine, causing an early decline in eGFR that does not represent nephrotoxicity.

Multiple Cardiac Risk Factors

Although the association between ABC and myocardial infarction remains controversial, guidelines recommend avoiding ABC in patients with high cardiac risk. In such patient, TDF is preferred, assuming normal renal function. Potential options would include EFV/COBI/FTC/TDF or DTG plus TDF/FTC.

Chronic Kidney Disease (CKD)

TDF should generally be avoided in patients with CKD. The one exception might be the patient who presents with HIV-associated nephropathy (HIVAN), which is expected to improve on ART. Patients with ESRD on hemodialysis can safely be treated with weekly TDF, provided no recovery of renal function is expected. In general, ABC is preferred in patients with CKD, though such

patients may also have multiple cardiac risk factors, in which case an NRTI-sparing regimen could be considered (see below). Potential options for the patient with CKD but low cardiac risk would include DTG/ABC/3TC or RAL plus ABC/3TC. Because DTG interferes with tubular creatinine excretion, an early decline in eGFR should be expected but does not represent worsening of CKD. If a PI-based regimen is chosen, DRV is preferred over ATV because of the potential nephrotoxicity of ATV. Either RTV or COBI can be used as pharmacoenhancers, although COBI will cause a somewhat greater decline in eGFR.

Questionable Adherence

When there are questions about the patient’s ability to adhere to therapy, a regimen with a high barrier to resistance has generally been preferred. Counterintuitively, this typically meant prescribing a PI-based regimen, which required taking multiple pills, as opposed to an easier to take single-tablet regimen (typically EFV/TDF/FTC). Today, PI-based regimens require only two pills

per day (either DRV/c or ATV/c plus either TDF/FTC or ABC/3TC). In addition, the single-tablet regimen DTG/TDF/FTC appears to have a high barrier to resistance. It remains to be seen whether it is as high as that of a boosted PI, but many clinicians are already extrapolating from clinical trials and using this regimen in patients with questionable adherence, assuming that resistance will not occur with failure. The investigational single-tablet regimen DRV/COBI/FTC/TAF will be another promising option for such patients. Patients who demonstrate good adherence on ART over time have the option of switching therapy.

Hepatitis B Coinfection

Whenever possible, patients with HIV/HBV coinfection should be treated with a regimen that includes TDF/FTC (or TDF plus 3TC), since all three drugs have activity against HBV. In patients who cannot take TDF, usually because of kidney disease, entecavir should be added to the regimen. An ABC/3TC-based regimen without entecavir is not sufficient, since it will lead to the development of 3TC-resistant HBV.

Hepatitis C Coinfection

There are no drugs that are contraindicated in patients with HCV coinfection, but the potential for interactions with drugs used to treat HCV makes RAL and DTG ideal choices for the third agent. Ledipasvir increases tenofovir levels. Since RTV-boosted PIs also boost tenofovir levels, there is concern that the combination could result in a higher risk of TDF toxicity, a concern that may also apply to COBI-boosted PIs and EVG. When changing the ART regimen is not possible, frequent monitoring of kidney function is advised during the course of HCV therapy.

Pregnancy

Current perinatal guidelines recommend an NRTI backbone of TDF/FTC, ABC/3TC, or ZDV/3TC plus either ATV/r, DRV/r, or RAL for the treatment of HIV-infected pregnant women. However, since ZDV is not recommended in any other setting and may exacerbate the nausea of pregnancy, TDF/FTC and ABC/3TC are generally preferred.

EFV has been associated with neural tube defects in the first trimester and should never be chosen in a woman planning to become pregnant or who is sexually active with men and not using birth control. However, EFV does not need to be discontinued in a woman who is found to be pregnant, since the neural tube will have already closed by that time, and it is now the preferred NNRTI after the eighth week of pregnancy. Worldwide, EFV is the most commonly used third agent, including in women of child-bearing potential.

Drug Interactions

Among the commonly used ART regimens, COBI and RTV have the greatest potential for drug interactions. There are too many interacting drugs to describe here, but clinicians should be especially aware of interactions with nasal and inhaled steroids (especially fluticasone), intra-articular steroid injections, statins, oral contraceptives, rifampin, and rifabutin. The third agents with the fewest drug interactions are RAL and DTG. The absorption of ATV and RPV is inhibited by proton pump inhibitors, H₂ blockers, and antacids, which should be avoided or taken only with careful dose separation, depending on the agent.

Food Restrictions

Some of the commonly used regimens should be taken with food for maximum absorption. These include regimens that include DRV, ATV, and EVG. RPV is especially dependent on food and should be taken with a meal. EFV is typically taken on an empty stomach, at least 2 h after a meal, to decrease CNS side effects. For patients who would have problems with these restrictions, DTG- or RAL-based regimens may be the best choices.

Transmitted Resistance

No generalizations can be made about the treatment of patients with baseline transmitted resistance mutations, since treatment must be individualized based on the resistance patterns. The NNRTI class is the one most likely to be affected by transmitted mutations, followed by

NRTIs. It would be unusual for a patient to be infected with a virus that was fully resistant to any PI, so boosted PI-based regimens have typically been used in such patients. The frequency of transmitted integrase mutations remains low, and baseline INSTI resistance testing is not currently recommended. Because of its high barrier to resistance, a DTG-based regimen might also be considered in a patient with some transmitted NRTI resistance, although there is limited experience with this approach.

Initiation of ART Without Baseline Resistance Data

This would typically be considered in a patient who needed immediate ART because of acute HIV infection, or in a patient with advanced HIV infection, especially if accompanied by a condition that could only be treated by ART (e.g., HIVAN, progressive multifocal leukoencephalopathy, or HIV dementia complex). PI-based regimens are often chosen, in part, because transmitted PI resistance is uncommon but also because of concerns about adherence in patients who present at these stages. As noted above, DTG-based regimens might also be considered in such cases. It is not known whether the more rapid viral suppression seen with INSTIs has clinical advantages. In patients with advanced disease, there is speculation that it could be a disadvantage, by potentially increasing the risk of immune reconstitution inflammatory syndrome (IRIS).

Side Effects or Desire for Simplification

Virologically suppressed patients who are experiencing side effects on ART or who desire simplification to a single-tablet regimen can easily switch therapy, provided there is no resistance to any of the components of the new regimen. There are strong safety and efficacy data from trials evaluating switches to RPV/TDF/FTC and EVG/COBI/TDF/FTC, and it is assumed that switches to DTG-based regimens would also be safe and effective. In the case of switches to RPV/TDF/FTC, the pretreatment viral load does not appear to affect efficacy the way it does when the regimen is used for initial therapy.

Patients Who Cannot Take Either TDF or ABC

The typical example is a patient with either kidney disease or osteoporosis who also has multiple cardiac risk factors or is positive for HLA-B*5701. There are currently no “NRTI-sparing regimens” recommended by guidelines, and selection of such regimens requires some extrapolation from existing data. The combination of a boosted PI and an NNRTI has been studied, but not with contemporary agents. The combination of LPV/r and EFV was effective but associated with unacceptable side effects and metabolic toxicity. Regimens containing DRV/r plus ETR have been studied but only in treatment-experienced patients. Thus, a combination of either DRV/r or DRV/c plus either ETR or RPV could be considered.

More commonly, clinicians tend to use boosted PIs plus INSTIs. To date, all studies evaluating such regimens have used RAL. For unclear reasons, the combination of DRV/r plus RAL has shown decreased efficacy compared to standard therapy in patients with high baseline viral loads (Raffi et al. 2014). Whether the results would have been different with DTG cannot be determined.

Patients who cannot take TDF or ABC can still take 3TC or FTC, which can be added to the regimens discussed above in the hope that this will increase efficacy. In fact, the data supporting “nuke-lite” regimens consisting of a boosted PI plus 3TC alone are stronger than those supporting boosted PIs plus INSTIs, offering a third potential approach to patients unable to take TDF or ABC.

At the present time, all NRTI-sparing regimens should include a boosted PI if possible. However, other approaches are under study, including DTG plus either RPV or 3TC, and an induction-maintenance strategy in which patients are switched to RPV plus the investigational INSTI cabotegravir after achieving virologic suppression.

Conclusion

There are now a number of outstanding options for initial therapy. The enthusiasm for INSTI-based regimens, which now comprise four of the five recommended regimens in the DHHS guidelines, is justified based on their established

efficacy, safety, and tolerability. Until more is known about the resistance profile of DTG, a PI-based regimen (usually with DRV/r or DRV/c) is a reasonable choice for patients with unreliable adherence.

The guidelines point out that while the number of recommended regimens for initial therapy has become relatively small, patients doing well on other agents do not necessarily need to switch therapy. A switch from older NRTIs (i.e., ZDV, d4T, ddI) is advisable because of their long-term toxicity, including damage to mitochondrial DNA. Switching from an older PI (e.g., LPV/r) to boosted DRV or ATV may improve lipids and decrease gastrointestinal toxicity. However, patients experiencing no side effects on NVP or EFV may safely remain on their existing regimen, since these drugs are not known to cause long-term toxicity if it is not clinically apparent.

Continued improvements and new treatment options can be expected in the future. TAF is likely to replace TDF for a large proportion of patients, mainly because of its apparent safety advantages and potential for coformulation with numerous other agents. Doravirine has the potential to return NNRTIs to the list of recommended regimens. BMS-955176, an investigational maturation inhibitor, is being studied for initial therapy. Long-acting injectable regimens such as cabotegravir plus rilpivirine appear promising, although the optimal patient population remains unclear since missed injections could lead to drug resistance. Long-acting regimens might be ideal for patients in supervised settings such as prisons or methadone clinics. They might also be considered for the small subset of patients who take no oral medications, who prefer shots over pills, or who are non-adherent with oral medications but who keep their clinic appointments.

The effect of drug cost and approval of generic medications cannot be underestimated or predicted. Currently, the only recommended and widely used antiretroviral agents available in generic form are ABC and 3TC, but the difference in cost between generic and brand name agents has often not been sufficient to force a breakup of coformulated branded products (ABC/3TC and DTG/ABC/3TC). However, the eventual

availability of generic EFV and TDF could have a significant impact on practice. EFV use has been declining steadily, and it is no longer a recommended agent in DHHS guidelines for reasons discussed above. However, it has a long track record for safety and effectiveness, and there may be considerable pressure on clinicians to start therapy with EFV, reserving other agents for those who do not tolerate it. Similarly, there may be pressure to start therapy with TDF rather than TAF, since TDF toxicity is not universal and generally only becomes apparent after prolonged use.

There is also interest in lower-cost, two-drug regimens. While there was brief interest in boosted PI monotherapy for initiation or simplification, this has been replaced by enthusiasm for the combination of a boosted PI plus generic 3TC, which looks promising in several clinical trials (Cahn et al. 2014). A particularly intriguing combination now under study is the combination of DTG and 3TC. If this regimen, which would presumably have an outstanding safety and tolerability profile, turns out to be effective with the same lack of resistance seen so far with other initial DTG-based combinations, it would be an extremely attractive choice for initial therapy, one that would be hard for any other regimen to compete with. However, such an approach – using a two-drug, non-PI-containing regimen – still seems radical today, at a time when we remain wedded to the same NRTI backbone plus third-agent approach that has been the standard of care for almost 20 years.

Cross-References

- ▶ [Integrase Inhibitors](#)
- ▶ [Non-Nucleoside Reverse Transcriptase Inhibitors for Treatment of HIV Infection](#)
- ▶ [NRTIs](#)
- ▶ [Protease Inhibitor](#)

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Integrase Inhibitors

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Definition

The fundamental approach to most antiretroviral therapy (ART) is based on a strategy of targeting the function of HIV enzymes critical to viral replication. Three such targets are HIV reverse transcriptase, HIV protease, and HIV integrase. After viral entry into the CD4+ T cell, viral RNA is reverse-transcribed into DNA by HIV reverse transcriptase after which HIV integrase forms a pre-integration complex comprised of virally encoded DNA and other cofactors. HIV integration is a two-step process catalyzed by HIV integrase, the first step consisting of the integrase enzyme removing a nucleotide from each 3' DNA terminus, resulting in the exposure of reactive hydroxyl groups. Next, the pre-integration complex enters the host cell nucleus where it binds to host DNA, after which the integrase enzyme nicks each strand of the host DNA, exposing the 5' phosphate end which allows bonding between host and viral DNA. After this step, called integrase strand transfer, is accomplished, host cellular enzymes repair gaps in the DNA.

The HIV integrase enzyme was shown to be susceptible to inhibition by oligonucleotides and synthetic peptides in 1995 (Ojwang et al. 1995) and thus became a target for ART development. In 2000, a seminal study described the first promising compound which targeted the strand transfer step of viral integration (Hazuda et al. 2000). Thus, the integrase strand transfer inhibitors (INSTIs) became the first drugs to target this critical enzyme in the HIV life cycle.

Introduction

Until recently, initial antiretroviral therapy (ART) typically consisted of two nucleoside

reverse transcriptase inhibitors (NRTIs) and a ritonavir-boosted protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI). While highly efficacious, these combinations often were limited by side effects, drug interactions, and complicated dosing regimens. Subsequent drug development focused on improving tolerability and convenience while maintaining or improving virologic efficacy. The emergence of HIV InSTIs as a new class of antiretrovirals has helped fulfill these drug development goals. Three InSTIs are currently available in the United States for the treatment of HIV infection: raltegravir (RAL), elvitegravir (EVG), and dolutegravir (DTG). Long-term safety and efficacy data have been shown for these drugs when used as part of combination therapy for the treatment of individuals infected with HIV. All have activity against a wide range of wild-type and drug-resistant HIV-1 isolates, including HIV-1 clades A, B, C, D, E, F, and G, and are also active against HIV-2. The current Department of Health and Human Services (DHHS) Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents include five different ART regimens as recommended for initial treatment of HIV-1-infected patients, four of which are InSTI based (DHHS 2015). In addition, InSTIs are increasingly used in the management of ART-experienced patients.

HIV-1 Integrase Inhibitors

Raltegravir

Description and Indications

Raltegravir was the first licensed HIV integrase inhibitor, FDA approved in 2007. It is indicated in combination with other antiretroviral agents for treatment of HIV infection in adult patients. Raltegravir plus emtricitabine/tenofovir is a recommended InSTI-based first-line regimen in the US and European treatment guidelines (DHHS 2015; EACS 2015). It can be used effectively in both treatment-naïve and treatment-experienced patients.

PK Parameters

Raltegravir is rapidly absorbed with a T_{max} of approximately 3-h postdose in the fasting state, and steady-state concentrations are achieved within 2 days. Serum half-life is about 9 h. C_{max} is 2.17 µg/ml, C_{min} is 68.6 ng/ml with 400 mg twice-daily dosing, and area under the curve (AUC) is 6.91 µg/ml.h. Bioavailability of raltegravir is approximately 32%. Raltegravir has been shown to achieve meaningful drug concentrations in cerebral spinal fluid (CSF) and genital compartments. It is metabolized through UGT1A1-mediated glucuronidation. Recommended dosing is 400 mg twice daily without food restrictions (Raltegravir package insert, 2014).

Adverse Event Profile

Clinical trials involving both treatment-naïve and treatment-experienced patients demonstrate that raltegravir is well tolerated. In treatment-naïve patients in the STARTMRK study, the rate of treatment discontinuation due to adverse events was 5% in subjects receiving raltegravir (plus emtricitabine/tenofovir) and 10% in subjects receiving efavirenz (plus emtricitabine/tenofovir) (Rockstroh et al. 2013). The most common adverse events of moderate to severe intensity ($\geq 2\%$) are insomnia, headache, dizziness, nausea, and fatigue. In addition, creatine kinase elevations, myopathy, and rhabdomyolysis have been occasionally observed in subjects receiving raltegravir (Raltegravir package insert, 2014).

Drug-Drug Interactions

Raltegravir is metabolized by a low-affinity, high-capacity pathway which helps limit the potential for drug-drug interactions. Interactions can occur when concomitant medications induce or inhibit the activity of UGT1A1. Rifampin is a potent inducer of UGT1A1, and thus a 100% increase in the dose of raltegravir (800 mg twice daily) is recommended if the two drugs are used together (Raltegravir package insert, 2014). In addition, coadministration or staggered administration of aluminum and/or magnesium hydroxide-containing antacids and raltegravir is not recommended. Other drugs that induce

UGT1A1 less strongly, such as phenytoin and phenobarbital, may also reduce concentrations of raltegravir, but this has not been fully characterized. Therefore, no dose modifications are currently recommended for these drugs.

Resistance

Resistance to HIV integrase inhibitors occurs with varying frequency depending on the drug being administered and the circumstances in which resistance is induced. It occurs with higher frequency with raltegravir (RAL) than with some other InSTIs and has been found in up to two-thirds of patients experiencing virological failure on raltegravir-based regimens, particularly if the drug continues to be administered after viral rebound occurs. The genotypic changes associated with raltegravir resistance usually include primary mutations Y143R/Y143H/Y143C, N155H, or Q148H/Q148K/Q148R, and most instances of high-level resistance are seen when the Q148 mutation is accompanied by additional secondary mutations (L74M + E138A, E138K, or G140S). In the STARTMRK trial of ART-naïve individuals, emergence of primary raltegravir resistance-associated mutations was observed in four of the 12 patients with virological failure (two with Y143H/Y143R and two with Q148H/Q148R). Continued administration of raltegravir after virological failure occurs is usually associated with ongoing evolution of resistance mutations.

InSTI resistance that occurs after raltegravir treatment failure is usually associated with resistance to a second InSTI, elvitegravir (see below). In contrast, a majority of raltegravir-resistant viruses remain susceptible to the most recently approved InSTI, dolutegravir.

Clinical Trials

Treatment-Naïve Patients The STARTMRK trial was the largest study of raltegravir among treatment-naïve patients; 563 patients were randomly assigned to raltegravir (given twice daily) or efavirenz in combination with tenofovir/emtricitabine. Raltegravir was found to be non-inferior to efavirenz with regard to viral load suppression at 48 weeks (86% in the raltegravir arm

vs. 82% in the efavirenz arm) with ongoing durability at weeks 96 and 240 (Rockstroh et al. 2013). In addition, the mean increase in CD4 cells was also significantly greater in the raltegravir arm (189 vs. 163 cells/mL). At week 240, there was a larger mean increase in CD4 cells and fewer neuropsychiatric side effects or other drug-related adverse events in the raltegravir arm. In an effort to determine if once-daily administration of raltegravir would be efficacious, the QDMRK study compared once- versus twice-daily raltegravir given with tenofovir/emtricitabine in a randomized, noninferiority trial of 775 patients. Once-daily raltegravir was found to be *not* non-inferior to twice-daily dosing with 83% patients taking once-daily raltegravir achieving viral suppression compared to 89% of those taking the drug twice daily (95% CI -10.7 to -0.83 , *p*-value for noninferiority = 0.044) (Eron et al. 2011).

Treatment-Experienced Patients The pivotal BENCHMRK trial was designed to assess the value of raltegravir in the treatment of HIV-infected patients with extensive prior antiretroviral treatment experience and resistance to at least three classes of ART. At study entry, the median CD4 cell count was 120 cells/mL, and 35% had a baseline HIV viral load of >100,000 copies/mL. When combined with an optimized background regimen, raltegravir demonstrated potent activity with 57% of raltegravir recipients (*n* = 462) achieving HIV suppression after 96 weeks of follow-up compared with 26% of those randomized to an optimized background regimen plus placebo (*n* = 237) (Steigbigel et al. 2010). Among patients experiencing virological failure while taking raltegravir (166 of 462 patients), 60% developed raltegravir resistance mutations (Eron et al. 2013b). At the 48-week evaluation visit, the prevalence of raltegravir resistance mutations was lower in isolates from individuals who were taking two or more active drugs in addition to raltegravir, compared to those for whom raltegravir was the only active agent.

Switch Studies In patients taking a virologically suppressive antiretroviral regimen, switching to

an alternative raltegravir-based regimen should be done with caution and generally only when the other agents in the regimen are fully active. This principle became clear based on results from the SWITCHMRK trials, two randomized studies involving 707 HIV-infected persons with HIV suppression achieved using an HIV protease inhibitor (lopinavir/ritonavir)-based regimen. These studies were designed to evaluate the effect of switching the protease inhibitor to raltegravir compared to continuation of the lopinavir/ritonavir regimen. Although lipid profiles significantly improved in patients who switched to raltegravir, the trial was terminated early because there were significantly more virological failures in those who switched to raltegravir compared to those who did not (16% vs. 9%) (Eron et al. 2010). Thus, switching to raltegravir was inferior to remaining on lopinavir-/ritonavir-based regimen, particularly in those with previous virological failure, suggesting that previously accumulated resistance predisposed patients to virological failure when raltegravir was substituted for a suppressive protease inhibitor.

Elvitegravir

Description and Indications

Elvitegravir was FDA approved in 2012 as part of a coformulated single-pill combination that also includes cobicistat, tenofovir, and emtricitabine. A stand-alone version of the drug was subsequently approved in September 2014. Cobicistat/elvitegravir/emtricitabine/tenofovir is one of the recommended initial therapy regimens for the treatment of HIV-1 infection, but, because of potential renal toxicity, it should only be prescribed to adult patients with an estimated creatinine clearance ≥ 70 mL/min (DHHS 2015; EACS 2015).

PK Parameters

With PK enhancement from cobicistat or ritonavir, elvitegravir is rapidly absorbed with a T_{max} of approximately 4 h. Steady-state concentrations are achieved within approximately 7 days. Serum half-life is ~ 3 h when dosed alone and ~ 9 h with PK enhancement. C_{max} is 1.7 ± 0.1 $\mu\text{g/ml}$, C_{min}

0.45 ± 0.26 $\mu\text{g/ml}$ with 150 mg once-daily dosing, and AUC 23.0 ± 7.5 $\mu\text{g/ml.h}$. Bioavailability has not been established. Elvitegravir CSF concentrations have not yet been established but are currently being studied, and penetration into genital compartments has only been evaluated in rhesus macaques (Mased et al. 2015). Metabolism is predominantly through cytochrome P450 (CYP3A4) with minor pathways via UGT1A1/UGT1A3 glucuronidation and oxidative metabolism. Standard dosing is 150 mg daily plus a booster (150 mg of cobicistat or 100 mg ritonavir) to be taken with meals (Elvitegravir package insert, 2014).

Adverse Event Profile

Large-scale clinical trials have shown that the once-daily combination pill that includes elvitegravir is generally well tolerated. The most common adverse drug reactions to coformulated elvitegravir (incidence greater than or equal to 10%, all grades) are nausea and diarrhea. When administered with tenofovir/emtricitabine, 6% of study subjects discontinued treatment due to adverse events with coformulated elvitegravir compared to 7.4% with tenofovir/emtricitabine/efavirenz and 8.5% with atazanavir plus ritonavir (Sax et al. 2012; DeJesus et al. 2012). In the same studies, elvitegravir/cobicistat was associated with a significantly greater median increase in serum creatinine than comparator arms, a finding previously shown to be due to cobicistat inhibition of tubular secretion of creatinine without effect on actual glomerular filtration rate (GFR). Still, 13 (1.9%) subjects in the coformulated elvitegravir arm and eight (2.3%) subjects in the atazanavir plus ritonavir arm discontinued study drug due to a renal adverse reaction, attributed primarily to tenofovir. Discontinuations were due to both true renal toxicity events and creatinine elevations from the effect of cobicistat when the cause was uncertain (Elvitegravir package insert, 2014).

Drug-Drug Interactions

Elvitegravir is principally metabolized through the cytochrome p450 pathway; therefore, drugs that induce or inhibit cytochrome P450 enzymes

are very likely to have drug-drug interactions with elvitegravir. Because elvitegravir is currently only used with the CYP3A4 inhibitor cobicistat, other drugs that are substrates for this enzyme may also have drug-drug interactions. Selected drug interactions are presented in Table 1.

Resistance

In clinical trials of elvitegravir as initial therapy, 57% of those with virological failure developed genotypic InSTI resistance, often accompanied by resistance to at least one of the nucleoside RT inhibitors being taken concurrently. The frequency of InSTI resistance among those with virological failure was similar to that seen with NNRTI-based ART (DeJesus et al. 2012; Sax et al. 2012). The primary integrase resistance mutations include T66I, E92Q, Q148R, and N155H with the latter three also conferring resistance to raltegravir. As noted above, given the high degree of cross-resistance between raltegravir and elvitegravir, switching between these two InSTIs is not recommended.

Clinical Trials

Treatment-Naïve Patients In treatment-naïve patients, a phase III trial among 708 individuals comparing elvitegravir, emtricitabine, tenofovir, and cobicistat with ritonavir-boosted atazanavir and the same NRTI backbone showed the elvitegravir-based regimen to be noninferior to the atazanavir-based regimen in terms of viral suppression (90% vs. 87%) (DeJesus et al. 2012). In addition, HIV suppression rates were also noninferior among the subset of patients with a baseline viral load >100,000 copies/mL (85% vs. 82%). Results at 96 weeks confirmed continued viral suppression in both treatment groups. Another study of 700 HIV-infected patients compared an elvitegravir-based ART regimen to an efavirenz-based ART regimen and found the elvitegravir regimen to be noninferior to the efavirenz regimen at 48 weeks, with viral suppression rates of 88% versus 84%, respectively. Viral suppression rates among patients with a baseline viral load of >100,000 copies/mL were comparable between the two treatment

arms (Sax et al. 2012). Increases in CD4 counts were significantly higher in the elvitegravir arm versus the efavirenz arm (239 vs. 206 cell/microL). Viral suppression was maintained at 96 weeks in both groups (84% vs. 82%).

Treatment-Experienced Patients A phase III noninferiority trial examined the efficacy and safety of ritonavir-boosted elvitegravir compared to raltegravir among 702 HIV-infected patients with a history of virological failure. Nearly half of the patients had baseline CD4 counts <200 cells/microL, and two-thirds had documented HIV resistance to two or more ART drug classes. The study demonstrated that HIV viral load suppression was similar in both arms (59% vs. 58%) with comparable efficacy among patients with a baseline viral load >100,000 copies/mL. Rates of serious adverse events were low overall, but diarrhea was more common in the elvitegravir arm, and liver abnormalities were more frequent in individuals taking raltegravir (Molina et al. 2012). Of note, the use of elvitegravir in treatment-experienced patients is uncommon since most use occurs in early therapy with the fixed-dose combination, and the requirement for pharmacologic boosting makes it more challenging to use in the complex regimens often necessary in treatment-experienced patients. Thus, raltegravir and dolutegravir are almost always preferred in treatment-experienced patients.

Switch Studies Elvitegravir administered as a component of the combination four-drug tablet (tenofovir, emtricitabine, elvitegravir, cobicistat) is indicated as a regimen switch strategy in patients who are virologically suppressed (viral load <50 copies/mL) on a stable antiretroviral regimen for at least 6 months with no history of previous treatment failure and no known resistance mutations to the individual components of the four-drug combination. In the STRATEGY-PI study, subjects were randomized 2:1 to switch to the four-drug combination elvitegravir pill (n = 293) or stay on their baseline antiretroviral regimen of a boosted PI plus tenofovir/emtricitabine (n = 140) (Arribas et al. 2014). The mean baseline

Integrase Inhibitors, Table 1 Selected drug interactions with elvitegravir in adults

Drug name	Concentration effect	Comment
Protease inhibitors		
Atazanavir	↔ Atazanavir ↑ elvitegravir	There are no data to make dosing recommendations for coadministration with doses of atazanavir/ritonavir other than 300/100 mg once daily
Lopinavir/ritonavir	↔ Lopinavir ↑ elvitegravir	There are no data to make dosing recommendations for coadministration with doses of lopinavir/ritonavir other than 400/100 mg twice daily
NNRTIs		
Efavirenz	↓ Elvitegravir	Coadministration decreases elvitegravir plasma concentration, which may result in loss of therapeutic effect and in development of resistance. Coadministration is not recommended
Nevirapine	↓ Elvitegravir	Coadministration decreases elvitegravir plasma concentration, which may result in loss of therapeutic effect and development of resistance. Coadministration is not recommended
Other agents:		
Acid-reducing agents: Antacids*	↓ Elvitegravir	Elvitegravir plasma concentrations are lower with antacids due to the formation of ionic complexes in the GI tract. It is recommended to separate elvitegravir and antacid administration by at least 2 h
Anticonvulsants: Carbamazepine Oxcarbazepine Phenobarbital Phenytoin	↓ Elvitegravir	Coadministration of phenobarbital, phenytoin, carbamazepine, or oxcarbazepine (CYP3A inducers) with elvitegravir may decrease elvitegravir plasma concentrations, which may result in loss of therapeutic effect and in development of resistance
Antifungals: Ketoconazole*	↑ Elvitegravir ↑ ketoconazole	Concentrations of ketoconazole may increase when used with elvitegravir in combination with protease inhibitors/ritonavir
Antimycobacterials: Rifampin, rifapentine	↓ Elvitegravir	Coadministration of rifampin or rifapentine, both potent CYP3A inducers, with elvitegravir may lead to decreased elvitegravir exposures, which may result in loss of therapeutic effect and in development of resistance. Coadministration is not recommended
Rifabutin	↑ Rifabutin ↓ elvitegravir	When rifabutin is used with elvitegravir and a protease inhibitor/ritonavir, dose reduction of rifabutin by at least 75% of the usual dose of 300 mg/day is recommended. Increased monitoring for rifabutin-associated adverse events is suggested. No dose adjustment of elvitegravir is required when coadministered with the reduced dose of rifabutin
Systemic corticosteroids: Dexamethasone	↓ Elvitegravir	Systemic dexamethasone may decrease elvitegravir plasma concentrations, which may result in loss of therapeutic effect and development of resistance
Herbal products: St. John's wort (<i>Hypericum perforatum</i>)	↓ Elvitegravir	Coadministration is not recommended
Hormonal contraceptives: Norgestimate/ethinyl estradiol	↑ Norgestimate ↓ ethinyl estradiol ↔ elvitegravir	Plasma concentration of ethinyl estradiol may be decreased when used with elvitegravir and a protease inhibitor/ritonavir

*Adapted from Elvitegravir 2014 package insert product information

CD4⁺ was 610 cells per mm³. Maintenance of virological suppression at 48 weeks occurred in 94% in the four-drug combination elvitegravir arm compared to 87% in the boosted PI arm with 1% in each arm having virological failure. In the STRATEGY-NNRTI study, subjects were randomized 2:1 to switch to the four-drug combination elvitegravir pill (n = 291) or to stay on their baseline antiretroviral regimen of an NNRTI plus tenofovir/emtricitabine (n = 143) (Pozniak et al. 2014). The mean baseline CD4⁺ was 588 cells per mm³. Maintenance of virological suppression at 48 weeks occurred in 93% in the four-drug combination elvitegravir arm compared to 88% in the NNRTI arm with 1% in each arm having virological failure.

Dolutegravir

Description and Indications

Dolutegravir is the most recently FDA-approved InSTI in August 2013 and is indicated for use in both ART-naïve and ART-experienced HIV-infected adults and children aged 12 and older in combination with other antiretroviral medications. In contrast to raltegravir and elvitegravir, dolutegravir remains active against many HIV strains with integrase mutations that cause resistance to raltegravir and elvitegravir.

PK Parameters

Dolutegravir is rapidly absorbed with a T_{max} of 1 h, and steady state is reached within approximately 5 days. Serum half-life is ~15 h. The absolute bioavailability of dolutegravir has not yet been established. C_{max} is 3.67 µg/ml, C_{min} 1.11 µg/ml, and AUC 53.6 µg/ml.h with 50 mg once-daily dosing. Dolutegravir achieves meaningful concentrations in the genital tract and colorectum, which support the evaluation of its use in HIV prevention and preexposure prophylaxis. It is metabolized primarily through UGT1A1-mediated glucuronidation with cytochrome P450 (CYP3A4) as a minor pathway. Recommended dosing is 50 mg daily in InSTI-naïve patients and 50 mg twice daily in InSTI-experienced patients with no food restrictions (Dolutegravir package insert 2014).

Adverse Event Profile

Side effects are uncommon with dolutegravir. In clinical trials, the most common adverse reactions of moderate to severe intensity (≥2%) were insomnia, fatigue, and headache (Dolutegravir package insert 2014). In clinical trials of treatment-naïve patients, rates of dolutegravir discontinuation due to adverse events were 2% in the SPRING-2 trial (Raffi et al. 2013) and 3% in the SINGLE study (Walmsley et al. 2013). It should be noted that dolutegravir inhibits creatinine secretion in the proximal tubule so small increases in serum creatinine after initiation are common and generally do not reflect decreased GFR.

Drug-Drug Interactions

Dolutegravir does not interact with many other drugs given its metabolism predominantly through UGT1A1-mediated glucuronidation. Potentially significant drug-drug interactions include some with antiretroviral, antimycobacterial, and anti-epileptic medications, as well as estrogens, and are listed in Table 2.

Resistance

Dolutegravir retains activity against most viruses that acquire resistance to raltegravir and elvitegravir. As such, it can be a useful agent as part of combination ART used to treat patients who have failed prior InSTI-containing regimens (Llibre et al. 2015) – (see also description of VIKING studies below). Probably because of its high potency, dolutegravir appears to have a high barrier to acquisition of integrase resistance similar to that seen with initial HIV protease inhibitor therapy. To date, treatment failure with InSTI resistance has not been seen when dolutegravir has been used as part of an initial ART regimen.

Clinical Trials

Treatment-Naïve Patients Four trials have supported the use of dolutegravir-based ART in treatment-naïve individuals. The SPRING-1 trial was a dose-ranging study of dolutegravir (10, 25, 50 mg) compared to standard doses of efavirenz, each administered in combination with two NRTIs (van Lunzen et al. 2012). Eighty-two percent of

Integrase Inhibitors, Table 2 Selected drug interactions with dolutegravir in adults

Drug name	Concentration effect	Comments
NNRTI		
Etravirine	↓ Dolutegravir	Use of dolutegravir with etravirine without coadministration of atazanavir/ritonavir, darunavir/ritonavir, or lopinavir/ritonavir is not recommended
Efavirenz	↓ Dolutegravir	Adjust dose of dolutegravir to 50 mg twice daily for treatment-naive and treatment-experienced, InSTI-naive patients
Other agents:		
Medications containing polyvalent cations (e.g., Mg or Al): Cation-containing antacids or laxatives Sucralfate Buffered medications	↓ Dolutegravir	Administer dolutegravir 2 h before or 6 h after taking medications containing polyvalent cations
Oral calcium or iron supplements	↓ Dolutegravir	Administer dolutegravir 2 h before or 6 h after taking supplements containing calcium or iron. Alternatively, dolutegravir and supplements containing calcium or iron can be taken together with food
Anticonvulsants: Carbamazepine Oxcarbazepine Phenobarbital Phenytoin	↓ Dolutegravir	Coadministration is not recommended
Herbal products: St. John's wort (<i>Hypericum perforatum</i>)	↓ Dolutegravir	Coadministration is not recommended
Antimycobacterials: Rifampin	↓ Dolutegravir	Adjust dose of dolutegravir to 50 mg twice daily for treatment-naive and treatment-experienced, InSTI-naive patients. Use alternatives to rifampin for InSTI-experienced patients with certain InSTI-associated resistance substitutions or clinically suspected InSTI resistance
Hypoglycemic agents: Metformin	↑ Metformin	Consider dose reductions in metformin when coadministered with dolutegravir

*Adapted from Dolutegravir 2014 package insert product information

all participants who received dolutegravir achieved an HIV viral load <50 copies/mL through week 96, and no patients who received the 50 mg dose developed a viral load of >400 copies/mL or resistance mutations. In the SINGLE trial, patients were randomized to dolutegravir plus abacavir/lamivudine (n = 414) versus tenofovir/emtricitabine/efavirenz (n = 419) (Walmsley et al. 2013). At week 48, those randomized to the dolutegravir arm had significantly higher rates of viral suppression <50 copies/mL than those treated with efavirenz (88% vs. 81%, p = 0.003). The difference between the two treatment arms was driven primarily by increased discontinuations due to adverse events related to efavirenz. The

SPRING-2 trial compared dolutegravir to raltegravir, each given with an investigator-selected NRTI background of either tenofovir/emtricitabine or abacavir/lamivudine. 411 patients were randomly allocated to receive dolutegravir and 411 to receive raltegravir and received at least one dose of study drug. At 48 weeks, the dolutegravir-based regimens were noninferior to the raltegravir-based regimens, with rates of viral suppression to <50 copies/mL of 88% and 85%, respectively (adjusted difference 2.5%, 95% CI -2.2 to 7.1) (Raffi et al. 2013). Finally, the FLAMINGO trial compared dolutegravir to the protease inhibitor darunavir/ritonavir, each administered with a tenofovir/emtricitabine or abacavir/lamivudine nucleoside RT inhibitor

backbone. In the analysis 484 patients were included, with 242 in each arm. Those randomized to dolutegravir had significantly higher rates of HIV suppression at study week 48 with the adjusted difference of 7% in viral suppression meeting criteria for superiority compared to darunavir-/ritonavir-based regimens (90% vs. 83%, $p = 0.025$) (Clotet et al. 2014).

Treatment-Experienced Patients In the SAILING study, 715 HIV-infected patients with HIV treatment failure (HIV RNA >400 copies/mL) and resistance to two or more classes of ART were randomized to optimized background ART and either dolutegravir ($n = 354$) or raltegravir ($n = 361$) (Cahn et al. 2013). At week 48, 71% patients on dolutegravir had viral loads <50 copies/mL versus 64% patients on raltegravir (adjusted difference 7.4%, 95% CI 0.7–14.2) with superiority of dolutegravir versus raltegravir also concluded ($p = 0.03$). The VIKING studies were designed to evaluate the efficacy of dolutegravir as rescue therapy in treatment-experienced patients infected with HIV strains with resistance mutations to raltegravir and/or elvitegravir. VIKING-1–VIKING-2 were a dose-ranging phase IIb trial which found that 50 mg twice-daily dolutegravir compared to once daily could significantly decrease HIV-1 viral load with more subjects achieving the primary end point in the twice-daily cohort (23 of 24 [96%]) compared with the once-daily cohort (21 of 27 [78%]) at day 11. In addition, at week 24, 75% in the twice-daily cohort versus 41% in the once-daily cohort attained viral loads <50 copies/mL (Eron et al. 2013a).

VIKING-3 was a single-arm phase III study in which therapy-experienced adults with INI-resistant virus received dolutegravir 50 mg twice daily while continuing their failing regimen (without raltegravir or elvitegravir) through day 7, after which the regimen was optimized with ≥ 1 fully active drug and dolutegravir continued. Mean change in HIV-1 viral load at day 8 was $-1.43 \log_{10}$ copies/mL, and 69% of subjects achieved <50 c/mL at week 24 (Castagna et al. 2014). However, VIKING-3 also revealed that the efficacy of dolutegravir 50 mg twice daily was reduced in patients with an InSTI resistance

Q148 substitution plus two or more additional InSTI resistance substitutions. Further, virological response rates declined significantly with fold changes between 4 and 10 and greater than 10. Finally, VIKING-4 was a phase III study of 30 therapy-experienced adults with raltegravir- or elvitegravir-resistant virus randomized to dolutegravir 50 mg twice daily or placebo while continuing their failing regimen (without raltegravir or elvitegravir) for 7 days (Akil et al. 2015). At day 8, all subjects switched to open-label dolutegravir 50 mg twice daily and optimized background therapy including ≥ 1 fully active drug. Adjusted mean change in HIV-1 viral load at day 8 was $-1.06 \log_{10}$ copies/ml for the dolutegravir arm and $0.10 \log_{10}$ copies/ml for the placebo arm (treatment difference $-1.16 \log_{10}$ copies/ml [$-1.52, -0.80$], $p < 0.001$). Overall, 47% and 57% of subjects had viral loads <50 and <400 copies/ml at week 24 and 40% and 53% at week 48, respectively. Longer-term efficacy and safety during the open-label phase were in line with the results of the larger VIKING-3 study.

Investigational Integrase Inhibitors

Cabotegravir (S/GSK1265744)

Cabotegravir, an investigational InSTI, was initially evaluated in a once-daily oral formulation but is currently being developed as a long-acting parenteral agent intended to be used for monthly or even quarterly administration. The Long-Acting Antiretroviral Treatment Enabling (LATTE) trial was a phase IIb study to determine if two antiretroviral agents suitable to be developed as very long-acting drugs would be sufficiently active as antiretroviral maintenance therapy in patients who had achieved and maintained viral suppression with an initial three-drug induction regimen. This trial of cabotegravir and the NNRTI rilpivirine included 243 patients randomized to the induction regimen of once-daily oral cabotegravir 10 mg, 30 mg, or 60 mg or efavirenz 600 mg with tenofovir/emtricitabine or abacavir/lamivudine through week 24, followed by a two-drug oral

maintenance regimen of a blinded cabotegravir plus rilpivirine 25 mg through week 96 (Margolis et al. 2014). Following induction therapy, oral cabotegravir and rilpivirine maintained virological suppression at a rate of 76% in the cabotegravir arm and 63% in the efavirenz arm. Results of this trial suggest the combination may be a future option for both HIV maintenance therapy and possibly preexposure prophylaxis (PrEP) for HIV-uninfected persons. In addition, the long-acting injectable cabotegravir nano-suspension has been shown to be protective against acquisition of simian/human immunodeficiency virus in a low-dose viral challenge monkey model (Andrews et al. 2014).

GS-9883

GS-9883 is an investigational once-daily oral InSTI, which does not require pharmacological boosting. It is early in the clinical development cycle with a phase Ib trial of safety, PK, and antiviral activity having been completed assessing doses ranging from 5 to 100 mg (clinicaltrials.gov 2015). Additional studies being done include evaluation of the PK of GS-9833 in persons with impaired renal function and a phase II study assessing the safety and efficacy of GS-9833 versus dolutegravir – each paired with emtricitabine/tenofovir alafenamide.

MK-0518

MK-0518 is an investigational reformulated version of raltegravir which is intended to be administered once daily. In a multidose, randomized, three-treatment, crossover PK study of 24 healthy male and female subjects, subjects received either 1200 mg once daily of the raltegravir formulation (3 × 400 mg tablets), 1200 mg once daily of MK-0518 (2 × 600 mg tablets), or 400 mg twice daily of raltegravir for 5 days. Results suggested high tolerability, less dependence on meal type, and high probability of MK-0518 achieving non-inferiority to 400 mg twice-daily raltegravir (Rizk et al. 2014). Data from this study supported the continued investigation of a once-daily dosing regimen with reformulated raltegravir. OnceMRK is an ongoing phase III, multicenter, non-inferiority RCT of an estimated 750 ART-naïve

adult patients designed to assess the safety and efficacy of MK-0518 1200 mg once daily compared to raltegravir 400 mg twice daily, both given in combination with emtricitabine/tenofovir (Merck 2015).

Clinical Use of HIV Integrase Inhibitors

Initial ART

In April 2015, the US Department of Health and Human Services (DHHS) Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents were changed to include five initial ART regimens as recommended for treatment-naïve HIV-1-infected patients: one protease inhibitor-based regimen and four InSTI-based regimens (DHHS 2015). The four recommended InSTI-based regimens are dolutegravir/abacavir/lamivudine, dolutegravir plus tenofovir/emtricitabine, elvitegravir/cobicistat/tenofovir/emtricitabine, and raltegravir plus tenofovir/emtricitabine. Increasingly, InSTI-based ART regimens are the default choices for initial antiretroviral therapy based on their favorable characteristics of high efficacy, infrequent adverse events, and (for raltegravir and dolutegravir) infrequent drug-drug interactions. Furthermore, in the head-to-head comparisons between darunavir/ritonavir and dolutegravir (FLAMINGO), the InSTI-containing regimen had a significantly higher viral suppression rate. While the selection of the optimal ART regimen must be individualized, InSTI-based ART represents an excellent option for most patients.

ART in Treatment-Experienced Patients

HIV integrase inhibitors have been demonstrated to have significant value in the treatment of HIV-infected patients failing ART with resistance to other antiretroviral agents. Raltegravir and dolutegravir both are indicated for treatment-experienced patients and are frequently critical components of successful ART in this setting, particularly in patients who are naïve to InSTI. Moreover, based on findings from the VIKING-3 study, twice-daily dolutegravir can provide significant benefit in many patients who have

acquired InSTI resistance due to failure of raltegravir- or elvitegravir-based regimens (Castagna et al. 2014). However, because patients with the Q148 integrase mutation plus two or more secondary InSTI mutations respond less well to dolutegravir, it is recommended that such patients undergo integrase genotypic or phenotypic resistance testing prior to initiating dolutegravir.

Regimen Switching

In patients on successful ART who are experiencing problems with tolerability or who are receiving complex regimens that challenge adherence, switching to a simpler InSTI-based regimen may be a good option. Review of any previously identified antiviral resistance is an important step in determining if such a switch can be done safely since the presence of accumulated nucleoside reverse transcriptase inhibitor resistance may diminish the likelihood of sustaining HIV suppression after such a switch. InSTI-based treatment regimens have become a popular option for switch strategies.

Use in Specific Clinical Scenarios

The use of HIV integrase inhibitor-based ART regimens may be particularly appealing in certain patient situations, though there are important caveats to consider:

1. **Patients requiring treatment with drugs affected by CYP3A4 inhibitors (pharmacologic boosters ritonavir or cobicistat)** should avoid elvitegravir since it requires boosting to be effective. Drug-drug interactions become problematic in this setting.
2. Raltegravir may be appealing for **patients already taking medications that require twice-daily dosing**. The resultant dose symmetry can facilitate improved adherence. On the other hand, in patients with adherence concerns based on the need for twice-daily dosing, raltegravir should probably be avoided.
3. **In patients who are HLA-B5701 positive**, abacavir is contraindicated. Thus, the use of the single-pill dolutegravir fixed-dose combination (with abacavir/lamivudine) must be avoided in this group. Screening for abacavir hypersensitivity risk (HLA-B5701 screen) is essential prior to initiating the dolutegravir single-pill combination.
4. **In patients with significant renal insufficiency**, tenofovir should generally be avoided. Thus, the use of elvitegravir as part of a fixed-dose single-pill combination treatment regimen (which contains tenofovir/emtricitabine) is not recommended for patients with a creatinine clearance of <70 mL/min.
5. **In patients with lipid disorders**, it is usually prudent to avoid the booster cobicistat due to drug interactions and its potential to impact lipid profiles. Therefore, the coformulated single-pill elvitegravir regimen should generally be avoided in this population.
6. Similarly for **patients with cardiovascular disease (CVD) or significant risk of CVD who require ongoing statin therapy**, it may be appropriate to avoid using cobicistat with certain statins including lovastatin and simvastatin to preclude possible harmful drug interactions. Concerns about associations between abacavir and CVD also make the use of the dolutegravir-based single-pill combination (which contains abacavir) problematic in this population.
7. Because of the relatively low risk of drug-drug interactions with both raltegravir and dolutegravir, these drugs are particularly good options for **patients coinfecting with hepatitis C virus (HCV)**. No clinically significant interactions have been found between these two medications and the most commonly used anti-HCV drugs, including ribavirin, simeprevir, and coformulated ledipasvir/sofosbuvir. The recently approved coformulated HCV drug ombitasvir/paritaprevir/ritonavir plus dasabuvir can be safely used with raltegravir, but its safety with dolutegravir has not yet been evaluated.
8. **In patients coinfecting with hepatitis B virus (HBV)**, all three InSTIs can be safely used with HBV therapy. However, most such patients should be on the dual HBV combination of tenofovir/emtricitabine. The combination tablet dolutegravir/abacavir/lamivudine would not be recommended in this setting since abacavir is not active against HBV, and an

additional antiviral agent would need to be added for adequate HBV treatment.

9. In **patients in whom poor adherence is a significant concern**, the usual preferred strategy has been to start a protease inhibitor-based treatment regimen since virological failure with boosted PIs does not engender PI resistance. A similar outcome has been noted with dolutegravir-based initial therapy, but there are too few observations to be certain of this effect. Many clinicians now prefer starting dolutegravir in such patients since its improved tolerability and single-tablet combination availability may facilitate adherence. Further data will help determine if this approach is optimal.

Conclusion

The addition of the HIV integrase inhibitor class of antiretroviral drugs has significantly strengthened available antiretroviral treatment regimens for all types of patients. With high potency and a low potential for drug-drug interactions, InSTIs are increasingly replacing the use of NNRTI- and PI-based ART regimens. As a result of their well-documented treatment efficacy, excellent tolerability, and simplicity and convenience, HIV InSTIs have emerged to become a mainstay of HIV-1 therapeutics.

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Integration

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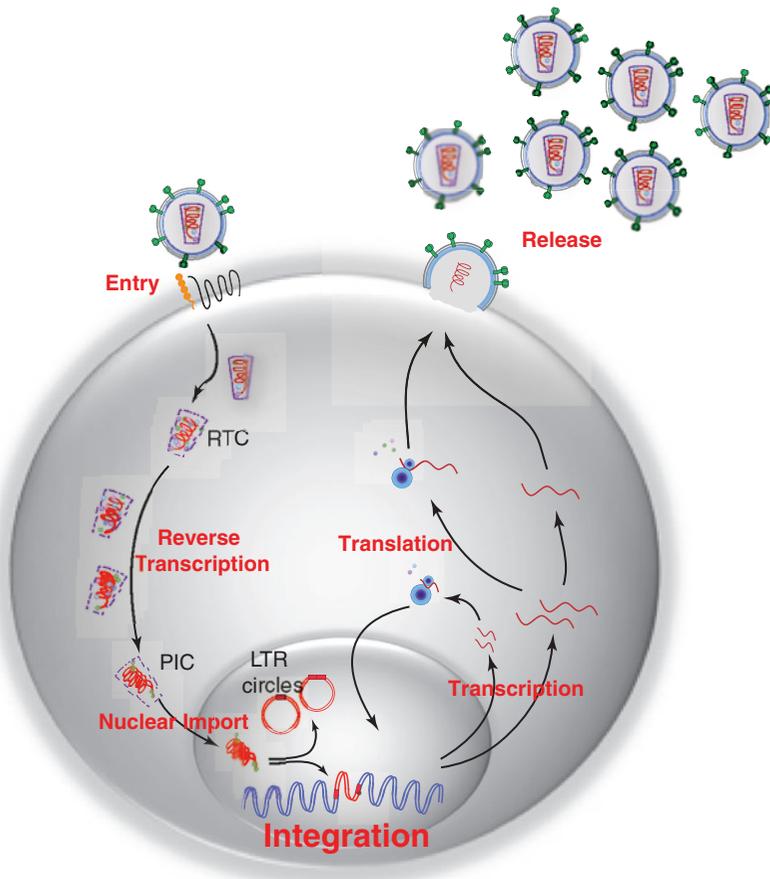
Definition

Integration refers to the stable connection of the viral DNA produced by reverse transcription to the host chromosomal DNA.

Introduction

Human immunodeficiency virus type 1 (HIV-1), like all retroviruses, is characterized by two steps in the replication cycle, reverse transcription and integration (Fig. 1; Suzuki and Craigie 2007; Flint et al. 2009; Nordkin 2009; Bushman et al. 2011;

► **Overview: Life Cycle**). HIV-1 particles contain two copies of the viral genome as single-stranded positive-sense RNA molecules. Upon entry and release of the viral core in the cytoplasm of the host cell, the viral RNA genomes are used as templates by the viral reverse transcriptase (RT) to generate a linear and blunt-ended double-



Integration, Fig. 1 HIV-1 replication cycle overview. HIV-1 particles attach to target cells through the subsequent interaction of viral envelope with CD4 and a chemokine co-receptor (CXCR4 or CCR5), triggering viral entry and core release in the cytoplasm of the host cell. Core-derived nucleoprotein complex, named reverse transcription complex (RTC), contains minimally matrix, capsid, nucleocapsid, RT, IN, and viral RNA genome. During reverse transcription, RTC evolves toward a preintegration complex (PIC) containing the viral linear cDNA genome

copy and migrates to the nuclear membrane along microtubules. Through interactions with nuclear pore proteins, the PIC translocates into the nucleus. The viral cDNA is then either degraded or circularized by host DNA repair proteins yielding 1-LTR or 2-LTR circles or integrated into the host chromosome through IN action. Once integrated, the viral genome uses the host cell machinery to transcribe and translate the viral components necessary to produce viral particle progeny

stranded cDNA copy (► [Fusion](#); ► [Uncoating and Nuclear Import](#); ► [Reverse Transcription](#)). This viral cDNA genome copy encodes the nine HIV-1 open reading frames (ORF) and at the ends contains ~634-bp direct repeats, the long terminal repeats (LTR). The LTRs contain cis-acting sequences needed for integration as well as sequences necessary for subsequent viral transcription. The viral cDNA genome is associated with multiple viral and cellular proteins, including the virally encoded integrase (IN), in a nucleoprotein complex called the preintegration complex (PIC). The exact composition of the PIC is still unclear but includes IN, matrix (MA), and capsid (CA) viral proteins as well as cellular proteins (Suzuki and Craigie 2007). Some PIC components, including CA, interact with nuclear pore proteins, thereby allowing PIC translocation into the nucleus of nondividing cells as well as dividing cells. Once in the nucleus, the viral DNA becomes integrated into the host genome. Integration typically occurs between 10 and 20 h post-viral exposure in susceptible CD4⁺ T cells. Only a minority of viral particles ultimately succeeds to integrate under most conditions. Integrated proviruses can affect the transcription of neighboring cellular genes by a number of pathways (insertional mutagenesis). The persistence of the integrated provirus is one of the biggest barriers to virus elimination from the infected individual (Siliciano and Greene 2011; Desfarges and Ciuffi 2012).

The Viral Integrase Enzyme

The HIV-1 *polymerase (pol)* gene codes for the three viral enzymes: protease (PR), reverse transcriptase (RT), and integrase (IN). The key protein required for viral genome integration is IN (Neamati 2011). IN is first incorporated into virions as part of a Gag-Pol precursor protein, which is processed by the viral PR to generate the mature protein products, including IN. A viral particle is estimated to contain about 40–100 molecules of IN.

The prototypical IN enzyme (HIV-1 group M subtype B strain HxB2, NCBI Reference Sequence NP_705928.1) is a 32 kDa protein consisting of 288 amino acids and is divided into three structural and functional domains (Fig. 2). These include the N-terminal domain (NTD) containing residues 1–50, the central catalytic core domain (CCD) encompassing residues 51–212, and the C-terminal domain (CTD) with residues 213–288 (Neamati 2011).

The NTD is a bundle of 4 α -helices that is stabilized by the coordination of a zinc via four conserved residues, H¹², H¹⁶, C⁴⁰, and C⁴³, constituting the characteristic HHCC motif. The NTD is involved in viral DNA binding as well as in IN multimerization.

The CCD is characterized by a conserved acidic triad motif, D,D-35-E, which constitutes the catalytic site motif that is highly conserved among retroviruses, retrotransposons, and DNA

1	FLDGIDKAQD	E HEKY H SNWR	AMASDFNLPP	VVAKEIVASC	DK C QLKGEM	50	NTD
51	HGQVDCSPGI	W Q L D CTHLEG	KVILVAVHVA	SGYIEAEVIP	AETGQETAYF	100	
101	LLKLAGRWPV	KTIHT D NGSN	FTGATVRAAC	WWAGIKQEFQ	IPYNPQSQGV	150	CCD
151	V ESMNKELKK	IIGQVRDQAE	HLKTAVQMAV	FIHNFKRKGG	IGGYSAGERI	200	
201	VDIATDIQT	KE				212	
213		L Q K Q ITKI	QNFVYYRDS	RNPLWKGPAP	LLWKGEHAVV	250	CTD
251	IQDNSDIKVV	PRRKAKIIRD	YGQMAGDDC	VASRQDED		288	

Integration, Fig. 2 HIV-1 IN sequence. The prototypical HIV-1 IN sequence (HIV-1 group M subtype B strain HxB2) consists in 288 amino acids and is divided into three functional structural and functional domains: (i) the N-terminal domain (NTD) consisting of residues 1–50, containing the HHCC motif (highlighted in *bold*) involved in zinc binding, (ii) the catalytic core domain (CCD)

encompassing residues 51–212, characterized by the D, D-35-E motif (highlighted in *bold*) required for Mg²⁺ coordination and catalytic activities of the enzyme, and (iii) the C-terminal domain (CTD) containing residues 213–288. The three domains are involved in IN multimerization and in DNA binding

transposons. The catalytic D⁶⁴D¹¹⁶E¹⁵² coordinates two divalent metal ions (Mg²⁺) necessary for the integration steps catalyzed by IN. Although Mg²⁺ is physiologically abundant and thus likely to be the divalent metal ion coordinated by IN in vivo, Mn²⁺ can also support integration in vitro. In addition to its catalytic activity, the CCD is also involved in DNA binding of both viral and DNA sequences, as well as in IN multimerization. In vitro studies identified multiple CCD residues that contact viral DNA ends, including Y143 and Q148. Overall, the fold of this domain is that of the RNase H superfamily of polynucleotidyl phosphotransferase enzymes.

The CTD is a less well-conserved domain consisting of five antiparallel β -strands arranged in a SH3-like β -barrel. It is implicated in IN multimerization and contains positively charged regions (mostly K residues clustered on the protein surface) involved in nonspecific DNA binding.

IN Structure

The full-length structure of HIV-1 IN is not yet solved at high resolution. However, the structures of the three individual IN domains, or pairs of IN domains, have been determined by nuclear magnetic resonance (NMR) or X-ray crystallography (Li et al. 2011; Neamati 2011; Craigie and Bushman 2012). More importantly, the structure of the complete retroviral prototype foamy virus (PFV) IN complexed with viral DNA (i.e., PFV intasome) has been solved both with and without target DNA (Cherepanov et al. 2011; Li et al. 2011). Although not identical to HIV-1 intasome, the PFV intasome provides valuable information on IN structure and function particularly that IN catalyzes strand transfer as a homotetramer, with only one active catalytic site in each IN dimer, and that the host target DNA is severely bent to bring host target DNA and viral DNA ends in close vicinity, which is required for strand transfer (ligation of viral and cellular DNA). Thus, the PFV intasome structure specifies IN residues in direct contact with viral and target DNAs. In addition, the PFV structure has been solved with integrase inhibitors bound, specifying key aspects

of the mechanism of action (section “[Compounds Inhibiting HIV-1 Integrase](#)” below).

IN Mutants

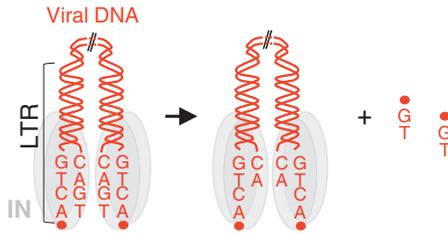
IN function has been probed extensively using site-directed mutagenesis. Two classes of mutants, class I and class II, have been identified (Engelman et al. 1999; Neamati 2011; Craigie and Bushman 2012). The first class of IN mutants defines enzymes that are selectively impaired in IN-mediated reactions. The second class of mutants, intriguingly, does not affect IN activity only but rather has pleiotropic effects on other HIV-1 replication steps, including reverse transcription and assembly/release of infectious viral particles. Indeed, IN can directly interact with RT, thereby improving RT efficiency. Mutations in the IN sequence of *pol* can affect PR-mediated processing of Gag-Pol polyproteins, thereby affecting the maturation of viral particles. These data suggest that HIV-1 IN protein has additional roles in viral infection in addition to the insertion of the viral genome into the host chromosome.

The Integration Reaction

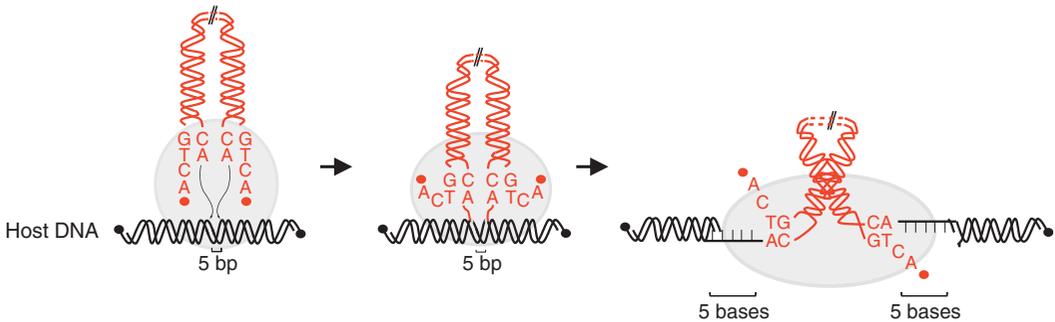
The integration process is divided in three steps (Fig. 3): (i) 3' processing, (ii) strand transfer, and (iii) repair (Desfarges and Ciuffi 2010; Neamati 2011; Arts and Hazuda 2012; Craigie and Bushman 2012; Hazuda 2012). The first two steps are catalyzed by IN; the last one is not fully clarified but probably carried out by the DNA repair machinery of the host cell.

The 3' processing reaction, also called terminal cleavage, consists in the removal of a dinucleotide at the 3' ends of the viral termini, generating CA-3' recessed hydroxyl ends. This reaction is catalyzed in the cytoplasm by a dimer of IN that is bound on each viral LTR end. The presence of the invariant 3' CA nucleotides in the LTR sequence is essential for efficient processing. In vitro this process can be recapitulated using purified IN and a duplex of oligonucleotides, usually of 21–32 bp long, mimicking the viral LTR ends. Experimentally, radioactive labeling of the 5' end of the cleaved strand of a duplex oligonucleotide

3' processing:



Strand transfer:



Repair (host):



Integration, Fig. 3 HIV-1 integration reaction. The integration process can be divided in three steps: 3' processing or terminal cleavage (*top panel*), strand transfer or joining (*middle panel*), and gap repair (*low panel*). 3' processing requires an IN dimer (IN depicted as ovals) at each viral end and consists in the IN-mediated cleavage of a dinucleotide at each 3' end of the viral cDNA genome, leaving recessed invariant 3' CA-OH ends. Strand transfer requires an IN tetramer (depicted as a *circle*) and consists in

IN-mediated breaking of the host DNA on opposite strands with a 5 bp stagger and simultaneous joining of the host DNA to the 3' recessed ends of the viral DNA. The last step is carried out by host DNA repair proteins, which fill in the 5 single-stranded nucleotide gap, remove the two protruding 5' viral nucleotides, and ligate the target host DNA to the viral DNA, ultimately leading to the stable integration of the viral DNA into the host genome. Filled circles at DNA ends represent 5' ends

model substrate allows monitoring of the reaction progression by gel electrophoresis.

The strand transfer or 3' joining reaction consists in the asymmetric break of the host target DNA 5 bp apart and simultaneous joining of the viral 3'-OH recessed ends by a one-step transesterification mechanism (Engelman et al. 1991). This process involves a tetramer of IN molecules and occurs in the nucleus. The reaction can be recapitulated *in vitro* with model substrates. In these, one viral end (half-site integration) or both viral ends (full-site or concerted integration) can

become joined to a target DNA. Half-site integration has been widely used and consists in mixing purified IN with a short oligonucleotide duplex (mimicking viral LTR end, as for *in vitro* 3' processing reactions) and subsequently with a target DNA, thereby reproducing the joining of one viral DNA end to a target DNA. A more faithful *in vitro* reproduction of strand transfer takes advantages of using longer donor DNAs containing mimics of viral 3' recessed LTR ends on both termini, thereby allowing full-site or concerted integration, i.e., the joining of both viral 3' recessed ends to

both strands of target DNA, with a 5 bp stagger. Although IN is able to carry out the 3' processing and strand transfer reactions alone as demonstrated by *in vitro* assays, its interaction with viral and cellular proteins may contribute to integration efficiency. This is best exemplified by lens epithelium-derived growth factor (LEDGF)/p75, a cellular protein interacting with IN, which improves integration efficiency both *in vitro* and *in vivo* and guides the viral genome integration into nonrandom sites of the host DNA (see section “[Integration Target Site Selection](#)”).

Proteins of the host DNA repair machinery probably fill in the five unpaired nucleotides of the host DNA on each side of the viral genome, remove the two viral 5' protruding nucleotides, and ligate the 5' viral ends to the repaired 3' host DNA ends. The specific host cell enzymes involved are unclear, though cocktails of known DNA repair enzymes can carry out the reaction on model substrates *in vitro*. For HIV-1, the repair process generates a 5-bp duplication at each host-virus DNA junction. The repair reaction completes the stable insertion of the viral DNA copy of the RNA genome into the host chromosome, forming the integrated provirus.

Circularization of the Viral DNA

In addition to correct integration, the viral cDNA is sometimes recognized by the host DNA repair machinery to generate 1-LTR or 2-LTR circles. 1-LTR circles are thought to arise from an aberrant RT product or from LTR-LTR homologous recombination. 2-LTR circles arise from the ligation of the two ends of the viral linear cDNA, through the action of the non-homologous end joining pathway (NHEJ), which is comprised of cellular Ku80, XRCC4, and ligase IV NHEJ components. Two-LTR circles are a useful marker for *in vitro* and *in vivo* studies because they are likely to reflect successful early steps, from entry to nuclear import. However, the LTR circles are considered to be dead-end products for viral replication, and only the correctly integrated viral genome (provirus) allows for productive infection, *i.e.*, for efficient transcription of all the viral genes and thus the assembly and release of infectious viral particles (Craigie and Bushman 2012).

Integration Target Site Selection

Although IN is able to catalyze the strand transfer of the viral genome into any host DNA phosphodiester bond *in vitro*, multiple *in vivo* studies have demonstrated that the integration of the viral genome into the host cell chromosome is not random (Desfarges and Ciuffi 2010; Craigie and Bushman 2012). Thanks to the publication of the human DNA genome sequence, it has become possible to map HIV-1-specific integration site distributions, revealing favored host DNA sites for integration. Preferential HIV-1 integration sites (i) display a weak GT^A/TAC DNA sequence consensus with AT-rich local target region; (ii) occur at distorted, bent DNA, at the outer surface of DNA wrapped nucleosomes and at major grooves; (iii) take place in gene-dense regions; (iv) are found in active transcription units, with no bias along the gene, and in both exons and introns; (v) are associated with epigenetic marks characterizing active transcription (histone acetylation, H3K4me1/2, H3K9me1, H3K27me1, H3K36me3); and (vi) occur frequently in regions rich in Alu repeats. HIV-1 integration is disfavored in intergenic regions, in long interspersed nuclear element (LINE) repeats, and in near epigenetic marks associated with repressed transcription (H3K9me2/3, H3K27me2/3, H3K79me3, DNA CpG methylation).

Host Cofactors Involved in Integration

IN is the major viral determinant of HIV-1 integration site targeting in the host DNA (Craigie and Bushman 2012; ► [Cellular Cofactors as Drug Targets, Overview](#)). However, many cellular proteins can interact with HIV-1 IN, thereby potentially affecting integration. Retroviral integration is favored on nucleosomal DNA *in vitro*, and analysis of integration site patterns *in vivo* suggests favored integration on outward-facing major grooves of nucleosome-wrapped DNA. Cellular factors may help to condense and organize the viral DNA. One candidate is the barrier-to-autointegration factor (BAF) which is involved in DNA condensation and prevents suicidal viral autointegration products, and another is LEDGF/p75 (more on the latter below) (Neamati 2011;

Craigie and Bushman 2012; Suzuki et al. 2012). Additional cellular proteins have been shown to interact with HIV-1 IN or assist integration in vitro, including high-mobility-group chromosomal protein A1 (HMGA1), integrase interactor 1 (Ini-1), Gemin2, importin 7, UNG2, VBP1, NUP153, NUP62, p300, DNA-PK, hsp60, EED, transportin 3 (TNPO3/TRN-SR2), and HRP-2 (Suzuki and Craigie 2007; Neamati 2011; Ao et al. 2012; Craigie and Bushman 2012; Suzuki et al. 2012; Taltynov et al. 2012). In general, to date the importance of these proteins to HIV-1 integration in vivo has not been fully clarified, but they do provide interesting hypothesis for further study. Similarly, genome-wide siRNA screens and mass spectrometry binding studies suggest additional potential cellular cofactors (for review and extensive references, see Desfarges and Ciuffi (2010) and Craigie and Bushman (2012)).

Recently, TRN-SR2 and RANBP2 were suggested to be involved in PIC nuclear translocation through an interaction with viral CA, and TRN-SR2 was also shown to interact with the HIV-1 intasome (Larue et al. 2012; ► [Transportin-SR2 \(TNPO3\)](#), [Nuclear Import](#)). Knockdowns of these two cellular factors or specific CA mutants resulted in altered integration site targeting. Although the detailed mechanism is still unclear, these data suggest a functional coupling between nuclear import and proper integration (Suzuki and Craigie 2007; Neamati 2011; Ao et al. 2012; Craigie and Bushman 2012; Suzuki et al. 2012; Taltynov et al. 2012).

LEDGF/p75

LEDGF/p75 is a 530 aa cellular protein belonging to the hepatoma-derived growth factor (HDGF) family that was first identified as a transcriptional co-activator ► [LEDGF/p75](#), [Cofactors of Integration](#). LEDGF/p75 contains an N-terminal chromatin-binding domain as well as a C-terminal integrase-binding domain (IBD), thereby allowing tethering the viral PIC to chromatin, facilitating integration and dictating integration target site selection (Desfarges and Ciuffi 2010; Neamati 2011; Craigie and Bushman 2012; Desfarges and Ciuffi 2012). Biochemical

and structural studies have identified residues involved in this interaction: critical LEDGF/p75 residues comprise I³⁶⁵, D³⁶⁶, F⁴⁰⁶, and V⁴⁰⁸, while IN key residues map to CCD and include L¹⁰², A¹²⁸, A¹²⁹, W¹³¹, and W¹³² on one IN monomer and residues D¹⁶⁷ to H¹⁷¹ on the second IN monomer. These IN residues participate in forming a hydrophobic pocket at the IN dimer interface into which a LEDGF/p75 protein loop structure can be inserted (Li et al. 2011; Neamati 2011).

Cell systems using LEDGF/p75 over-expression, knockdown or knockout, have shown that (i) small amounts of LEDGF/p75 are sufficient for contribution to HIV replication, (ii) LEDGF/p75 protects HIV-1 IN from proteosomal degradation, (iii) LEDGF/p75 improves integration efficiency, and (iv) LEDGF/p75 is the major cellular determinant of HIV-1 integration site targeting preferences.

HIV-1 integration site distribution can be successfully modified by generating LEDGF/p75 constructs in which the chromatin-binding domain of LEDGF/p75 was replaced with other domains which have different chromatin-binding specificities. Expression of such constructs in cells depleted for wild-type LEDGF resulted in altered HIV-1 integration site distributions in cells. This provides strong evidence that a simple tethering mechanism mediates HIV-1 integration targeting by LEDGF/p75. Hepatoma-derived growth factor-related protein 2 (HRP-2), another member of the HDGF family, also contains an IBD and interacts with HIV-1 IN. Recent studies investigated the role of HRP-2 in HIV-1 integration, in the absence of the major cofactor LEDGF/p75, and confirmed that HRP-2 could function as an alternative tether for HIV-1 IN, although less efficiently than LEDGF/p75 (Desfarges and Ciuffi 2010, 2012; Neamati 2011; Craigie and Bushman 2012).

Consequences of HIV-1 Integration

Retroviral integration can affect cells by altering the activity of host genes surrounding the integrated provirus (Craigie and Bushman 2012; Desfarges and Ciuffi 2012). HIV-1 may have evolved to target integration preferentially in

active transcription units in order to allow efficient transcription after integration, thereby improving the chances of productive infection and viral progeny production (Craigie and Bushman 2012). On the host cell side, gene disruption provoked by viral genome insertion may lead to altered transcription of the host gene. Retroviral integration is well-documented to be associated with cancer in numerous animal models, and a rich literature documents the mechanisms by which retroviral integration can cause transformation. Gammaretroviruses, which have different integration site preferences than does HIV-1, have been used as viral vectors in successful gene therapy clinical trials but have been associated with tumor development correlated with insertion of the viral vector genome in the vicinity of proto-oncogenes.

In the case of HIV-1, however, though HIV+ individuals may develop HIV-associated cancers, these tumors do not appear to be mediated directly by HIV-1 – analysis of tumor cells does not show integrated HIV-1 proviruses. Thus other causes appear to mediate HIV-related malignancies, such as loss of immune surveillance due to the immunodeficient state or activation of cancer-associated infectious agents. The lack of insertional activation by HIV-1 is likely linked to the propensity to infect postmitotic cells, and the presence of at least two cytotoxic/cytostatic HIV-1 gene products (HIV-1 *vpr* and *env*).

HIV-1 Latency

The stable integration of the viral genome into the host cell chromosome ensures a lifelong association of the virus with the infected cell. The long-term persistence of the integrated provirus is one of the main barriers to eradicating HIV-1 from infected individuals (Siliciano and Greene 2011). HIV+ patients treated effectively with antiretroviral therapy (ART) have no detectable plasma viremia, but cessation of ART leads to viral rebound, establishing the existence of long-lived latently infected cells. Extensive efforts are currently being devoted to understanding the repressive mechanisms responsible for latency (viral

transcriptional silencing). Multiple factors likely influence the efficiency of HIV-1 transcription – for example, the physiological reduction of T-cell transcription associated with formation of the memory compartment is one likely mechanism (► [Transcription \(Initiation, Regulation, Elongation\)](#)). Another potential factor effecting transcriptional efficiency is the genomic site of viral DNA integration. The effects of chromatin environment on transcription are well known, for example, through studies of position-effect variegation in model organisms. Although HIV-1 integrates preferentially into active transcription units, this is only a statistical trend, so that a minority of proviruses can be found in less favored regions. Studies of HIV-1 integration site positions for poorly transcribed proviruses have suggested several possible effects of integration site. Integration in highly transcribed host transcription units may be associated with poor transcription of HIV-1 due to transcriptional interference (conflict between cellular and viral gene transcription). Integration within centromeric heterochromatin, as indicated by integration within centromeric alphoid repeats, or in gene deserts, may lead to repression by heterochromatin formation (Siliciano and Greene 2011; Craigie and Bushman 2012).

Compounds Inhibiting HIV-1 Integrase

HIV-1 viral genome integration is a key step for productive infection, and thus an attractive drug target for inhibition (Neamati 2011; Arts and Hazuda 2012; Hazuda 2012). Identifying inhibitors of HIV-1 IN was challenging but ultimately succeeded with the approval of raltegravir by the US Food and Drug Administration (FDA) in 2007. These compounds bind to the active site formed upon completion of the terminal cleavage reaction, making contacts to both the enzyme and cleaved viral DNA ends. The compounds thus selectively block the DNA strand transfer reaction. Importantly, structural studies using the PFV model further suggest that compound binding displaces the viral DNA end from its correct position in the enzyme active site, thereby interfering

with the chemistry of the strand transfer step (Cherepanov et al. 2011; Li et al. 2011).

Viral escape mutants have arisen that contain amino acid substitutions in the IN protein that reduce sensitivity to raltegravir and related molecules. Two primary pathways have been documented, one involving N155H, the other Q148H. A variety of further substitutions optimize replication of viruses containing the primary substitutions.

Due to the emergence of drug-resistant viruses, identification of novel integrase inhibitors is an important next goal. The resolution of the PFV intasome structure and the identification of essential HIV-1 IN cofactors open new opportunities for inhibiting integration through structure-based design of small molecules and through different mechanisms, such as blocking IN-LEDGF/p75 interaction or IN multimerization (Taltynov et al. 2012).

Conclusion

HIV-1 DNA integration is a key step in viral replication, allowing establishment of a productive infection. Although the enzymatic reactions catalyzed by the HIV-1 IN have been elucidated, many cellular proteins contributing to successful viral integration remain to be validated or uncovered, particularly at the level of cellular repair proteins. Recently, novel cellular proteins (TRN-SR2, RANBP2) highlighted a role for nuclear import in integration targeting, suggesting a functional coupling between these two viral steps. LEDGF/p75 and HRP-2 tether the HIV-1 integration complexes to chromatin and dictate integration site selection. The solved structure of the PFV IN in complex with viral and target DNAs represents a major advance in the molecular understanding of integration. The possible role of integration target site selection in HIV latency is another important topic for further study. A detailed and complete picture of HIV-1 integration process, including cellular players, is starting to be unveiled, and may help identifying novel drug targets and new inhibitors of this essential step of HIV-1 replication.

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Integrin Alpha4beta7

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Definition

The integrins are a superfamily of cell adhesion receptors that play central roles in the biology of metazoans by controlling cell adhesion to the extracellular matrix, cell migration, growth, differentiation, and apoptosis. Integrins are prime examples of physiologically important receptors that have been usurped by viruses for attachment and/or cell entry, including HIV. Various integrins have been recognized as host factors that influence HIV replication. Integrin $\alpha 4\beta 7$, the gut-homing receptor, can serve as a receptor and a signaling molecule for HIV, by interaction with HIV gp120 protein. The specific affinity of gp120 for $\alpha 4\beta 7$ provides a mechanism for HIV-1 to target activated cells that are critical for efficient virus propagation and dissemination following sexual transmission.

The Integrin Superfamily

The integrins are a superfamily of transmembrane receptors that bind to extracellular matrix ligands, cell-surface ligands, and soluble ligands, to mediate adhesion of cells and intercellular interactions. Integrins are the main receptor proteins that cells use to both bind to and respond to the extracellular matrix (Takada et al. 2007).

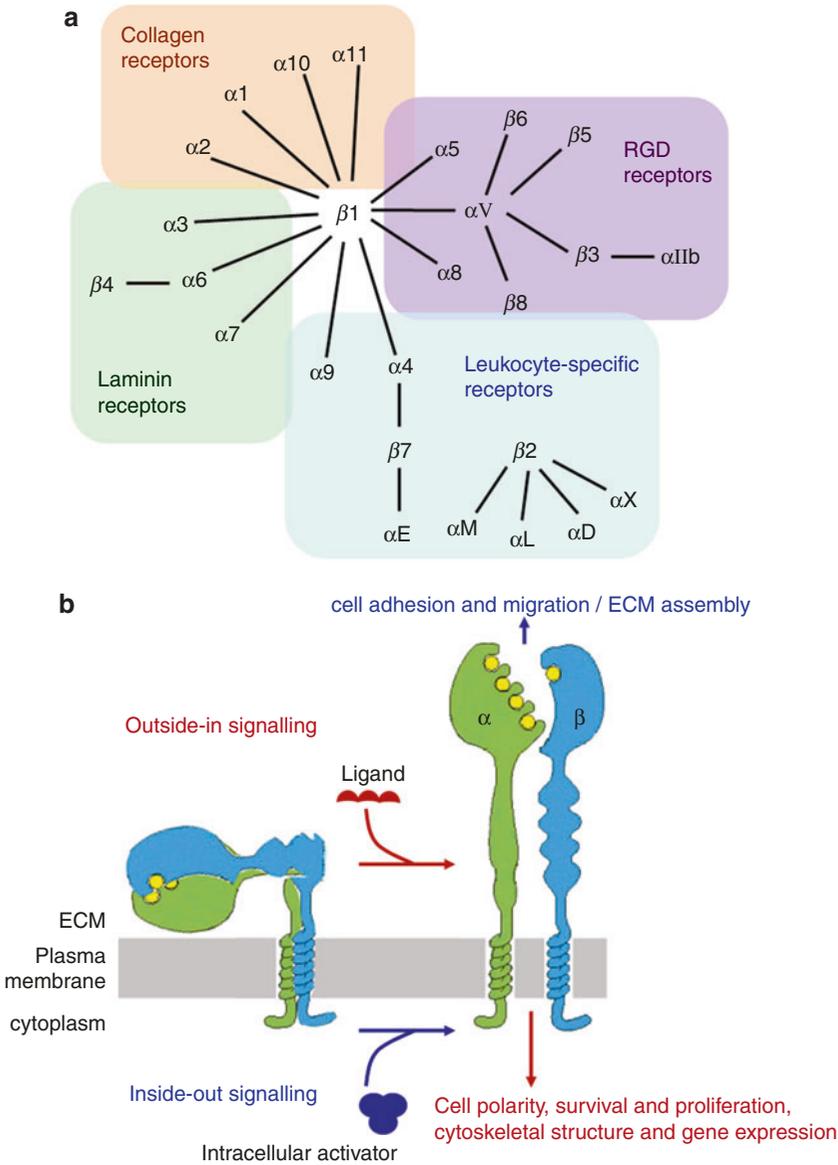
An integrin molecule is composed of two non-covalently associated transmembrane glycoprotein subunits, named α - and β -subunits. In humans, at least 18 α - and eight β -subunits are known, generating 24 different heterodimers, each of them with different cell expression patterns and ligand specificities (Fig. 1a) (Takada et al. 2007). Members of this family have been found in mammals, chicken, and zebra fish, as

well as lower eukaryotes (sponges, the nematode *C. elegans*, and the fruit fly *D. melanogaster*). The α - and β -subunits have distinct domain structures, with extracellular domains from each subunit contributing to the ligand-binding site of the heterodimer (Fig. 1b).

A given integrin can have different ligand-binding specificities in different cell types, mainly due to the existence of specific factors that by interacting with integrins modulate their binding activity (Fig. 1a). On the other hand, many matrix proteins are recognized by multiple integrins, for example, at least eight integrins bind fibronectin and at least five bind laminin (Takada et al. 2007). The sequence arginine-glycine-aspartic acid (RGD) was identified as a general integrin-binding motif, although individual integrins are also specific for particular protein ligands. The binding of integrins to their ligands depends on extracellular divalent cations (Ca^{2+} or Mg^{2+} , depending on the integrin), reflecting the presence of divalent cation-binding domains in the extracellular part of the α - and β -subunits (Shattil et al. 2010). The type of divalent cation can influence both the affinity and the specificity of the binding of an integrin to its ligands.

Integrins contribute to the regulation of development, immunity, inflammation, and hemostasis and to the development of diseases including autoimmunity, atherothrombosis, and neoplasia (Takada et al. 2007). Integrins function as traction receptors that can both transmit and detect changes in mechanical force acting on the extracellular matrix. They serve as transmembrane links between extracellular contacts and the actin microfilaments of the cytoskeleton, whose behavior integrins also regulate and modulate.

There are two directions of integrin signaling, which have different biological consequences. Signals generated inside the cell can either enhance or inhibit the ability of integrins to bind to their ligand outside the cell (Fig. 1b). The ability of a cell to control integrin-ligand interactions from within is termed *inside-out* signaling. On the other hand, integrins also behave like traditional signaling receptors in transmitting information into cells by *outside-in* signaling (Shattil et al. 2010).



Integrin Alpha4beta7, Fig. 1 Human integrin structure and function. (a) The members of human integrin family and how they combine to form heterodimeric integrins. Eighteen α - and eight β -subunits have been identified, which are able to generate 24 different integrins. Integrin subunits that bind to each other to form a heterodimer are connected by *solid lines*. Although each integrin has distinct ligand-binding specificity, they can be grouped with respect to the similarity of their ligands and function. (b) Schematic representation of integrin function. The inactive

integrin is in a V shape with the ligand-binding domain close to the membrane. There is close association between the α - and β -subunits in the membrane-proximal region. There are two directions of integrin signaling. A cell can regulate the adhesive activity of its integrins from within (inside-out signaling, *blue lines*). Integrins also function as signal transducers, activating various intracellular signaling pathways when activated by matrix binding (outside-in signaling, *red lines*)

During *inside-out* signaling, an intracellular activator binds to the β -integrin tail, leading to conformational changes that result in increased affinity for extracellular ligands (integrin

activation). *Inside-out* signaling controls adhesion strength and enables sufficiently strong interactions between integrins and extracellular matrix proteins to transmit the forces required for cell

migration and extracellular matrix remodeling and assembly. The integrin affinity for a given ligand directly regulates the nature of the binding and appears to tune the degree and kinetics of cell adhesion.

On binding extracellular ligands, mammalian integrins cluster in the membrane and transduce signals inside the cell (*outside-in* signaling). Extracellular ligand binding induces conformational changes that lead to the interaction of the cytoplasmic tail with intracellular signaling molecules. The intracellular signals are responsible for the control of cell polarity, cytoskeletal structure, gene expression, and cell survival and proliferation.

Both signaling processes are often closely linked; integrin activation can increase ligand binding, resulting in *outside-in* signaling. Conversely, ligand binding can generate signals that cause *inside-out* signaling, i.e., integrins transduce signals into the cell, but they can also receive intracellular signals that regulate their ligand-binding affinity (Shattil et al. 2010). In most cells, integrins are usually maintained in an adhesion-competent state, with the exception of platelets and leukocytes, where *inside-out* signaling is particularly important as integrins usually have to be activated before they can mediate adhesion. Regulated adhesion allows leukocytes to circulate unimpeded until they are activated by an appropriate stimulus. Moreover, because the integrins do not need to be synthesized de novo, the response is extremely fast.

Integrins in Virus Infections

When searching for their host tissues, animal viruses frequently attach to cell-surface receptors that have key roles in normal cell function. Integrins are a prime example of physiologically important receptors that have been usurped by viruses for attachment and/or cell entry. Using integrins as an “entrance door,” viruses take advantage of the wide integrin expression, as well as of the variety of cells expressing integrins throughout the body. Moreover, integrin ligation by pathogens elicits potent signaling responses that promote cytoskeletal reorganization and/or cell entry (Stewart and Nemerow 2007).

Both enveloped and nonenveloped viruses utilize integrins for cell invasion, and they do so with a variety of mechanisms (Stewart and Nemerow 2007). Viruses such as adenoviruses elicit potent signaling events via ligation of integrins that enhance infection of different host cells. Adenoviruses interact with αV integrins via a long flexible RGD loop on the surface of the penton base. Several large enveloped DNA viruses, such as herpesviruses, use also integrins to invade host cells. Several members of the Hantaviruses family (enveloped viruses containing a negative-stranded RNA genome) use also integrins as receptors; pathogenic strains utilize $\beta 3$ integrins, whereas $\beta 1$ integrins are commonly used by nonpathogenic Hantaviruses. $\beta 3$ integrins, rather than $\beta 1$ integrins, have a significant role in vascular permeability; therefore, the use of $\beta 3$ integrins by pathogenic Hantaviruses can explain why infection by these strains results in severe disease.

One of the most commonly used integrin receptors by members of diverse virus families is integrin $\alpha V\beta 3$, which is known to exhibit substantial diversity in ligand recognition. Thus, multiple viruses have evolved to take advantage of the promiscuous attributes of integrin $\alpha V\beta 3$. Because integrins are capable of recognizing relatively small peptide sequences, this may provide a convenient pathway for virus evolution as integrin-ligand sequences can be readily incorporated without drastically altering virus structure.

Integrins and HIV

Infection by HIV-1 causes a profound depletion of CD4⁺ T cells. This depletion eventually leads to the progression of HIV disease ultimately resulting in AIDS. To enter and infect a permissive cell, HIV-1 requires CD4 and the chemokine receptors CCR5 or CXCR4. Besides the normal association between the external envelope gp120 and the appropriate cell-surface receptors, other interactions between the virion and receptors on the target cell surface also contribute to the formation of a replication-competent environment. Adhesion molecules including integrins have been recognized as host factors that influence HIV replication. Several integrins have been

involved as host factors affecting HIV replication at different steps of the virus life cycle.

LFA-1 Integrin

Besides interactions between the viral envelope glycoproteins with cell-surface receptors, the interaction between cell-derived molecules incorporated into virions and their ligand could also modulate the HIV replication cycle. HIV-1 incorporates a vast array of host cell membrane molecules during budding, including the intercellular adhesion molecules (ICAM-1), which is an immunologically important integrin ligand, generally expressed on inflamed endothelium and antigen-presenting cells (APC).

Engagement of ICAM-1 with the integrin lymphocyte function-associated antigen 1 (LFA-1), its natural ligand (also known as CD18 or integrin $\beta 2$), increases viral infectivity by several folds. In $CD4^+$ T lymphocytes that express functional LFA-1, this phenomenon is associated with an enhancement of both HIV-1 attachment and virus-cell fusion (Tardif and Tremblay 2005).

LFA-1-mediated adhesion plays a key role in immune surveillance and the mounting of a potent immune response. More specifically, LFA-1 is involved in the arrest of rolling lymphocytes along blood vessels facilitating extravasation and migration of the activated T cells to infection sites. The integrin LFA-1 participates in the formation of the immunological synapse between T cells and APCs (Bromley et al. 2001). These events require a rapid modulation of adhesion/deadhesion and the adhesion needs to be stable for hours to sustain the immunological synapse. Hence, activation of LFA-1 must be tightly regulated. Engagement of ICAM-1 induces *outside-in* signaling that triggers lateral diffusion of heterodimers in the plasma membrane, a process regulating the adhesion strengthening. Upon T-cell activation, the *inside-out* signaling generates an intermediate affinity state and activates the cysteine protease calpain that releases LFA-1 from its cytoskeleton constraint and favors lateral diffusion of LFA-1, triggering cell adhesion. In the context of HIV infection, LFA-1-mediated cytoskeleton remodeling and signaling are key events to achieve an increase in HIV-1 entry in primary $CD4^+$ T lymphocytes (Bromley et al. 2001).

αV Containing Integrins and HIV Infection in Macrophages

Human blood monocytes, originated from hematopoietic precursors in the bone marrow, give rise to mature macrophages which are distributed ubiquitously in all tissues. Adhesion molecules, particularly integrins, have been shown to upregulate when monocytes differentiate into macrophages, suggesting that integrins play an important role in macrophage adhesion, migration, and tissue infiltration. Blood monocytes and tissue resident macrophages are important *in vivo* cell targets of HIV infection. Macrophages may become chronically infected or serve as reservoirs of latent virus. Furthermore, HIV may sequester macrophage immunoregulatory function, that is, inducing secretion of proinflammatory cytokines that lead to recruitment and activation of new target cells for HIV, including $CD4^+$ T cells.

Monocytes, but not monocyte-derived macrophages (MDM), are difficult to infect by HIV. A productive viral infection may only occur after monocytes are differentiated following serum or cytokine stimulation, a process that leads to the upregulation of different cell-surface markers and receptors, including αV integrins (De Nichilo and Burns 1993). Conditional αV knockout mice models have shown that the absence of αV did not affect lymphocyte migration to lymphoid organs or *in vitro* activation of lymphocytes. On the contrary, these models indicate an important role for αV integrin on myeloid cells (macrophages, dendritic cells, and/or neutrophils) in immune regulation.

In macrophages, blocking αV integrin interaction with its ligands initiates a signaling cascade, mediated by MAP kinases, that finally compromises HIV-1 replication by downregulating NF- κB -dependent HIV-1 transcription (Ballana et al. 2009). Integrin αV can be coupled with various β -integrin subunits ($\beta 1$, $\beta 3$, $\beta 5$, $\beta 6$, and $\beta 8$), forming heterodimeric proteins that could differently affect HIV replication. A significant role in HIV-1 infection of macrophages has been reported in the case of the $\alpha V\beta 5$ integrin dimer (Ballana et al. 2011).

The complex interactions between monocytes/macrophages and HIV are of special importance,

since these cells are relatively refractory to the cytopathic effects. Adhesion to the substrate and differentiation state enhance HIV replication in macrophages. Although several α - and β -subunits are expressed in macrophages, integrin $\alpha V\beta 5$ is one of the mediators of the replication enhancement seen in macrophages.

Integrin $\alpha 4\beta 7$ Role in HIV Infection

Lymphocyte recirculation depends on specific interactions between the lymphocyte cell surface and the surface of the specialized endothelial cells lining the postcapillary venules in the peripheral lymphoid organs. Many cell types in the blood come into contact with these endothelial cells, but only lymphocytes adhere and then migrate out of the bloodstream. The lymphocytes initially adhere to the endothelial cells via homing receptors that bind to specific ligands on the endothelial cell surface. Expression of the integrin $\alpha 4\beta 7$, together with the chemokine receptor CCR9, is essential for a preferential homing of T cells to the gut. The vitamin A metabolite, retinoic acid, enhances the expression of $\alpha 4\beta 7$ and CCR9 on T cells upon activation and imprints them with the gut tropism.

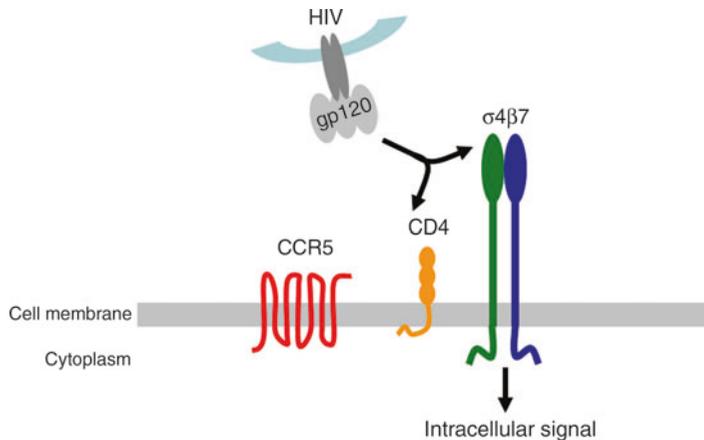
Regardless of the route of transmission, HIV-1 rapidly establishes infection in gut-associated lymphoid tissue (GALT), destroying the majority of CD4+ T cells during the acute phase of infection. This structural and functional damage is accompanied by nonspecific systemic immune activation and cell death, and it has been proposed that this depletion represents an irreversible insult to the immune system, which ultimately results in AIDS.

The $\alpha 4\beta 7$ integrin functions principally as a gut-homing receptor, facilitating the migration and retention of lymphocytes from gut inductive sites (Peyer's patches and mesenteric lymph nodes) to the lamina propria. These sites within GALT play central roles in the initial phases of infection. In the acute phase of HIV infection, following mucosal transmission, the bulk of HIV replication occurs in Peyer's patches and mesenteric lymph nodes. Concurrently, HIV mediates a massive depletion of lamina propria CD4+ T cells. Integrin $\alpha 4\beta 7$ facilitates the migration of

lymphocytes from gut inductive sites to the lamina propria. Thus $\alpha 4\beta 7$ is functionally linked to the major sites of HIV replication and CD4+ T-cell depletion during acute infection.

A specific biochemical interaction between the HIV-1 envelope protein gp120 and $\alpha 4\beta 7$ on CD4+ T cells has been described (Arthos et al. 2008). The HIV-1 envelope protein gp120 binds and signals by means of an activated form of integrin $\alpha 4\beta 7$ on CD4+ T lymphocytes. The explicit linkage between $\alpha 4\beta 7$ and Peyer's patches, mesenteric lymph nodes, and lamina propria and the early phase of acute infection suggest that gp120- $\alpha 4\beta 7$ interaction plays an important role at an early point in HIV infection in vivo (Fig. 2). Different facts support the role of $\alpha 4\beta 7$ (Arthos et al. 2008). First, the highest frequencies of HIV-1 infection occur in the memory CD4+ T-cell compartment in the gut, in which, unlike in almost all other tissue compartments, memory CD4+ T cells express activated $\alpha 4\beta 7$. Second, the gp120- $\alpha 4\beta 7$ interaction is conserved across the four major HIV-1 subtypes. gp120 binding to $\alpha 4\beta 7$ is mediated by the Leu-Asp-Val tripeptide in the V2 loop, which effectively mimics a similar epitope on the natural ligands of $\alpha 4\beta 7$. Despite the fact that the tripeptide resides in one of the most variable domains of gp120, it is highly conserved. Finally, gp120 activates LFA-1 in a $\alpha 4\beta 7$ -dependent way. As mentioned above, LFA-1 is capable of increasing the efficiency of HIV infection. This structural mimicry, along with the high degree of conservation across subtypes, implies that binding to $\alpha 4\beta 7$ confers a replication advantage to HIV.

Transmission of CCR5 viruses is strongly favored over CXCR4 in sexual transmission. The subset of CD4+ T cells localized within mucosal tissues that express high levels of $\alpha 4\beta 7$ are also distinct in expressing high levels of CCR5 and relatively low levels of CXCR4 and therefore represent an ideal target population for productive infection. On these cells $\alpha 4\beta 7$ is closely associated with both CD4, the entry receptor, and CCR5, the fusion coreceptor (Cicala et al. 2009). The marked coexpression of CCR5 and $\alpha 4\beta 7$ and the close physical association of these two surface markers with CD4, on cells that are highly



Integrin Alpha4beta7, Fig. 2 Schematic representation of $\alpha 4\beta 7$ interaction with HIV-1 virions. $\alpha 4\beta 7$ demarcates a distinct subset of CCR5/CD4⁺ T cells that are prone to productive infection. $\alpha 4\beta 7$ is closely associated with CD4,

and therefore, the affinity of HIV gp120 for $\alpha 4\beta 7$ would increase the likelihood of establishing a productive infection by specifically targeting this highly susceptible subset of cells

susceptible to productive infection, provide at least a partial explanation for the strong bias toward R5 virus transmission across mucosal surfaces (Fig. 2).

Unlike CCR5, $\alpha 4\beta 7$ is not hidden from HIV when a virion engages a CD4 receptor. $\alpha 4\beta 7$ is a prominent receptor, approximately three times the size of CD4, that gp120 can engage independently of CD4 (Cicala et al. 2010). However, both receptors reside in close proximity on the CD4⁺ T-cell surface. The gp120 V2 loop, which mediates binding to $\alpha 4\beta 7$, is positioned near the apex of an envelope spike. In this way, both $\alpha 4\beta 7$ and its binding epitope on a virion spike are well positioned for the initial engagement between the virion and the target CD4⁺ T cell. Expression pattern is also different for CD4 and $\alpha 4\beta 7$ receptors; CD4 exhibits a near-uniform expression regardless of whether they are metabolically active or resting, whereas $\alpha 4\beta 7$ is highly expressed on activated cells (Arthos et al. 2008). In this manner, $\alpha 4\beta 7$ provides a structural mechanism for HIV-1 to target activated cells that express high levels of CCR5.

The interaction between gp120 and $\alpha 4\beta 7$ triggers a signal that is not yet fully defined. However, gp120-mediated signal transduction is known to impact viral replication in several cellular subsets. HIV-1 gp120 is a ligand that can mediate signals

at least through CD4 and the chemokine receptors, CCR5 and CXCR4 (Berger et al. 1999). The capacity of gp120 to trigger signals that promote viral replication in both activated and resting cells may facilitate infection, an activity that may be particularly important during mucosal transmission. gp120 signals may provide the necessary metabolic stimulus to achieve a productive infection, and it has been speculated that gp120- $\alpha 4\beta 7$ signal transduction may play an important role and could be a major factor in the transmission of HIV at the mucosal surface.

Virological synapses allow efficient transfer of viral proteins from an infected cell to an uninfected target cell (Piguet and Sattentau 2004). A virological synapse is a structure formed between an APC and T cells and precedes T-cell activation. The formation of an HIV-1 virological synapse is facilitated by the interaction of envelope with CD4. Integral to the virological synapses are adhesion molecules including LFA-1, whose activation is one of the consequences of gp120- $\alpha 4\beta 7$ interaction (Arthos et al. 2008). gp120- $\alpha 4\beta 7$ mediate a rapid activation of LFA-1, and therefore, it is plausible that gp120-mediated signaling through host cell-expressed $\alpha 4\beta 7$, CD4, and CCR5 works together to promote infection by reiterating events that are associated with the formation of a virological synapse.

In summary, the explicit linkage between $\alpha 4\beta 7$ and the major sites of HIV replication following mucosal transmission suggests that this interaction plays an important role at an early phase in the HIV infection cycle. $\alpha 4\beta 7$ is not required for infection but appears to increase the efficiency of infection. The capacity of the HIV envelope to bind to $\alpha 4\beta 7$ represents a strategy whereby HIV is able to more efficiently infect a highly susceptible subpopulation of CD4⁺ T cells. In this manner the probability of a productive infection following sexual/mucosal transmission is increased.

Conclusion

It is not surprising that HIV, like other viruses, have usurped integrins for cell invasion, because integrins are expressed on a wide variety of cells throughout the body. Moreover, integrin ligation by viruses elicits potent signaling responses that promote cell entry and viral replication and transmission. HIV takes advantage of various integrins that recognize different ligands and are expressed in different cell types, being an example the case of LFA-1, αV , and $\alpha 4\beta 7$. LFA-1 stabilizes the cell-to-cell contacts, αV -containing integrins are relevant in macrophages, and $\alpha 4\beta 7$ plays a key role in GALT during the initial phases of infection. Although none of them is required for HIV infection, all of them have important roles in specific settings and contribute to HIV pathogenesis.

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Interactions Between HIV-2 and Host Restriction Factors

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Definition

Host restriction factors mount a first line response to viral infection, yet, are often hampered by specific viral proteins which directly resist their

action. The HIV-2 proteins *gag*, *vif*, *env*, and *vpx* serve to modify the actions of four major host restriction factors: TRIM5 α , APOBEC, Tetherin, and SAMHD1, respectively. The HIV-2 proteins utilise no specific enzymatic function in their roles as antagonists, but rather act as “linker molecules”; connecting host restriction factors to the proteins of cellular pathways that facilitate their sequestration or degradation, and in so doing-enabling viral replication and transmission. This article explores the complex interplay between the host restriction factor and the HIV-2 protein antagonist.

Introduction

Human Immunodeficiency Virus Type 2 (HIV-2) arose during the zoonotic transfer of sooty mangabey Simian Immunodeficiency Virus (SIV_{sm}) on at least eight occasions. Of the resulting eight lineages, HIV-2 A-H, only A and B are endemic, resulting in an estimated one to two million cases, mainly across West Africa. Like HIV-1, HIV-2 is a blood-borne virus, primarily transmitted via heterosexual contact. However, the rate of transmission is substantially lower than that of HIV-1. The majority of HIV-2-infected persons exhibit a phenotype similar to that of HIV-1 long-term non-progressors. This phenotype is characterized by low to undetectable viral load, with little to no symptoms of immunodeficiency. Nevertheless, 20% of people living with HIV-2 progress to AIDS in a manner identical to that of HIV-1-infected persons.

Box 1 Defining Host Restriction Factors

Restriction factors are host proteins that mount a first line response to viral infection. HIV specifically antagonises the action of human restriction factors, yet is severely hampered by analogous restriction factors in non-human primates, indicating the pivotal role of restriction factors in cross-species transmission. Restriction factors
(continued)

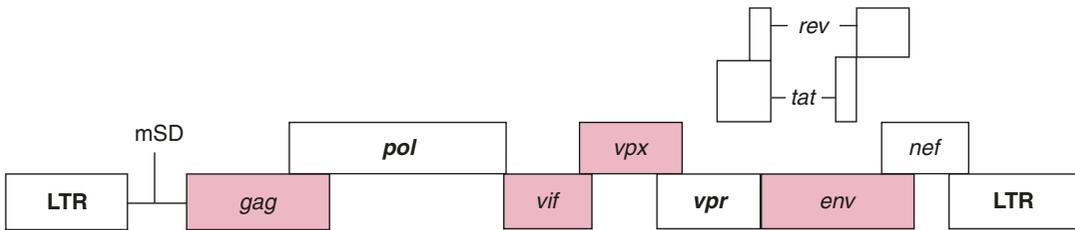
Box 1 (continued)

in the context of HIV-2 infection are characterised by:

- Derivation from functionally autonomous genes that are unusually variable
- Sensitivity to immune activation and viral infection resulting in notable changes in their expression
- The identification of specific viral proteins which directly resist their action
- A significant decrease in retroviral activity during their overexpression, or attenuation of antagonist-deficient retroviral infection

Experimental infection of macaques has been the dominant animal model for HIV research, resulting in the discovery of several gene expression signatures that interfere with virus transmission and disease progression. The discovery that HIV-1 could not replicate in rhesus macaques despite its similarity to SIV led to the identification of endogenous factors that restrict a number of retroviruses. These proteins, termed host restriction factors (Box 1), are encoded by functionally autonomous genes and tend to be unusually variable. In fact, their counterparts in nonhuman primates are also unusually variable, suggesting that they have evolved under diversifying selection. They are believed to have a role in immunity and inflammation because transcription of the genes encoding these proteins is in most cases stimulated by the innate immune system. The distinctive feature of host restriction factors is that several retroviruses have evolved to resist their action by, in most cases, encoding accessory genes that antagonize their function.

HIV-2 bears more similarity to nonhuman primate lentiviruses than to HIV-1. However, HIV-2 has a highly similar protein structure to HIV-1, and yet is a less pathogenic virus. A better understanding of the interaction between HIV-2 and the human host could play an important role in determining methods to counteract the highly pathogenic HIV-1. Furthermore, the ability of host



Interactions Between HIV-2 and Host Restriction Factors, Fig. 1 HIV-2 Genes which antagonise the function of Host Restriction Factors. Structural proteins

gag and *env*, and accessory proteins *vif* and *vpx*, are involved in HIV-2 antagonism of host restriction factors

restriction factors to counteract HIV-2 infection should yield important insights into mechanisms that may reduce virulence of the human immunodeficiency viruses.

Like all primate lentiviruses, HIV-2 possesses the major structural genes *gag*, *pol*, and *env*, which encode the essential proteins for infection and replication. The genome also contains the regulators of viral expression *tat* and *rev*, along with four accessory genes, *vif*, *vpx*, *vpr*, *nef* (Fig. 1) The HIV-2 proteins *gag*, *vif*, *env*, and *vpx* serve to modify the actions of four major host restriction factors: TRIM5 α , APOBEC, Tetherin, and SAMHD1 respectively. The HIV-2 proteins utilize no specific enzymatic function in their roles as antagonists, but rather act as “linker molecules,” connecting host restriction factors to the proteins of cellular pathways that facilitate their sequestration or degradation, and in so doing facilitating viral replication and transmission.

Gag Versus TRIM5 α

The HIV Capsid encoded by Gag was previously thought to be an inert packaging shell that protects the genomic material. However, it has emerged that it has several domains that allow interaction with host proteins, such as Cyclophilin A and the cleavage and polyadenylation specific factor 6 (CPSF6). Recent studies into host restriction factors have shown that the Capsid serves as a pathogen-associated molecular pattern (PAMP) for TRIM5 α which is a pattern recognition receptor (PRR) (Pertel et al. 2011).

The species-specific retroviral restriction properties of TRIM5 α were discovered because HIV-1 could not grow in certain Old World monkey cells. TRIM5 α is a member of the *Tripartite Motif* family of proteins that have the three protein domains: RING, B-Box, and Coiled Coil. TRIM5 α has an additional domain, the PRYSPRY/B30.2 domain, that interacts with the retroviral capsid and is responsible for species specificity (Stremlau et al. 2005). Depending on the mammalian species or cell line, TRIM5 α may mediate a postentry block of lentiviral replication either before or after reverse transcription. Although the exact mechanism of retroviral restriction is not known, several mechanisms have been proposed with supporting experimental data. Oligomeric TRIM5 α recognition of and binding to the incoming intact capsid destabilizes the capsid core, leading to accelerated or premature uncoating, which perturbs reverse transcription. The E3 ligase activity of the RING domain can add ubiquitin molecules to itself and to other proteins, leading to proteasome-mediated protein degradation of the capsid-TRIM complex. TRIM5 α is also a pattern recognition receptor that recognizes and binds to the viral capsid, causing a cascade of innate immune signaling that leads to an antiviral state, restricting retroviral replication.

Human TRIM5 α has very limited effect on HIV-1 but can restrict N-tropic murine leukemia virus (N-MLV) potently and can limit HIV-2 replication to certain extent. HIV-1 restriction by human TRIM5 α maps to the v1 region of the PRYSPRY domain, where a change from arginine or any positively charged residue at position

332 to a proline or any uncharged residue results in potent restriction of both HIV-1 and SIVmac (Yap et al. 2005; Li et al. 2006). Restriction of N-MLV is different from that of HIV-1, in that a larger area of the PRYSPRY domain is involved as well as the coiled coil for restriction of N-MLV mutant L117H (Yap et al. 2005). Human TRIM5 α restriction of HIV-2 was also mapped to a single amino acid at position 119 of HIV-2 ROD capsid. HIV-2 viruses with a proline at this position were much more sensitive to human TRIM5 α restriction than those without (glutamine or alanine) (Song et al. 2007). Further studies showed that presence of hydrophobic amino acids or those with ring structures were associated with sensitivity to TRIM restriction and those with small side chains or amide groups were linked to TRIM5 α resistance (Miyamoto et al. 2011). Three-dimensional modeling of the HIV-1 and HIV-2 capsids showed that the N terminal domains each consist of 7 α -helices from which 3 loops protrude. Position 119 of the HIV-2 capsid is located in the loop between helices 6 and 7. When a proline is present at position 119, the loop between helices 6 and 7 (L6/7) is closer to the loop between helices 4 and 5 (L4/5), a region that directly interacts with cyp A in HIV-1 (Miyamoto et al. 2011). The presence of hydrophobic or ring-structure residues at position 119 of the capsid maintained a certain conformation at L4/5 that is characteristic of sensitive viruses. Curiously, the equivalent position in the N-tropic Murine Leukemia Virus (N-MLV), at position 110, determined viral susceptibility to Human TRIM5 α ; it was suggested that while HIV-1 and HIV-2 are restricted by different mechanisms, human TRIM5 α utilizes a similar mechanism of recognition for N-MLV and HIV-2 (Miyamoto et al. 2011). Further information on TRIM5 α in the context of HIV-2 infection is available elsewhere in this chapter (“► TRIM5 Alpha and HIV-2 Infection”).

Vif Versus APOBEC

Viral infectivity factor (Vif) is an accessory protein conserved across all primate lentiviruses.

Vif-deficient HIV-1 is unable to spread physiologically in relevant macrophage and T-cell cultured cells (Ribeiro et al. 2005). Instead, Vif-deficient virions are produced in normal quantities, but the ability of these virions to infect the subsequent target cell is compromised. This is due to the presence of APOBEC proteins. The APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) proteins have several members, A-H, that have been studied in the context of HIV infection. The APOBEC protein most explored in HIV-1 research, APOBEC3G (A3G), is expressed in the majority of cell types. In the absence of Vif, A3G is packaged into nascent virions and acts during reverse transcription to hypermutate viral DNA and decrease cellular cDNA levels. The expression of A3G is sensitive to viral infection and interferon, and so the amount of A3G incorporated into nascent virions is proportionate to its expression level in the producer cell. The net effect of its increased expression, and packaging into nascent virions, is a restriction of HIV-1 infection.

APOBEC proteins mediate their effect utilizing two zinc-coordinating deaminase domains (Z domains) at each end of the protein:

The carboxy-terminal Z domain is responsible for deamination.

The amino-terminal Z domain mediates A3G's incorporation into viral particles and is also recognized by HIV-1 Vif.

In order to package itself into assembling virions, A3G uses its amino terminal to bind RNA and attach the Z domain to the NC region of Gag. Following its incorporation into assembling virions, it is transported from the producer cell to the target cell. Once the virion has fused with the target cell, A3G dimerizes and associates with the viral reverse transcriptase complex. A3G then deaminates cytidine residues to uridine in nascent, single-stranded, negative strand cDNA. These uridine-rich transcripts can be degraded. However, if salvaged, during the synthesis of the DNA plus strand, adenosines are incorporated instead of the original guanines resulting in G to A mutations. Up to 10% of cytidines may be

edited resulting in G to A hypermutation of the plus strand sequence, and the loss of genetic integrity. However, not all of the hypermutation occurring in Vif-deficient HIV-1 restriction can be attributed to A3G. A3G has a substantial preference for mutating the second C of CC but not TC. APOBEC3F (A3F), a sister cytidine deaminase to A3G with high sequence identity, favors TC and also serves to induce G to A hypermutations resulting in nonfunctional HIV provirus. The combined effect of A3G and A3F restriction results in hypermutated proviruses that are largely nonfunctional, and to decreased levels of cDNA during Vif-deficient HIV infection. In the case of wild-type HIV-1, the expression of APOBEC proteins has no effect on viral infectivity. Vif is able to bind A3G and A3F, and recruit them to a cellular ubiquitin ligase complex that comprises cullin5, elongins, Rbx2, and an E2 conjugating enzyme. The polyubiquitination of APOBEC proteins results in their proteosomal degradation, and thus thwarts their incorporation into nascent viral particles.

Conversely, A3G and A3F do not play a major role in the replication of Vif-deficient HIV-2. The Vif-deficient HIV-2 virus is able to replicate in the majority of cell lines, including the lymphocyte and macrophage cell lines that are not permissive to Vif-deficient HIV-1. In fact, the Vif of SIVsm (the parent strain of HIV-2) is able to rescue the replication of Vif-deficient HIV-1 in human H9 cells. Though Vif is conserved across all primate lentiviruses, HIV-2 Vif has only 30% amino acid identity with HIV-1 Vif, and it is evident that APOBEC proteins interact with the two very differently. Regardless of the amount of A3G expressed in a cell, the infectivity of Vif-deficient HIV-2 remains very high (Abada et al. 2005). A substantial reduction in infectivity is only observed in the presence of HIV-2 Vif mutants. These Vif mutants confer a reduction in Vif expression and occur in small motifs essential for Vif function and stability. It is possible that such mutants make Vif more susceptible to APOBEC proteins; however, the infectivity of these mutants is unaltered by increases in A3G expression. Furthermore, regardless of whether a reduction in infectivity is observed in HIV-2 Vif mutant studies,

no alteration is observed in the steady state of deaminase. This indicates that the deamination conferred by A3G is ineffective against HIV-2.

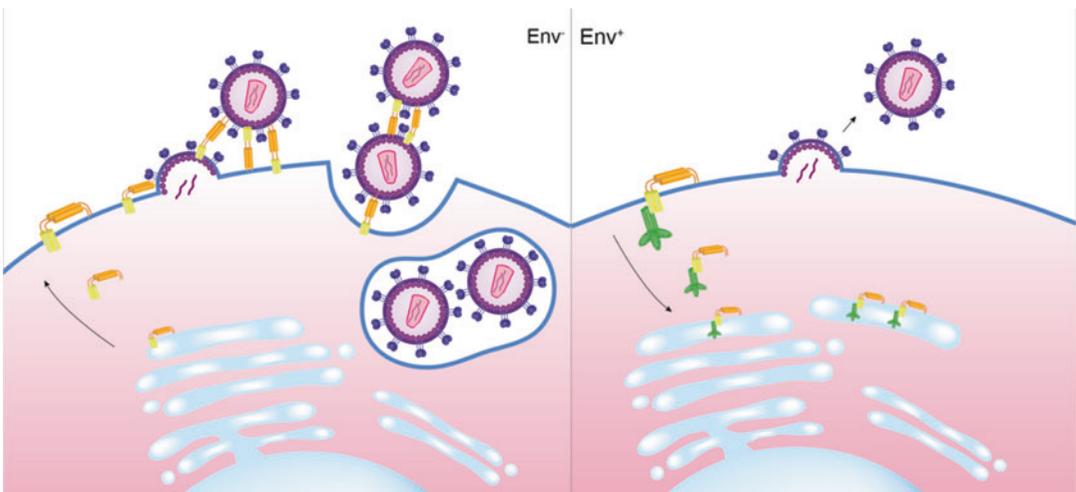
Paradoxically, even though A3G is ineffectual for Vif-deficient HIV-2 infectivity, HIV-2 Vif strongly degrades A3G. Furthermore, the effect of HIV-2 Vif is not limited to A3G. HIV-2 Vif can also antagonize A3F, A3H haplotype II, and A3B, with intermediate to high sensitivity, and A3D with low sensitivity. Despite the variation in sensitivity of HIV-2 Vif induced degradation of APOBEC proteins, all known APOBEC proteins except A3A bind to HIV-2 Vif. Now, HIV-2 Vif is better at restricting both A3G and A3F than HIV-1 Vif. In fact, experiments show that HIV-1 Vif reduces A3G by 90% and HIV-2 Vif reduces A3G by almost 100%. The effect of HIV-2 Vif antagonism on A3F is even more disparate, showing a 100% reduction compared with only 83% by HIV-1 Vif. This is because HIV-2 Vif interacts with separate and distinct domains on APOBEC proteins from those that HIV-1 Vif interacts with. For example, HIV-1 Vif targets a short motif in A3G at position 128–130. This region is essential for HIV-1 Vif's antagonism of A3G. However, HIV-2 Vif targets multiple, non-neighboring residues found between positions 163 and 321 of A3G (Smith et al. 2014). This indicates that HIV-2 Vif has a broader range of interaction sites, which may result in a greater propensity to degrade A3G than HIV-1 Vif.

It is evident that APOBEC proteins are less able to resist HIV-2 Vif than HIV-1 Vif, and that HIV-2 Vif is better at antagonizing APOBEC proteins than HIV-1 Vif (Wiegand et al. 2004). But despite the complex interplay between HIV-2 Vif and APOBEC proteins, to date there is no physiologically relevant evidence for their interaction in the context of HIV-2 infection of the human host. It is possible that the deamination resulting from APOBEC proteins is countered by another virus mechanism or accessory protein which is presently unknown. Finally the mechanism by which APOBEC proteins are incorporated into nascent HIV-2 virions has yet to be confirmed, and it is very possible that this mechanism may yield important insights into how the effect of APOBEC proteins is managed by HIV-2.

Env Versus Tetherin

The Env protein which forms the HIV-2 envelope comprises transmembrane protein gp36 and glycoprotein gp125 and serves to bind and initiate a conformational change in the host cell membrane facilitating virus to cell fusion. HIV-2 env is not only able to utilize a greater range of coreceptors, but its interaction with these coreceptors leading to virus/cell fusion occurs twice as fast, when compared to that of HIV-1 Env. In addition to its role in virus entry, HIV-2 Env is responsible for a four to sixfold increase in virus export, a function analogous to that of HIV-1 vpu (Abada et al. 2005). Infection of CD4+ T-cells with HIV-1 mutants lacking the accessory gene vpu leads to poor HIV-1 production, and the accumulation of mature virions on cell surfaces and within vacuolar structures. It was observed that protein tetherin was responsible for this retention of vpu-defective HIV-1 virions. The envelope glycoprotein of some HIV-2 isolates is able to stimulate the release of vpu-defective HIV-1 virions from tetherin positive cells.

Tetherin is a type II transmembrane protein which is constitutively expressed on several cell types and is upregulated in response to pro-inflammatory cytokines. Tetherin is found at the cell membrane from which wild-type HIV-2 is exported in infected cells, and also in intracellular compartments such as the trans-Golgi network and endosomes which package proteins into membrane-bound vesicles. Tetherin is able to facilitate transport between the cell membrane and the Golgi apparatus and endosomes using clathrin adaptors. Anchored at both the N and C terminals to the plasma membrane, the extracellular coiled coil domain protrudes. Acting as a dimer, tetherin cross-links viral membranes, and viral and cellular membranes to each other. In this manner, it is able to tether nascent virions, preventing their release (Fig. 2). Instead, they remain tethered to the infected cell. The virions are then internalized into endosomal compartments where they are degraded. In addition, it is also highly likely that tetherin serves an additional function in cell signaling. As antigen-presenting cells are targets for HIV-2, the



Interactions Between HIV-2 and Host Restriction Factors, Fig. 2 Env-mediated Down Regulation of Cell Surface Tetherin. In Env deficient HIV-2 infection, tetherin moves from the ER, through the Golgi network, to the plasma membrane through the anterograde trafficking pathway where it is recycled between the TGN and the cell surface. Tetherin is available to cross link viral membranes,

and viral and cellular membranes to each other, resulting in the tethering of nascent virions to the cell. The virions are internalised and degraded. In wild-type HIV-2 infection, HIV-2 Env attaches to tetherin redirecting it away from the plasma membrane and causing its retention in the trans-golgi network. Nascent HIV-2 virions are able to bud from the infected cell

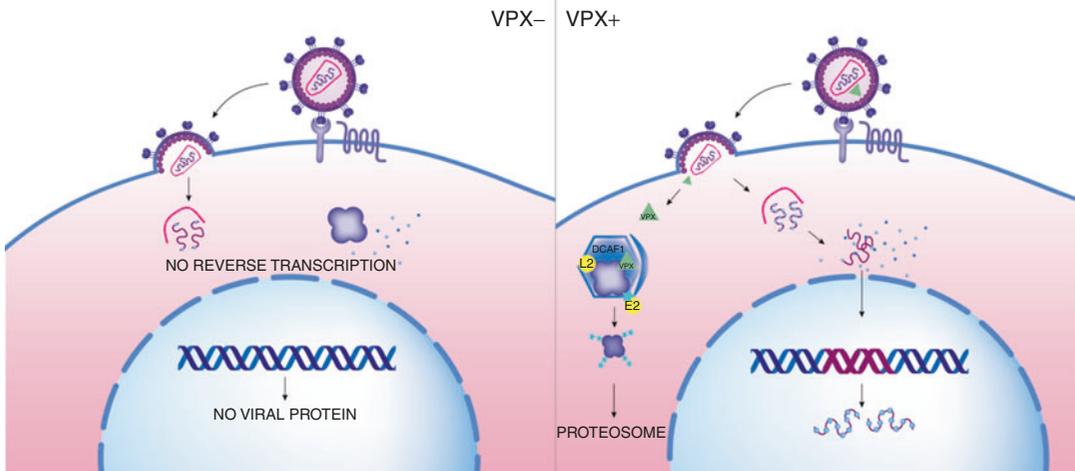
degradation of nascent virions in phagosomes likely leads to the presentation of viral particles to the adaptive immune system. Further, the tetherin promotor contains binding sites for NF-AT and NF- κ B (IFN and interleukin response elements). As tetherin is induced by signals that also trigger type I IFN production, the induction of the type I IFN response and tetherin is linked. Recent data show that tetherin may act as an innate sensor of viral infections that activates NF- κ B to induce an inflammatory response (Hotter et al. 2013).

As observed with TRIM and APOBEC proteins, HIV-2 is able to antagonize host restriction factors by the recruitment of ubiquitin ligases, targeting them for degradation. An alternative method employed by HIV-2 to counteract host defenses is by altering the trafficking pathways used by the host factors to prevent their expression at the cell surface. HIV-2 Env does not reduce total cellular levels of tetherin, as studies have yet to find evidence of tetherin degradation. However, HIV-2 Env acts to downregulate the total level of cellular expression of tetherin in two ways: firstly the downregulation of cell-surface tetherin and secondly through tetherin accumulation in the trans-Golgi network, with the net effect of excluding tetherin from virus assembly and export sites. HIV-2 Env attaches its cytoplasmic tail to tetherin, forming a link between it and the clathrin adaptor complex AP-2 and serves to redirect *de novo* or recycling tetherin away from the plasma membrane and to perinuclear compartments via clathrin-mediated endocytosis (Le Tortorec et al. 2011). However, while Env promotes the release of virions and facilitates subsequent viral infection, this may come at a cost to the virus. In the case of HIV-1, the anti-tetherin effect is mediated by an accessory protein: vpu. However, HIV-2 Env is a major structural protein which accompanies tetherin along the clathrin-mediated endocytosis pathway, and into its sequestration. This reduction in cellular Env may decrease the levels of Env available during assembly of virus, and thus the amount of virus which can be exported. This is one theory for the reduced virulence of HIV-2.

Vpx Versus SAMHD1

HIV-2 encodes two accessory proteins that are homologous to HIV-1 Vpr: Vpr and Vpx. Vpx is unique to HIV-2, SIVsm, and SIVmac, and arose from gene duplication of Vpr during the evolution of primate lentiviruses (Planelles and Barker 2010). Vpx is considered a paralogue of HIV-1 Vpr. They are both packaged into budding viral particles and are able to assemble into an E3 ligase complex with cellular proteins (Fujita et al. 2010). These functions suggest that Vpx plays an important role in early infection by targeting certain proteins for proteosomal degradation. Early studies showed that Vpx is dispensable for HIV-2 infection of lymphocyte or monocyte derived immortalized cell lines. However, Vpx-deficient HIV-2 shows impaired replication in monocyte-derived macrophages, PBMCs, and primary T-cells. This indicates that Vpx is essential for HIV-2 replication only in nondividing/slow dividing cell lines. Further, though human cells of myeloid lineage and resting CD4 T-cells possess the necessary receptors to allow HIV-1 capture and entry, they are not readily infected by HIV-1. In this case, HIV-1 infection is rescued by the intracellular delivery of Vpx (Sharova et al. 2008). The reason HIV-1 encounters a blockade in non- and slow-dividing cells was found to be due to a host restriction factor: Sterile alpha motif (SAM) and histidine/aspartic acid (HD) domain protein 1 (SAMHD1).

SAMHD1 is found in all cell types and localizes to the nucleus. It possesses nuclease and deoxyribonucleoside triphosphate phosphohydrolase (dNTPase) activity. As a dNTPase, SAMHD1 forms a tetramer and acts to sequester dNTPs, thus keeping them at very low levels outside of the S phase of the cell cycle (Yan et al. 2013). This reduction in cellular dNTP halts reverse transcription (Fig. 3). SAMHD1 also possesses exonuclease activity against single-stranded DNAs and RNAs, and preferentially cleaves 3' overhangs in double-stranded substrates. This function further contributes to virus restriction by direct cleavage of viral nucleic acids (Beloglazova et al. 2013). SAMHD1 is



Interactions Between HIV-2 and Host Restriction Factors, Fig. 3 SAMHD1 Blocks Reverse Transcription in the Absence of Vpx. In Vpx-deficient HIV-2 infection, SAMHD1 reduces the amount of dNTP available for reverse transcription halting the production of viral protein.

In wild-type HIV-2 infection, Vpx couples SAMHD1 with the Cul4/DDB1/DCAF1 ubiquitin ligase complex facilitated by cyclin L2, resulting in proteasomal degradation of SAMHD1. As a result, dNTP is available for viral reverse transcription leading to production of viral proteins

regulated by phosphorylation at position T592. In cycling cells, SAMHD1 is phosphorylated, and while its dNTPase activity is unaltered, it is unable to block retroviral restriction (White et al. 2013). Phosphorylation is mediated through SAMHD1's interaction with the cell cycle regulator cyclin-dependent kinase (CDK1). CDK1 is inactive in resting cells, hence explaining why Vpx is only essential for HIV-2 replication in non- or slow-dividing cells. The C-terminal region of SAMHD1 contains a Vpx binding motif, which is important for the ability of Vpx to degrade SAMHD1. Vpx counteracts SAMHD1 restriction by binding to the restriction factor, and coupling with the Cul4/DDB1/DCAF1 ubiquitin ligase complex. This results in the proteasomal degradation of SAMHD1 (Fig. 3). Vpx requires DCAF1 interaction for efficient macrophage infection by HIV-2. Studies on other DCAF-1 interacting proteins revealed that SAMHD1 forms a complex with cyclin L2 which facilitates SAMHD1 interaction with DCAF1. In fact, knockdown of cyclin L2 resulted in a five-fold, threefold, and fourfold reduction in the replication of HIV-1, HIV-2, and Vpx deficient HIV-2, respectively (Kyei et al. 2015). While in HIV-2 infection, Vpx tags SAMHD1 to DCAF resulting in its proteasomal

degradation, it is evident that cyclin L2 assists Vpx in proteasomal degradation of SAMHD1.

Vpx also antagonizes APOBEC3A (A3A) and interferon regulatory factor 5 (IRF5), providing insight into why A3A is the only APOBEC protein which does not bind to HIV-2 Vif. Unlike A3G, A3A exerts its effect in myeloid cells, monocytes, dendritic cells, and macrophages. Silencing of A3A in primary macrophages, dendritic cells, and monocytes results in an increased susceptibility to HIV-1 and augmented replication of R5 HIV-1 tropic viruses. Further, treatment of myeloid cells with IFN α results in increased resistance to HIV-1 infection which coincides with a threefold increase in A3A expression. The antiviral effect mediated by A3A results in a decrease in viral DNA accumulation. Like A3G, A3A is believed to act as a cytidine deaminase, destroying viral DNA through extensive deamination of cytosine. In addition, A3A exerts a cytotoxic effect by:

- Inducing strand breaks in genomic DNA which results in cell-cycle arrest
- Degrading foreign DNA
- Blocking retrotransposition of LINE-1, Alu, and TLR

Experimental evidence shows that A3A, but not A3G, co-immunoprecipitates with Vpx proteins derived from HIV-2 and SIVmac. That same study concluded that SIVmac Vpx antagonizes A3A by inducing its degradation in the early stages of infection (Berger et al. 2011). Biochemical analysis of A3A activity reveals its enclosure in autophagosomal membrane compartments and cell nuclei (Pham et al. 2013). In the case of IRF5, Vpx inhibits IRF5-mediated transactivation by a mechanism that is not completely understood. IRF5 is involved in the production of pro-inflammatory cytokines and Type 1 interferon. Expression of Vpx can reduce mRNA levels and protein production of Toll-like receptor-dependent IL6, IL12, and TNF α , which would interfere with the upregulation of interferon-sensitive host genes (Cheng and Ratner 2014).

The interaction of Vpx with SAMHD1, while fascinating, has yet to result in substantial clinically relevant findings. Vpx contains a highly conserved polyproline motif (positions 103–109) which is critical for its effective translation. Mutation of multiple prolines to alanines in this motif resulted in minimal expression of Vpx. Yet, a study of Vpx sequences from HIV-2 progressors and nonprogressors revealed that only one mutation at position 68 reduced SAMHD1 antagonism in vitro. It is possible that further studies of the interaction between Vpx and SAMHD1 in multiple populations may reveal clinically relevant mutations in host or virus that have a clinical impact on the course of HIV-2 disease progression.

Conclusions

It is widely believed that there are many more host restriction factors to be uncovered, and further roles of HIV-2 proteins to be described. It is plausible that the reduced virulence of HIV-2 when compared to HIV-1 may be due to modulation of viral replication by host restriction factors. Furthermore, several HIV-2 proteins alter the course of mutant HIV-1 infection, and in addition, the mutation of HIV-1 amino acids to achieve greater identity to their HIV-2 counterparts substantially

alters retroviral replication in several instances. Proteins such as MOV10 and TRIM22 are presently under investigation for their role in retroviral disease progression. Research into TRIM5 α , APOBEC, tetherin, and SAMHD1 in the context of HIV-2 infection presents significant scope for therapeutic exploitation. Gene therapies that modify host restriction factors to better restrict retroviral infection and agents that block interactions between antagonistic viral proteins and host restriction factors are attractive approaches for the development of new antiretroviral therapies.

Cross-References

- ▶ [APOBEC3F/G and Vif: Action and Counteractions](#)
- ▶ [Counteraction of SAMHD1 by Vpx](#)
- ▶ [Nef/Env/Vpu/Tetherin](#)

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J

Jail and Prison Populations, Epidemiology of HIV/AIDS

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Definition

Human immunodeficiency virus (HIV) is the virus that causes acquired immunodeficiency syndrome (AIDS) and can lead to a diminished immune system – opening one up to the risk of developing opportunistic infections. Individuals in jail or prison have higher rates of HIV, and HIV and incarceration share many risk factors (e.g., illicit substance use). Often jails and prisons are thought of as synonymous, but it is important to note that there are important differences. Jails are facilities in which individuals are housed while either serving short-term sentences (typically no longer than one year) or are being detained pre-trial. In contrast, prisons house those who are incarcerated for longer time periods (e.g., greater than a 1 year).

Introduction

Whereas the last several years have had a slight decline in the number of individuals incarcerated, the rate of incarceration in America continues to soar (Kaeble and Glaze 2015). Today over 6.7 million people are involved in the criminal justice (Carson 2014). Those who are incarcerated have a disproportionate burden of health issues, including HIV (Brinkley-Rubinstein 2013). Incarcerated individuals are three times greater more likely than their non-incarcerated counterparts to have HIV (Maruschak 2001–2010). Approximately 14% of all HIV-positive individuals will go to jail at some point in any given year (Spaulding et al. 2009). Rates of AIDS are also elevated among incarcerated individuals with the rate of AIDS being about 2.4 times that of the general population (CDC 2012).

HIV testing in jails and prisons provides a unique opportunity for screening that might not otherwise be accessible. Access to HIV treatment may increase while incarcerated. For example, incarcerated HIV-positive individuals may be maintained on antiretroviral medications and often experience subsequent viral load suppression (Meyer et al. 2014). Therefore, correctional settings can provide a unique opportunity to address HIV among a particularly vulnerable population.

Demographics and Risk Factors of Incarcerated HIV-Positive Individuals

Low-income communities of color are disproportionately impacted by both the criminal justice system and HIV. According to the CDC (2012), black men are seven times more likely to be incarcerated than their White counterparts. Mirroring this trend is the disparate rate of HIV among minorities. Blacks account for 44% of all new HIV-positive individuals while only accounting for about 12% of the general population (CDC 2014). While the reasons for why minorities have a disproportionate risk of both HIV and incarceration can be difficult to isolate, scholars have pointed to both structural and individual-level risk factors (Brinkley-Rubinstein 2013). Macro-level policy related to the war on drugs, the limiting of access to treatment for those with mental illnesses, poverty, and lack of access to insurance and medical treatment have been cited as contributing to disproportionate minority contact in prison and jails and to disparate rates of HIV in communities of color (Brinkley-Rubinstein 2013; CDC 2012).

HIV Criminalization

HIV criminalization adds a unique layer of complexity to the possible health impact of the criminal justice system. In many states, the sexual transmission of HIV from one person to another is viewed as a criminal act if the HIV-positive person knows that he or she has HIV and does not tell the other person. Often such laws go further than just criminalizing sexual transmission of HIV and extend to include additional incidents of person-to-person contact in an attempt to minimize risk of transmission via fighting, spitting, or other routes. Galletly and Lazzarini (2013) found that from 2000 to 2010, 44% of all HIV exposure charges in their study area were for nonsexual modes of transmission.

Moreover, in 13 states, prostitution charges can be modified for those who are HIV positive. For example, in some states, if a person is charged with prostitution and is known or later found to be

HIV positive, his or her charge is upgraded from a misdemeanor to a felony offense. HIV-positive individuals who are convicted of a prostitution charge are sometimes required in select states to register as sex offenders for the rest of their lives. For example, Galletly and Lazzarini (2013) surveyed data on HIV-specific charges from 2000 to 2010 in a midsized Southern city and identified 25 arrests for HIV exposure and 27 for aggravated prostitution, most of which did not involve allegations of transmission.

HIV Transmission in Prisons and Jails

While a majority of HIV-positive individuals in prison/jail acquired HIV in the community, there are routes of HIV transmission that are unique to correctional settings (CDC 2006; Federal Bureau of Prisons 2006; National HIV/AIDS Strategy 2010). The prison environment can include overcrowding and lack of access to and low quality of healthcare and access to medications (Brinkley-Rubinstein and Turner 2013). In addition, prisoners often do not have access to clean needles that would lower the risk of HIV after engaging in intravenous drugs use or tattooing. Additional risks include lack of access to condoms and engagement in unprotected sex. However, as Hammett (2006) reports, correctional settings vary from place to place and depend on the type of facility (e.g., prison or jail). As such, questions remain about just how much transmission of HIV is occurring in the carceral setting.

In the early years of the HIV epidemic, there were many attempts to lower both the incidence of HIV among prisoners and the mortality rate of inmates who were already infected. The initial correctional response to HIV prevention in jails included mandatory testing and segregation of HIV-positive inmates. This, in part, was an attempt to stem transmission of HIV from prisoner to prisoner *in the correctional setting*. However, over the years many more comprehensive and effective strategies to HIV prevention in correctional settings have been developed (Hammett 2006).

HIV Testing in Prisons and Jails

HIV exploded onto the public health scene in the late 1970s, and the first cases of HIV/AIDS were discovered in a New York State correctional facility in the early 1980s. A study by Hammett and his colleagues (Hammett 1986) found that, of 766 inmates who were diagnosed with AIDS, nearly half of them died by the time the study period was over. This shocking mortality rate highlighted the dire impact the epidemic was having on correctional populations. However, over the years correctional facilities have begun to normalize HIV testing and screening.

In 2009, most states had some form of HIV testing implemented in jails and prisons/carceral settings. There are three types of possible testing options. Voluntary testing encompasses testing policies that are both active and passive. This type of testing is recommended by the National Commission on Correctional Health Care, the American Public Health Association, and the World Health Organization. However, very few states test exclusively upon prisoner request. Routine testing is an approach to testing in which prisoners are tested unless they refuse. Finally, mandated testing is conducted by several prison and jail systems including the Federal Bureau of Prisons. Mandatory testing can take place at intake, discharge, or in the event of a high-risk incident.

Institutional barriers to testing may restrict the effectiveness of testing and screening. For instance, jail facilities often have high turnover rates of inmates with short sentences or those who are being transferred to other facilities. These logistical constraints can make it hard to administer tests *and* provide results since HIV testing can sometimes take longer than one might be incarcerated. Rapid tests may lessen this barrier but come at an increased financial cost. Regardless of the barriers, HIV screening and testing have had a great impact on linkage to care during incarceration and continuation of care post-release by easing the ability to identify HIV-positive inmates. HIV testing has also caused a major decline in the number of inmates dying AIDS-related deaths. To illustrate: the number of

AIDS-related deaths in 2008 was 120, while in 1995 it was 1110 (Maruschak 2005; CDC 2012).

HIV Treatment in Correctional Facilities

Incarcerated individuals are the only population that has a constitutional guarantee of healthcare (Estelle vs. Gamble 1976). However, research related to treatment and medication access in correctional settings is mixed, demonstrating both positive and negative experiences. For instance, Meyer et al. (2014) reported that viral suppression during incarceration was attained due to regular access to antiretroviral medication in the correctional setting. However, other studies have illuminated barriers to well-being that are salient to HIV-positive populations, including stigma, restricted access to medical care, and lack of trust of medical providers (Brinkley-Rubinstein and Turner 2013). Federal funding for linkage to care post-release is available for low-income individuals via the Ryan White Care Act. Funds from this act are treated as funds of last resort meaning that they are available for those who could not otherwise afford treatment and other related medical and social services.

Linkage to Services and Health Impact of Incarceration Post-Release

While HIV treatment during incarceration can have health benefits, the impact of incarceration post-release may be more negative. During community re-entry linkage to HIV services can be challenging. In addition, HIV outcomes post-release can be worse than even before incarceration (Iroh et al. 2015). For example, in Maryland, HIV-positive drug users who had recently been briefly incarcerated were seven times more likely to experience virological failure than HIV-positive drug users who had not been incarcerated (Wilson et al. 2012). However, some linkage services post-release have been demonstrated as an effective way to keep those who have been incarcerated healthy and linked to medical care. The

Health Resources and Services Administration funded EnhanceLink, which provides augmented linkage services to medical and social services for people just released from jail (Spaulding et al. 2012). EnhanceLink included 10 cities and 20 jails. The initiative linked participating individuals to medical care and other transitional services post-release. This is but one example of a successful intervention aimed at linkage post-release.

Conclusion

While much has changed over the years, those in correctional facilities continue to have very disparate rates of HIV. Prevention and treatment initiatives such as screening and testing, treatment while in jail or prison, and linkage to services post-release have been effective at reducing mortality rates of those who have experienced incarceration. However, incarceration is often a factor that is overlooked as a contributor to the health outcomes of HIV-positive individuals. There is preliminary evidence of effective practices to help maintain individual health, stem transmission, and ease the post-release experience; but, existing research also highlights the need for continued research and innovation to limit the intersectional impact of HIV/AIDS and the incarceration experience on health.

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K

Kaposi Sarcoma-Associated Herpesvirus (KSHV) or Human Herpesvirus 8 (HHV-8)

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Definition

Kaposi sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV-8) is a virus that is associated with the original AIDS-defining tumor, Kaposi sarcoma (KS). This virus is necessary for the development of KS. Expression of the viral protein, LANA, represents the definitive diagnostic marker for KS. In addition to KS, KSHV is also associated with primary effusion lymphoma and a plasmablastic variant of multicentric Castlemann disease.

KSHV is a double-stranded DNA virus. There exists no vaccine against this virus. Viral DNA replication is sensitive to ganciclovir. The virus establishes lifelong latency in the infected host.

Introduction

The development of Kaposi sarcoma (KS) is linked to infection with Kaposi sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus 8). This association was established when Drs. Yuan Chang and Patrick Moore discovered viral DNA in KS biopsies, but not in the skin from healthy controls. KSHV is present in essentially every tumor cell within a KS lesion (Chang et al. 1994). Within each tumor cell, the viral latent proteins, as well as the viral micro-RNAs, are expressed. Other viral proteins may be expressed as well depending on tumor subtype and the particular signals that emanate from the tumor microenvironment, e.g., hypoxia can activate lytic replication and gene expression of specific KSHV genes (Davis et al. 2001). In addition to KS, KSHV also drives the pathology of primary effusion lymphoma (PEL) (Cesarman et al. 1995) and a plasmablastic variant of multicentric Castlemann disease (MCD) (Carbone et al. 2009; Soulier et al. 1995). In KS and PEL the virus predominantly exists in its latent form and does not replicate to high levels. MCD may be an exception, since it is associated with high-level expression of the viral IL6 homologue and other lytic proteins. Primary infection or reactivation from tumor cells and latently infected CD20⁺ B cells leads to systemic viremia, salivary

shedding, and person-to-person transmission. In extremely severe cases, high-level viremia can lead to KS-associated inflammatory cytokine syndrome (KICS) (Polizzotto et al. 2012).

Transmission

KSHV is transmitted orally or by sexual routes, blood transfusion, or the transplantation of infected organs (reviewed in Bagni and Whitby (2009)). In endemic areas such as sub-Saharan Africa and the Mediterranean region, the prevalence of KSHV is high. KSHV is detected in breast milk and maternal saliva suggesting an oral route of transmission in children. Close family relatives are positive for viral DNA in their saliva, also suggesting an oral transmission route. By comparison to endemic and epidemic regions, Western Europe and the USA have a low prevalence of KSHV. Here the virus is thought to be transmitted sexually, particularly in men who have sex with men (MSM). The mechanism of transmission here may be largely through the use of saliva as a lubricant. The reason for this apparent geographic difference in transmission pattern is unknown. Blood-borne transmission, as well as transmission by a transplanted organ, can also occur. At present the blood supply even in endemic areas or for high-risk donors is not routinely screened for KSHV. It is noteworthy that unlike HIV, KSHV appears to be an old virus that coevolved with humans.

Kaposi Sarcoma

The incidence of KS reflects the prevalence of KSHV. In certain high-prevalence regions, such as sub-Saharan Africa, KS is not uncommonly seen in children, the elderly, and transplant recipients independent of HIV infection. Whether there exists a genetic susceptibility locus for KSHV infection or for the development of KS is currently unknown. KS is one of the most common cancers in sub-Saharan Africa where it causes significant morbidity and mortality (Jemal et al. 2012). HIV

coinfection substantially increases the risk for developing KS. In Western Europe and the USA, the number of KS cases has declined since the introduction of combination antiretroviral treatment (cART), but KS still occurs even in the presence of high CD4 counts and low-HIV viral load (Krown et al. 2008). For example, KS rates in the San Francisco area for white men were ~30 per 100,000 during 1987–1991 (pre-cART era) and declined to 2.8 in 1998 (post-cART era). A further decline however did not happen and KS incidence in the US has stabilized since 2000. There are multiple epidemiological forms of KS:

- (i) “Classic” KS afflicts elderly men of Mediterranean or eastern European origin. Classic KS occurs in the absence of HIV coinfection.
- (ii) “Endemic” KS occurs in Central and Eastern Africa in the absence of HIV coinfection. It is often a disease of children or young adults.
- (iii) “Transplant-associated” or iatrogenic KS develops in immunosuppressed individuals e.g., organ transplant patients. This also occurs in the absence of HIV coinfection.
- (iv) “Epidemic” KS also known as AIDS-KS is the most common AIDS-defining cancer, which predominantly afflicts HIV-infected MSM, although women can also develop KS.

These forms intermix, as HIV is now prevalent in KS endemic areas, even in children, and HIV+ patients undergo organ transplantation. In addition KS has been noted to flare up shortly after the start of cART, a phenomenon that is called KS immune reconstitution inflammatory syndrome (KS-IRIS). KS is also seen in some cases of KSHV-associated herpesvirus inflammatory syndrome (KICS), which is a recently described clinical entity that is associated with high-level KSHV replication (Polizzotto et al. 2012).

Systemic KSHV viral load is associated with KS development and often precedes it. It is important to consider, however, that KSHV levels in the plasma of KS patients are low (200–30,000 copies) compared to other herpesviruses. There is no

direct correlation between the severity of disease or number of lesions and KSHV viral load. This suggests that KS lesions result from the seeding of infected cells (analogous to traditional metastasis) as well as de novo infection of peripheral, lymphatic endothelial cells (reviewed in Ganem (2010)). In addition, local seeding and autocrine stimulation may help local KS lesions grow once they begin.

Kaposi Sarcoma-Associated Herpesvirus (KSHV): Genetic Structure

KSHV is a double-stranded DNA virus. Its genome is approximately 130,000 bp in length. A long unique region encodes all known viral proteins and is flanked on either end by a varying number of terminal repeat sequences. Upon circularization the terminal repeat sequences fuse and serve as the latent origin of replication and as the anchor point by which the viral extrachromosomal plasmid is tethered to the host chromosome. During latency the host cell DNA-dependent DNA polymerase is used to replicate the latent episomes. By contrast, lytic viral replication initiates at two conserved regions (oriLyt) and is dependent on the viral DNA-dependent DNA polymerase, as well as a complex of core replication proteins. The viral DNA polymerase is sensitive to ganciclovir, azidothymidine, foscarnet, and cidofovir, but not acyclovir. Two viral kinases (Orf36 and Orf21) mediate susceptibility to ganciclovir and azidothymidine.

KSHV encodes approximately 84 open reading frames. These can be categorized in multiple ways. Blocks of co-regulated proteins exhibit homology to other herpesviruses and mediate viral DNA replication, entry, capsid, envelope, and tegument formation. A second set of genes (K genes) are unique to KSHV or only present in the lymphotropic lineage of herpesviruses, which includes ► [Epstein-Barr-Virus \(EBV\)](#), as well as the monkey and mouse homologues of KSHV (reviewed in Chang and Moore (1996), and Damania (2004)). Several KSHV genes are homologues of human cellular genes and appear to have been acquired from the human genome.

KSHV Structure

KSHV virions exhibit an electron-dense capsid surrounded by a lipid bilayer envelope. In between the capsid and the envelope is a morphologically amorphous but highly organized proteinaceous layer called the tegument. The envelope is studded with viral glycoproteins, which engage host cell surface receptors and participate in viral entry. KSHV encodes seven glycoproteins: ORF22 (gH), ORF39 (gM), ORF47 (gL), ORF53 (gN), ORF68 (gB), and K8.1. Three of these, gB, gH, and gL, are required to mediate membrane fusion. The tegument contains multiple proteins and RNA transcripts. The herpesvirus tegument proteins are important as they are delivered into the target cell upon primary infection and may thus contribute to early reprogramming events before the synthesis of immediate-early proteins. The KSHV capsid architecture and polypeptide composition have been determined. Cryo-EM reconstruction has revealed that the icosahedral capsid is symmetric ($T = 16$) with 20 triangular faces. The building blocks are composed of hexamers and pentamers of the major capsid protein (MCP/ORF25) and interconnected by heterotrimer structures comprised of the minor capsid proteins ORF62 and ORF26 (Deng et al. 2008).

KSHV Entry

The virus enters target cells through multiple receptors and coreceptors (reviewed in Chandran (2010)). The $\alpha V\beta 3$ and $\alpha V\beta 5$ integrins serve as receptors for KSHV (Akula et al. 2001). The viral gB protein contains the signature integrin-binding RGD motif. Antibodies to either integrins or RGD peptides block entry. xCT is another receptor for KSHV. It is part of the cell surface CD98 (4F2 antigen) complex. Expression of xCT restores permissivity for KSHV and antibodies against xCT block infection. DC-SIGN (CD209) is also a receptor for KSHV. KSHV infection can be blocked by an anti-DC-SIGN monoclonal antibody and soluble DC-SIGN. Most recently, ephrin receptor A2 has been shown to act as a coreceptor

of KSHV by binding to the viral gH and gL proteins (Hahn et al. 2012). Lastly, heparin sulfate increases the efficiency of infection. Since this virus enters multiple cell types (B lymphocytes, monocytes, endothelial cells, epithelial cells), different receptors may be utilized in different cell lineages; they may be essential for some cells but only serve auxiliary roles in others.

Upon entry, the virus immediately triggers an innate host response and induces interferon. In monocytes, KSHV activates TLR3 resulting in its upregulation and induction of downstream mediators, including IFN- β 1 and the chemokine CXCL10 (also called IP-10). In plasmacytoid dendritic cells (pDCs), which are the chief IFN-producing cells in the body, KSHV activates TLR9 (reviewed in West et al. (2012)). In endothelial cells KSHV downregulates TLR4.

KSHV Gene Regulation

Many of the studies of KSHV gene regulation have been done in PEL cell lines. These studies have shown that viral reactivation, replication, egress, and infectious virion production can be triggered by a variety of signaling pathways (reviewed in Mesri et al. (2010)). The initial trigger event leads to the coordinated transcription of three kinetic classes of transcripts: those encoding immediate-early proteins (RTA/Orf50, K-bZIP), those encoding early proteins (MTA/Orf57, polymerase and its associated factors, vGPCR/Orf74), and those encoding late genes (capsid and tegument proteins).

The viral immediate-early transactivator RTA is necessary and sufficient to initiate KSHV viral replication (reviewed in Deng et al. (2007) and Staudt and Dittmer (2007)). The KSHV *Rta* gene encodes a 691-amino-acid protein that is highly phosphorylated and localizes to the nucleus of mammalian cells. Deletion of 160 amino acids in the C-terminal activation domain of the KSHV Rta/ORF50 results in the production of a truncated but stable Rta/ORF50 protein. This truncated Rta/ORF50 protein forms multimers with wild-type Rta/ORF50 in PEL cells and functions as a dominant negative inhibitor of Rta/ORF50.

A KSHV deletion mutant missing the *Rta* gene is unable to reactivate upon chemical treatment. Rta/ORF50 is present in KSHV virions, i.e., it can be considered a virion transactivator and thus ensures lytic replication upon primary infection. Rta/ORF50-responsive promoters fall into one of two subgroups: those where Rta/ORF50 directly binds promoter DNA and those where Rta/OrRF50 does not directly bind promoter DNA, but rather transactivates the promoter by establishing protein-protein interactions with cellular transcription factors that mediate sequence-specific DNA binding. Most important among these interactions is the binding of Rta/ORF50 to cellular RBP-J κ to modulate KSHV transcription. RBP-J κ is a sequence-specific DNA-binding protein that is a downstream effector of cellular Notch signal transduction. KSHV can usurp the function of cellular RBP-J κ without the requirement for Notch-ligand interaction, as the binding of KSHV Rta/ORF50 protein to RBP-J κ converts RBP-J κ from a transcriptional repressor to a transactivator.

Of note, the pattern of KSHV gene transcription is not as rigid as for other herpesviruses. Specific signaling stimuli and cellular environments can induce specific and sporadic transcription patterns. Stimuli that sporadically activate the transcription of oncogenic viral proteins, such as K1, viral interleukin-6 (vIL-6), or vGPCR, in the absence of complete replication (which would destroy the infected cell) are thought to contribute to oncogenesis in a paracrine fashion. Periodic reactivation and reinfection cycles are also thought to contribute to viral persistence in endothelial lineage cells.

Histone deacetylase inhibitors (vorinostat, valproic acid, sodium butyrate), signaling inducers such as TPA (Renne et al. 1996), and ligands for Toll-like receptors (TLRs) such as TLR7/8 agonists can induce KSHV reactivation from latency in culture. Interferon-alpha does not induce KSHV in its entirety, but only the expression of specific viral genes, e.g., the vIL-6 protein.

KSHV Carcinogenesis

All KS and PEL cells consistently express the viral latent proteins LANA/Orf73, vCyclin/

Orf72, vFLIP/Orf71, kaposin, the viral microRNAs, and one or more of the viral interferon regulatory factor (vIRF) homologues (Dittmer 2011). These exhibit transforming activities in specialized assays. Other viral proteins like K1, vIL6, and vGPCR are strongly transforming in multiple assays in culture. They are expressed at low and varying levels in latent KSHV-infected cells but are highly upregulated during the lytic replication cycle. Abrogation either of latent proteins, latent protein-induced signaling, receptor-induced signaling, or purging of the viral episome is incompatible with tumor growth, demonstrating that KSHV is required for KS.

LANA is the major latency protein involved in latent viral replication and maintenance of the latent genome. LANA tethers the viral episome to histones on the host chromosome. During normal cell division, viral genomic DNA is replicated and segregated along with host chromosomes, thereby ensuring that each of the daughter cells also contain viral genomes. LANA also functions to augment cell proliferation and survival. LANA has been shown to bind the tumor suppressors p53 and Rb (reviewed in Ballestas and Kaye (2011) and Damania and Pipas (2009)).

LANA, vCyclin, and vFLIP are expressed on a polycistronic transcript. vFLIP is a viral homologue of cellular FLIP (FLICE [protein FADD-like interleukin-1 beta-converting enzyme, now called caspase-8] inhibitory protein). vFLIP strongly activates the NF κ B signaling pathway and is thought to contribute to KSHV-associated oncogenesis (reviewed in Mesri et al. (2010)). Another latency-associated protein is vCyclin; vCyclin is a homologue of cellular cyclin D. vCyclin binds and activates CDK6 and is thought to promote S-phase entry. vCyclin transgenic mice develop lymphomas only in the context of p53 deficiency (reviewed in Damania and Pipas (2009)).

A working model of how the different molecular effectors that are encoded by KSHV work together to bring about the molecular phenotypes that are associated with KSHV infection can be constructed based on our knowledge of homologous viruses. The related gammaherpesvirus, herpesvirus saimiri (HVS), only requires two proteins,

STP and TIP, to transform human T cells in culture, yet it encodes homologues of many of the genes of KSHV, which function primarily in modulating host interactions, in vivo persistence, and pathogenesis. The KSHV K1 protein can functionally substitute for STP and engages signaling pathways, principally PI3K/AKT/mTOR, through its ITAM motif. Whereas K1 is located on the left end of the KSHV genome, K15 another viral receptor signaling protein is located on the right side. K15 engages TRAFs 1, 2, and 3, which leads to the activation of NF κ B and NF κ B-regulated cytokines. It also triggers mitogen-activated protein kinase (MAPK) signaling. The KSHV K1 and K15 proteins appear to phenocopy the two principal EBV-transforming genes LMP1 and LMP2A (reviewed in Damania (2004)), thus establishing a receptor-initiated signaling environment as a common theme for all lymphotropic herpesviruses.

KS is arguably one of the most angiogenic tumors that arises in the human population. It is thought that KSHV viral proteins expressed in endothelial (and surrounding epithelial cells) induce the overexpression of angiogenic factors like vascular endothelial growth factor (VEGF). vGPCR, K1, and vIL-6 have been shown to induce VEGF and function to stimulate angiogenesis in a paracrine fashion (reviewed in Mesri et al. (2010)). KSHV infection can also reprogram endothelial cells creating a gene expression phenotype that is intermediate between blood and lymphatic endothelium (reviewed in Dimaio and Lagunoff (2012) and Hong et al. (2004)).

Please note that the clinical and systemic manifestations of KSHV infection and KSHV-associated cancers are discussed elsewhere (Ablashi et al. 2002; Dittmer and Krown 2010).

KSHV MicroRNAs

The most recent addition to our understanding of KSHV biology has been the discovery of the KSHV microRNAs (miRNA). KSHV encodes 12–20 mature miRNAs (reviewed in Cullen (2011) and Skalsky and Cullen (2010)). Each mature miRNA is made from a looped, double-stranded pre-miRNA. Depending on cell type and

the exact RNA sequence, one or the other strands are preferentially processed and incorporated into the active RISC complex. Hence, there exists extensive variation in the count of mature miRNAs. KSHV miRNAs are expressed in KS and PEL and together with the cellular miRNA profile can be used to distinguish stages of KSHV infection. At present the function of all the miRNAs is not completely known. Thrombospondin 1, TGF-beta, and Bach1 are examples of some proteins known to be targeted by the KSHV miRNAs. Analogous to viral proteins, which mimic functions of host proteins, some viral miRNAs also share seed sequence (and therefore the same target range) as cellular miRNAs, most notably miR-K12-11 and miR-155. Downregulation of cellular miR-155 has been implicated in terminal plasma cell differentiation and it can be reasoned that by ectopically expressing an ortholog, KSHV can stall this process.

KSHV Immune System Interactions

Equally important to carcinogenesis is the means by which KSHV proteins modulate the immune system (reviewed in Lee et al. (2010) and Moore and Chang (2003)). These events may have systemic effects long before the development of clinically apparent lymphoma and KS. For instance, KSHV encodes a homologue to CD200/Ox2. Cellular CD200 is a negative regulator of inflammation. CD200 knockout mice exhibit increased susceptibility to experimentally induced autoimmune disease. The viral homologue of CD200, K14, is soluble and can bind to the CD200 receptor. How exactly this event modulates target cell function is not currently known.

KSHV also encodes homologues to cellular interferon regulatory factors (IRFs). The cellular IRFs transmit the activating signals from TLR or IFN alpha/beta receptors to the nucleus. This initiates and subsequently increases interferon production in a positive feedback loop. KSHV encodes four viral IRFs, vIRF1–4. The vIRF3 protein is constitutively expressed in latently infected PEL; vIRF1 is expressed in latent KS cells, whereas vIRF2 and vIRF4 have thus far

only been seen upon lytic infection. vIRF1, 2, and 3 interfere with IFN signaling. The molecular mechanism by which these proteins function is quite varied. For example, vIRF1 binds to cellular IRF-1 and IRF-2 and inhibits these proteins in a classical dominant negative mechanism. However, vIRF1 also binds to CBP/p300, p53, and Bim. For a more detailed review of the function of the four vIRFs, please see review article Jacobs and Damania (2011).

As mentioned above, KSHV encodes for a viral IL6 homologue, vIL-6 (reviewed in Sin and Dittmer (2012)). Unlike human IL-6, vIL-6 does not need to bind to the gp80 subunit of the IL-6 receptor complex to activate signal transduction. vIL-6 has been shown to activate cell signaling in an intracrine, autocrine, and paracrine fashion. vIL-6 can augment cell survival, prevent apoptosis, and activate angiogenesis through the upregulation of the proangiogenic factors, VEGF and angiopoietin 2. Depletion of vIL-6 in PEL has also been shown to inhibit their ability to proliferate.

In addition to coding for a viral IL6 homologue as described above, KSHV also encodes homologues to cellular inflammatory cytokines. These are vCCL1/vMIP-I/ORFK6), vCCL-2/vMIP-II/ORFK4), and vCCL-3/vMIP-III/ORF K4.1). KSHV vCCL-1 signals through CCR8; vCCL-2 signals through CCR8 and CCR3; and vCCL-3 signals through CCR4. Thus, these KSHV chemokines activate chemokine receptors that are present on CD4+ Th2 cells. vCCL-2 can also bind to multiple other chemokine receptors, but this binding is nonproductive and therefore diminishes signaling through the cognate, cellular ligand.

Conclusion

KSHV encodes an arsenal of viral proteins that control cell proliferation, cell survival, and angiogenesis. Moreover, KSHV viral proteins help the virus evade both adaptive and innate immune responses in the infected host. Several KSHV proteins are homologues of cellular proteins, while others are uniquely encoded by KSHV. By

modulating cellular signaling, apoptotic, and immune pathways in the infected cell, KSHV creates an environment that maintains virus survival and allows for virus dissemination and spread within the infected individual, as well as viral transmission from person to person. By commandeering the host environment in the aforementioned ways, KSHV infection may inadvertently result in cellular transformation and subsequent malignancy in some infected and susceptible hosts.

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KIR Locus Variation

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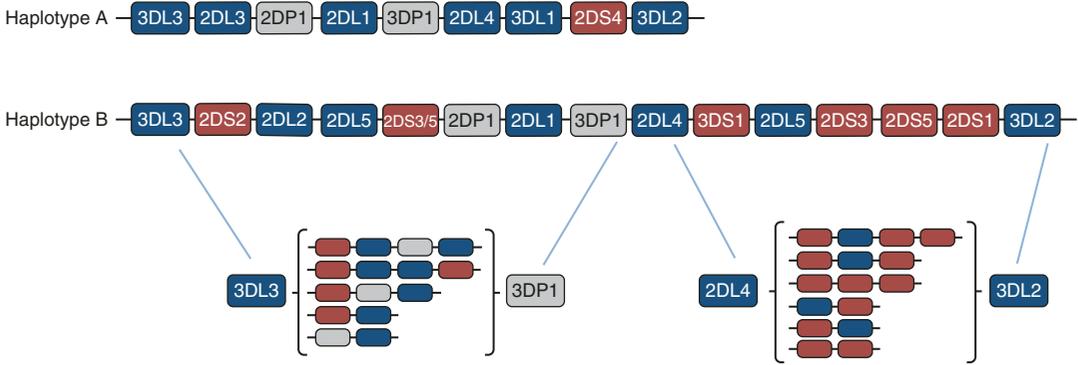
Definition

The killer cell immunoglobulin-like receptors (KIRs) belong to the immunoglobulin (Ig) superfamily of receptors and consist of a group of regulatory molecules that are expressed on natural killer (NK) cells and a subset of T cells. *KIR* genes are arranged in a “head-to-tail” cluster on human chromosome 19q13.4 within the leukocyte receptor complex (LRC) (Fig. 1). To date, 16 *KIR* genes have been identified, of which two are pseudogenes. *KIR2DL* and *KIR3DL* genes with long cytoplasmic tails are inhibitory by virtue of the immunoreceptor tyrosine-based inhibition motifs (ITIMs) present in their cytoplasmic domains, whereas the short-tailed *KIR2DS* and *KIR3DS* encode activating receptors. Short-tailed KIRs transmit activating signals through their

interaction with the adapter molecule DAP-12 (DNAX activation protein of 12 kDa.), which contains an immunoreceptor tyrosine-based activation motif (ITAM). There is substantial *KIR* gene content and allelic variation observed in humans. Two basic groups of *KIR* haplotypes termed A and B have been described (Fig. 1). Haplotype A is uniform in terms of gene content and is composed of nine genes that encode predominantly inhibitory receptors. The B haplotypes on the other hand are a diverse group that contain variable numbers of activating and inhibitory receptors. Diversity of the B haplotypes appears to be a function of unequal crossing-over, and the number of genes present on B haplotypes ranges from 4 to 17. Multiple alleles exist for each *KIR* gene, which can result in differences in expression level or functional capacity of a given gene. The likelihood that unrelated individuals share identical *KIR* haplotypes is exceedingly low. Another notable feature of the *KIR* locus is that expression of *KIR* genes is variegated on NK cells and clonally restricted.

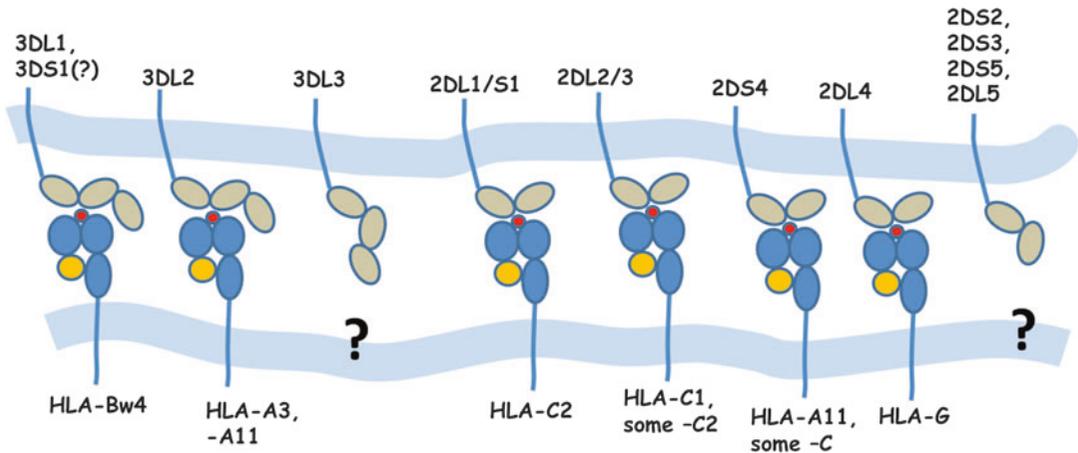
KIR Ligands

Thus far, only HLA class I molecules have been identified as ligands for KIRs (Fig. 2). Receptor-ligand relationships between HLA and KIR are allotype specific. *KIR3DL1* recognizes HLA-B molecules and a subset of HLA-A molecules that have the serologically defined Bw4 motif (amino acid positions 77–83). Some *KIR3DL1* subtypes exhibit a stronger inhibitory effect in the presence of HLA-B Bw4 subtypes that have isoleucine at position 80 (Bw4-80I) as opposed to threonine at the same position (Bw4-80 T). The alternative HLA-B Bw6 allotypes do not serve as ligands for KIR. The activating *KIR3DS1* and inhibitory *KIR3DL1* segregate as alleles of the same locus, and they share >97% similarity in their extracellular domains. There is no direct evidence of interactions between *KIR3DS1* and Bw4 allotypes, although genetic epidemiological, functional, and population genetic data suggest that some form of interaction, either direct or indirect, occurs between them. On the basis of



KIR Locus Variation, Fig. 1 Haplotypic diversity of the KIR gene cluster. KIR haplotypes vary extensively in gene content. The A haplotype is fixed in terms of gene content. The B haplotypes are characterized by variable gene numbers (shown in parentheses). The centromeric (*KIR3DL3*,

KIR3DP1) and telomeric (*KIR2DL4*, *KIR3DL2*) framework genes are present on virtually all haplotypes. Genes encoding activating KIR are shown in red, inhibitory KIR in blue, and pseudogenes in grey



KIR Locus Variation, Fig. 2 KIRs and their HLA ligands. HLA-C2 = HLA-C allotypes with Asn77/Lys80. HLA-C1 = HLA-C allotypes with Ser77/Asn80. Bw4 = HLA allotypes with the serologically defined Bw4 motif

dimorphisms in the HLA-C $\alpha 1$ domain that are characterized by Ser77/Asn80 and Asn77/Lys80, all HLA-C allotypes can be divided into two distinct groups (C group 1 and group 2, respectively). The inhibitory KIR2DL1 interacts with group 2 allotypes, while KIR2DL2 and KIR2DL3, which segregate as alleles of the same locus, interact with group 1 allotypes. KIR3DL2 interacts with HLA-A3 and HLA-A11, and KIR2DL4 recognizes the nonclassical class I molecule HLA-G. KIR2DS4 binds to subsets of HLA-C groups 1 and 2 and HLA-A*11. The activating KIR2DS1 demonstrates weak binding to

C group 2, but its high-affinity ligand has not been identified. The ligands for the activating KIR2DS2, KIR2DS3, and KIR2DS5 and the inhibitory KIR2DL5 and KIR3DL3 have not been identified.

Natural Killer Cells

► **Natural killer (NK) cells** are components of the innate immune system, and they represent the first line of defense against virally infected cells and tumor cells. They comprise about 10–15% of

peripheral blood lymphocytes. KIRs are mainly expressed on CD56^{dim}CD16^{pos} NK cells, which constitute the bulk of peripheral blood NK cells and have high cytotoxic potential. Through the interaction of inhibitory KIR with HLA class I, healthy cells are protected from spontaneous destruction by NK cell-mediated cytotoxicity, but they eliminate cells that express either nonself or aberrant levels of class I (altered or missing self). Thus, downregulation of class I on tumor cells or virally infected cells, a mechanism that allows these cells to escape T cell recognition, theoretically confers vulnerability to NK cell-mediated killing. The importance of NK cell receptors in defense against viral infections can be inferred from the complex mechanisms used by viruses to evade immune recognition. The HIV-1 Nef protein, for example, selectively downregulates HLA-A and HLA-B as a means of evading T cell recognition while preserving HLA-C expression as a means of avoiding destruction by NK cells. The *HLA* class I and *KIR* loci exhibit extensive genetic polymorphism and likely influence functional capacities of NK cells across individuals.

KIR in HIV Disease Outcomes: Overview

The diversity of clinical outcomes after HIV infection is broad, ranging from progression to AIDS within 1–3 years after seroconversion to the control of HIV without drugs for more than two decades. A small fraction of individuals are also resistant to HIV infection, even upon repeated exposure, and there is abundant information regarding host genetic variation and its consequence on outcome of HIV infection. Most genetic variants associated with HIV outcome are within or near genes encoding molecules involved in the acquired or innate immune response. The *HLA* and *KIR* molecules are encoded by two of the most diverse gene families in the human genome. Given the involvement of these molecules in fundamental immune processes, such as antigen presentation to T cells and regulation of NK cell responses, it is no surprise that the extreme diversity characterizing the *HLA* and *KIR* loci impacts HIV disease pathogenesis

KIR Locus Variation, Table 1 *KIR* associations with HIV^a

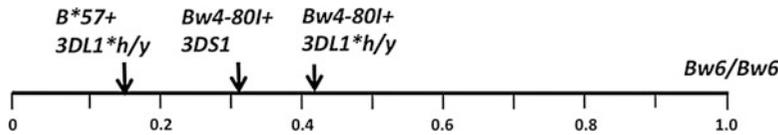
Genotype	Effect
<i>KIR3DS1/HLA-B Bw4-80I</i>	Delayed progression to AIDS Protection against AIDS-defining opportunistic infections
<i>KIR3DS1</i> homozygosity	Reduced risk of infection
<i>KIR3DL1* h/y/HLA-B Bw4-80I</i>	Delayed progression to AIDS
<i>KIR3DL1* h/y/HLA-B* 57</i>	Reduced risk of infection
↑Effective <i>KIR3DS1/L1</i> count	Lower viral load set point
<i>KIR2DS2</i>	Rapid progression to AIDS
Group B <i>KIR</i> haplotypes	Lower CD4 ⁺ T cell counts
<i>KIR2DS4* 001</i>	Increased risk of transmission
<i>KIR2DL2/2DL3/absence of C grp1</i>	Reduced risk of infection
<i>KIR3DL1/absence of Bw4</i>	

^aSee Bashirova et al. (2011) and Sobieszczyk et al. (2011) for specific citations

differentially across individuals (Table 1; Reviewed in Bashirova et al. 2011; Sobieszczyk et al. 2011).

KIR3DL1/S1

The combination of the activating *KIR3DS1* and *HLA-B* alleles having isoleucine at position 80 (*HLA-B Bw4-80I*) has been shown to exert a protective effect against AIDS progression after HIV infection based upon genetic association analyses of ART-naïve AIDS cohorts (Martin et al. 2002). This was the first study describing an epistatic interaction between *KIR* and *HLA* in disease association and indicated the likelihood that activating *KIR* may have biological significance in viral infections. The *KIR3DS1/HLA-B Bw4-80I* compound genotype also correlates with lower viral load and protection from opportunistic infections. The proposed hypothesis is that *KIR3DS1* binds *HLA-B Bw4-80I* allotypes on target cells, thereby signaling the NK cell to kill the HIV-infected target. Although there is no direct



KIR Locus Variation, Fig. 3 *KIR3DL1/S1+ HLA-B Bw4* continuum of HIV viral load control. Odds ratios of protective genotypes relative to the Bw6/Bw6 control group in terms of their distribution in the <2,000 versus the

>10,000 HIV RNA mean viral load groupings are shown. KIR3DL1*h/y = KIR3DL1 allotypes with high cell surface expression (Martin et al. 2007)

evidence for a KIR3DS1: HLA-B Bw4-80I interaction, in vitro studies have demonstrated that NK cells expressing KIR3DS1 specifically expand in acute HIV-1 infection in the presence of Bw4-80I and are able to effectively suppress HIV-1 replication in Bw4-80I⁺ target cells in vitro (Alter et al. 2009). It should be noted, however, that other studies have failed to replicate the synergistic protective effect of KIR3DS1 + HLA-B Bw4-80I, which could be the result of differences in the characteristics of the cohorts as well as differences in analytical methodologies (Gaudieri et al. 2005; Barbour et al. 2007).

KIR3DL1 allotypes can be divided into those with high expression and high inhibition and those with lower expression and lower inhibition. Ironically, the strongly inhibitory *KIR3DL1* alleles + *HLA-B* alleles with the *Bw4-80I* motif are also very protective. Notably, the most protective *HLA-B* allele, *B*57*, belongs to the *Bw4-80I* group, and individuals carrying both the strongly inhibitory *KIR3DL1* allotypes and *B*57* were shown to have the strongest genetic protection both in terms of AIDS progression and viral load (Martin et al. 2007; Fig. 3). Involvement of NK cells in this protection is supported by data indicating high functional potential of NK cells isolated from individuals with strongly inhibitory *KIR3DL1* allotypes and *B*57* (Boulet et al. 2010). These observations imply that the *B*57* protection is due at least, in part, to its interaction with KIR in addition to its well-characterized role in the adaptive immune system involving the presentation of HIV epitopes to CTL (see ► [MHC Locus Variation](#)). Overall, the data suggest that efficient engagement of both activating and inhibitory KIR is beneficial for the host in viral infection, which may confer benefit at different times after HIV infection. Interactions between

inhibitory KIR and HLA class I play a key role in establishing tolerance to healthy cells as well as determining the activation potential of mature NK cells. Thus, protection by highly inhibitory allotypes may be the result of more effective tuning of the high-expressing KIR3DL1-positive NK cells during their maturation process, resulting in stronger NK cell activation when their ligand is down-regulated, as is HLA-B by HIV-1 Nef.

KIR3DL1/S1 Copy Number Variation (CNV)

Copy number variation (CNV) is defined as a segment of DNA that is 1 kb or larger and is present at variable copy numbers in comparison with the reference genome. They are present abundantly in the human genome and have been shown to associate with disease susceptibility. Because of their homology and tandem arrangement on chromosome 19q13.4, *KIR* genes are susceptible to nonallelic homologous recombination, which causes deletions or duplications of genes within the locus and formation of novel hybrid genes in some cases. A recent genome-wide screen in a large HIV-1 positive cohort identified a CNV in the *KIR* region that associates with HIV control as measured by plasma viral load at set point, specifically the *KIR3DL1/S1* locus. The vast majority of *KIR* haplotypes has a single copy of either *KIR3DL1* or *KIR3DS1*. Subsequent quantification of *KIR3DS1* and *KIR3DL1* copy numbers by quantitative PCR showed that increased *KIR3DS1* CNV was associated with lower viral load set point, but only if its putative ligand *HLA-B Bw4-80I* was present (referred to as effective *KIR3DS1* count), regardless of *KIR3DL1* status. In contrast, an increase in

effective *KIR3DL1* count (i.e., *KIR3DL1* + *HLA-B Bw4-80I* or *Bw4-80 T*) was protective only when at least one effective copy of *KIR3DS1* (i.e., *3DS1* + *Bw4-80I*) was also present. Functional studies demonstrated that individuals with a single effective copy of *KIR3DL1* and *KIR3DS1* had an increased capacity to inhibit HIV replication in vivo, and this effect was even more pronounced in individuals with multiple copies of *KIR3DL1*. In addition, there was a significant expansion in the frequency of *KIR3DS1*⁺ NK cells, which was also more pronounced in individuals with multiple copies of *KIR3DL1* (Pelak et al. 2011). This suggests that there is a beneficial interaction between *KIR3DL1* and *KIR3DS1*, which may reflect more robust NK cell licensing in the presence of *KIR3DL1*. In support of a contribution of *KIR3DL1* through NK cell licensing, another study reported stronger NK cell responses to HLA-deficient K562 cells among HIV-infected slow progressor individuals with *KIR3DL1* and *HLA-Bw4* as compared to individuals without this receptor-ligand combination (Kamya et al. 2012). Interestingly, increasing copy numbers of the activating *KIR3DH* also associate with the control of SIV replication in rhesus macaques during primary infection (Hellmann et al. 2011).

Additional KIR Genes and Haplotypes

Presence of the activating *KIR2DS2* has been shown to associate with more rapid progression to AIDS. In addition, the carriage of group B *KIR* haplotypes that contain varying numbers of activating *KIR* (including *KIR2DS2*) is associated significantly with lower CD4⁺ T cell counts (but not viral load levels) in the absence of *HLA-Bw4* and *HLA-C* group 1 (which serve as ligands for the inhibitory *KIR3DL1* and *KIR2DL2/3*, respectively) in chronically HIV-1-infected subjects (reviewed in Sobieszczyk et al. 2011). An earlier study also found that *KIR3DS1* in the absence of its putative ligand *HLA-B Bw4-80I* was associated with accelerated disease progression (Martin et al. 2002). Aberrant immune activation is a feature of late-stage HIV disease, so the most parsimonious

explanation for these findings collectively is that group B *KIR* haplotypes (with varying numbers of activating *KIR*) and absence of ligand for inhibitory *KIR* favor NK cell activation, which is deleterious in the late stages of HIV-1 infection.

KIR-Mediated Immune Escape in HIV Infection

There is strong evidence for selection pressure of CD8⁺ T cells on HIV sequence evolution over the course of infection. Following the peak in CD8⁺ T cell response, the virus begins to show dramatic changes in sequence. Most of these changes arise within the HLA class I-restricted CD8 T cell epitopes and therefore disrupt binding of viral peptide to HLA class I or impair recognition by the T cell receptor as a means of viral escape. It is now becoming apparent that NK cells can also mediate immune pressure on the virus in part via *KIR*-associated HIV-1 sequence polymorphisms. A recent report described several amino acid polymorphisms within the HIV-1 sequence that were significantly associated with the presence of specific *KIR* genes (Alter et al. 2011). Functional analyses showed that polymorphisms in a region encoding the carboxy-terminal end of Vpu and the amino-terminal end of Env enhanced the binding of the inhibitory *KIR2DL2* to HIV-1 infected targets, thereby reducing the antiviral activity of NK cells. Interestingly, common HIV-1 peptide variants have also been shown to mediate differential binding of *KIR3DL1* to its HLA-Bw4 ligand. For example, point mutations that are frequently selected by CD8⁺ T cells within dominant HLA-B*57-restricted HIV-1 epitopes abrogate binding of *KIR3DL1* to HLA-B*5701, including one within the well-characterized TW10 epitope (T242N) (Fadda et al. 2011). In line with these data, an early emerging mutation in the TW10 epitope (G9E) was identified in two HLA-B*5703+ patients acutely infected with HIV-1 (Brackenridge et al. 2011). This change was not associated with strong escape from CD8 T cell recognition but was found to abrogate *KIR3DL1* binding.

KIR in HIV-1 Infection/Transmission

The innate immune response, which involves NK cells, could potentially be important in protection from HIV infection in the absence of vaccination. NK cell activity in a group of highly exposed uninfected (EU) intravenous drug users (IVDU) was found to be significantly greater than that from infected patients and unexposed individuals. Subsequently, it was shown that the *KIR3DS1/3DL1* transcript ratio was higher among the protected individuals (Ravet et al. 2007). A role for *KIR3DS1* in the protection from HIV infection is further supported by the demonstration that exposed HIV-1 seronegative individuals have a higher frequency of *KIR3DS1*, while the frequency of *KIR3DL1* plus/minus *Bw4* was decreased. The *KIR3DL1* h/y + HLA-B Bw4-80I* genotype is also associated with reduced risk of infection, which is consistent with the effect of this compound genotype on viral load and disease progression (Boulet et al. 2008). Overall, these results emphasize the need to consider not only the presence or absence of each *KIR* gene but also the expression levels and allelic differences. The data support the idea that strong NK cell responses may contribute to the protection from HIV infection, though additional support is necessary. Additional *KIRs* have also been found to associate with mother to child transmission as well as heterosexual transmission, but these data need to be confirmed in larger studies (Table 1).

Conclusion

Genetic diversity of immune response genes, such as *HLA* and *KIR* loci, holds promise for explaining, in large part, the variability in outcome to HIV infection among exposed individuals. Understanding how this diversity influences the immune response presents new opportunities for the development of effective therapeutics and vaccines, especially in light of the failure of recent HIV-1 vaccine trials to induce protective immunity in humans.

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Latin America and the Caribbean: Specific Characteristics of the HIV/AIDS Epidemic

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Introduction

Latin America is the region in the Americas where Spanish or Portuguese are the main national languages. It includes islands in the Caribbean, a region consisting of islands of the Caribbean Sea, and surrounding coasts (Oxford Dictionary Definition of Latin America in English 2015). Other smaller nations and colonies in proximity to Latin America have Creole, French, English, or Dutch as their national languages, along with other local languages.

The population of this region is around 600 million, approximately 8% of the world's population, with Brazil the most populous country (Central Intelligence Agency Country Comparison: Population 2014). According to the Economic Commission for Latin America and the Caribbean, despite poverty reduction and economic growth in the last 5 years, this region harbors immense economic and health disparities

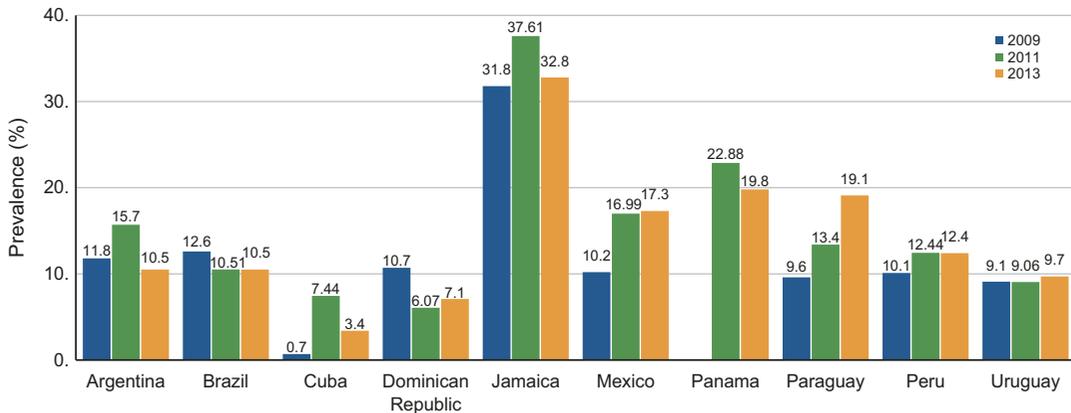
(Cecchini and Rodrigo Martínez 2012). Similarly, there are disparities in the prevalence of HIV/AIDS among countries and within their regions as well.

Epidemiology

The first cases of HIV in Latin America were reported in Haiti between 1979 and 1982. Of the first 61 cases reported, 85% were men. High-risk exposure such as bisexual activity or blood transfusions were identified in 17% of male and 22% of females (Pape et al. 1983). Demographically there has been a substantial change in the pattern of the population at risk. An infection that was used to be most prevalent in men who have sex with men (MSM), sex workers, and persons who inject drugs or had frequent blood transfusions is now somewhat more widely distributed in the general population including heterosexual men and women with few sexual partners (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2008).

Incidence and Prevalence

The Joint United Nations Program on HIV/AIDS (UNAIDS), in its 2014 report, estimated that in Latin America, there were 1.6 million people living with HIV (PLHIV) on 2013. New infections in 2013 numbered 94,000, a decline of 3% between 2005 and 2013. Children represented 1,800 of these new cases of HIV infection.



Latin America and the Caribbean: Specific Characteristics of the HIV/AIDS Epidemic, Fig. 1 Prevalence (%) of HIV infection in men who have sex with men in Latin America and the Caribbean (UNAIDS 2015)

In the Caribbean the prevalence of HIV is estimated to be 1% of the population, higher than in any other region besides sub-Saharan Africa (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2008) (Fig. 1). According to the UNAIDS 2014 fact sheet, in 2013, there were 250,000 PLHIV in the Caribbean, including 12,000 new infections. This reflects a decline of 40% from 2005 to 2013. Of these new cases, fewer than 1,000 were children (UNAIDS Communications and Global Advocacy 2014).

Women make up around 31% and 52% of the adults living with HIV in Latin America and the Caribbean (LAC), respectively (UNAIDS Communications and Global Advocacy 2014). By 2013, the Pan American Health Organization (PAHO) estimated that in Latin America and the Caribbean (LAC), an estimated of all PLHIV, 71% were aware of their diagnosis. Approximately 35% of newly diagnosed cases are identified later in the course of the infection (UNAIDS Communications and Global Advocacy 2014).

Mortality

In Latin America, AIDS-related causes accounted for around 47,000 deaths in 2013, a fall of 31% between 2005 and 2013. In the Caribbean, in 2013, 11,000 people died of AIDS-related causes,

representing a decrease of AIDS-related deaths by half. Within the Caribbean region, Haiti alone accounted for 59% of all these deaths in 2013 (UNAIDS Communications and Global Advocacy 2014).

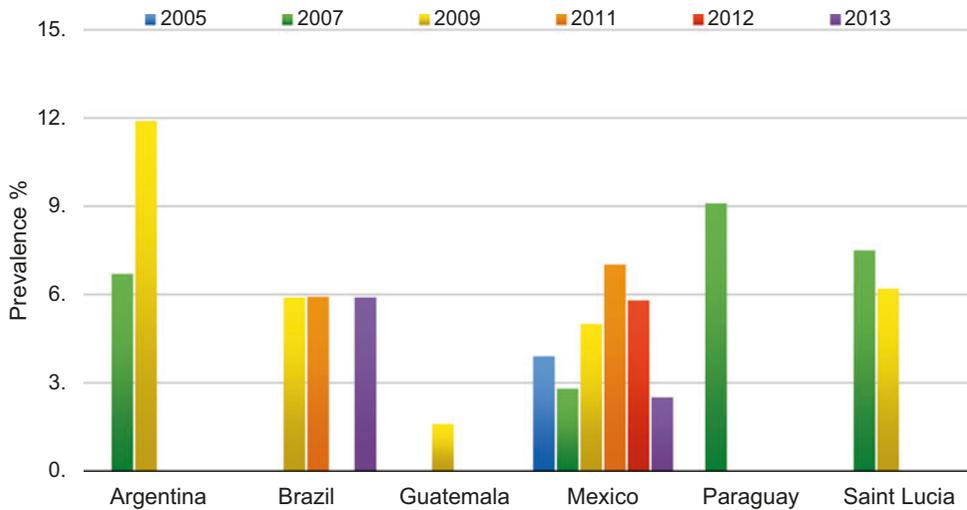
Patterns of Transmission

Men Who Have Sex with Men

Men who have sex with men (MSM) represent a population defined by their sexual behaviors regardless of their sexual orientation or gender identity. Worldwide, the HIV prevalence is highest among MSM and transgender persons and, this population shows, an increasing prevalence over time. Despite this, there is a lack of high-quality data on MSM HIV prevalence in LAC (Beyrer and Baral 2012).

Prevalence among MSM ranges from 3.4% in Cuba to 32.8% in Jamaica. These rates are far higher than the overall HIV prevalence of 1% in LAC.

Unprotected anal intercourse is the principal route of transmission in this group. Factors such as the use of illicit drugs and having multiple partners increase this risk. Furthermore, a 2001–2008 review found that 40–70% of MSM in developing countries had never been tested for HIV, which translates into increased risk of transmission, lower access to HIV care, and a



Latin America and the Caribbean: Specific Characteristics of the HIV/AIDS Epidemic, Fig. 2 Prevalence (%) of HIV infection in people who inject drugs in Latin America and the Caribbean (UNAIDS 2015)

need for reinforcement of prevention strategies (Geibel 2010).

Persons Who Inject Drugs (PWID)

Another route of HIV transmission is the injection of intravenous drugs. The United Nations Office on Drugs and Crime (UNODC) estimated in 2013 that there were 14 million PWID worldwide, 1.6 million of them were living with HIV, an 11.5% of all the PWID population. In LAC, the prevalence of HIV among PWID was estimated to be 6.9% (UNODC 2013).

As shown in Fig. 2, the prevalence is particularly high in Mexico, especially along the border with the United States as well as in the southern countries of America (Argentina, Brazil, and Paraguay). Once again, data regarding the number of PWID and the proportion infected with HIV are scarce (WHO, Global HIV/AIDS response 2011).

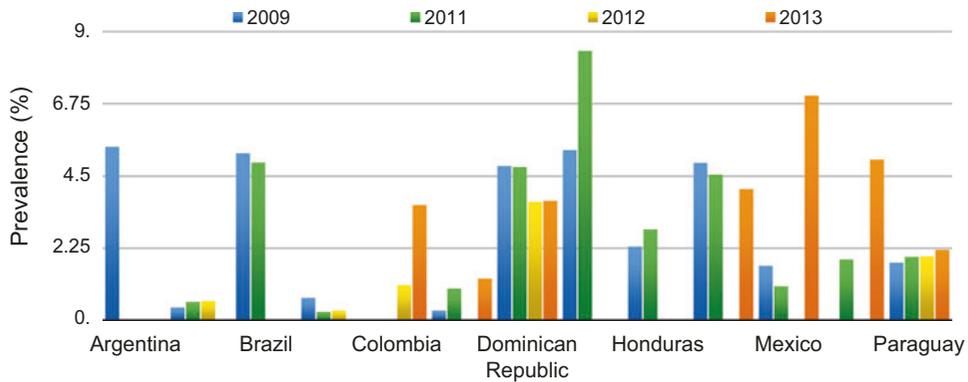
Countries which have instituted aggressive HIV control campaigns have seen a reduction in high-risk behaviors and as a consequence a markedly drop in the incidence of HIV (Geibel 2010). Policies such as disposable and clean needles and syringes exchange programs and opiate substitution therapy with buprenorphine or methadone have reduced the likelihood of HIV transmission (UNODC 2013).

Female Sex Workers

A meta-analysis, in 2012, showed that the prevalence among female sex workers (FSW) on 11 countries in LAC was 6.1% (5.7–6.6, 95% CI) (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2013). As shown in graph N° 3 (Fig 3), prevalence is variable among the countries in the region, with Haiti as the country with highest rates. Once again, the lack of quality data is detrimental, and the real prevalence may be much higher than reported. FSW have 12 times higher odds of being infected with HIV in comparison to the female population of low- and middle-income countries (Baral and Beyrer 2012).

Heterosexual Population

According to the 2007 epidemic update from UNAIDS, in Latin America, there is an increasing number of heterosexual men and women living with HIV. Monogamous women get the infection from their male sexual partners who at the same time got infected from unprotected sex with another man or woman. In Argentina on 2005, four out of five new HIV diagnosis were attributed to unprotected sex, mainly heterosexual. Furthermore, in Uruguay, unprotected sex (mostly heterosexual) causes around two thirds of the



Latin America and the Caribbean: Specific Characteristics of the HIV/AIDS Epidemic, Fig. 3 Prevalence (%) of HIV infection in female sex workers in Latin America and the Caribbean (UNAIDS 2015)

reported HIV cases. This pattern is also seen in Chile, Peru, and Brazil (Joint United Nations Programme on HIV/AIDS (UNAIDS) 2008).

Introduction of ART

Antiretroviral therapy (ART) was started in LAC in 1990 in Argentina and in 1991 in Brazil. The number of people infected with HIV on ART in LAC has progressively increased. The PAHO reports that by the end of 2013, around 720,000 people, 75% of the eligible population, were taking ART. LAC is the region with the highest ART coverage; the worldwide coverage of ART for eligible PLHIV is 61% (WHO/UNAIDS/UNICEF 2013).

According to the UNAIDS 2013 report and using all PLHIV as the denominator, 44% [34–50%] of all PLHIV in LAC were receiving treatment. This shows a 31% increase from the previous report in 2011. In children younger than 14 years old, the coverage is estimated to be 51% [38–63%] (WHO/UNAIDS/UNICEF 2013). Furthermore, the PAHO and WHO in their 2014 Public Health Analysis in LAC, reported that by 2013, of the population eligible for ART in LAC, 56% were estimated to be receiving ART and, of the people on ART, 77% had achieved viral suppression (Pan American Health Organization 2014).

Estimates from Mexico, El Salvador, and Paraguay suggest that more than 30% of the HIV-positive population remains unaware of their diagnosis (Table 2). A higher risk of transmission can be expected from this subgroup.

Multiple factors need to be considered in order to assess optimal access to HIV care and treatment. Among these are (1) the proportion of PLHIV who do not know their status, (2) the proportion of diagnosed persons who do not get effectively linked to care, (3) the criteria used in each country to start ART, (4) the public policies that guide the accessibility of ART, and (5) retention and adherence of persons on ART.

The CD4+ cell cutoff has increased throughout the years often following PAHO and WHO guidelines. Earlier initiation of ART is being encouraged in order to improve survival. The CD4 cutoff for HIV treatment is 500 cells/ μ L in most countries according to the 2013 WHO recommendations (Table 1). An exception is Brazil, where there is no CD4 cutoff for ART initiation. Other criteria to start ART include active tuberculosis (TB) disease, hepatitis B virus (HBV) confection with chronic liver disease, and pregnant or breastfeeding women with HIV (World Health Organization 2013).

As shown in Table 3, the percentage of PLHIV on ART is variable among countries due to differing public policies on ART coverage, strength of

Latin America and the Caribbean: Specific Characteristics of the HIV/AIDS Epidemic, Table 1 Number of people living with HIV, prevalence and mortality in adults

in selected countries of Latin America and the Caribbean between 2012 and 2013 (UNAIDS 2015)

	People with HIV that know their diagnosis (%)	People with HIV on ART (%)	People with HIV on ART with suppressed viral load (%)	CD4 cutoff for ART (cells/uL)
Argentina	70	55	36	500
Barbados	92	46	39	350
Brazil	80	48	40	Irrespective of CD4
Costa Rica	67	47	40	500
Cuba	92	51	37	500
El Salvador	69	31	22	500
Guatemala	^a	31	19	500
Jamaica	72	27	12	500
Mexico	63	49	33	350
Nicaragua	71	19	14	500
Panama	81	49	28	350
Paraguay	69	26	18	500
Uruguay	70	35	23	350

^aInformation not reported in the reference

the underlying health system, and social factors like stigma, isolation, and discrimination. Some of the barriers that the countries must face include medication shortage and limited services for persons without coverage through the national health and social security systems. Another barrier is the cost of the drugs, since generic and low-cost drugs are scarce in LAC. Furthermore, although the drug prices have decreased significantly, there are still disparities between countries (Chequer et al. 2002).

ART and Mortality

Mathematical models for the HIV epidemic in Latin America showed that in the year 2008, a 16% HIV annual mortality was likely, substantially lower than the estimated 21% mortality modeled for 2004. This likely due to the increased availability and access of ART in the region, albeit with imperfect coverage and adherence (World Health Organization 2009).

Gonzales et al. in (2011), analyzed the available data in 11 countries in Latin America regarding ART and mortality. They found that the standardized mortality rates before the implementation of public policies with wider availability of

ART ranged from 2.2 to 15.6 per 100,000 (mean 5.7 CI 4.1–7.2). After the adoption of stronger ART policies, the mortality rates had similar ranges: 2.5–14.0 per 100,000 (mean 5.1 CI 4.5–5.7; $p = 0.4$). Although, there has been an increase in the ART coverage over the time, the mortality had not yet decreased significantly in the study.

However, when analyzed by countries, the mortality rates showed two different patterns. Argentina, Brazil, Chile, Costa Rica, and El Salvador reported a significant decrease in HIV mortality rates after ART was made available. Panama also presented a decrease in mortality, though it was not significant. The second pattern was seen in Colombia and Ecuador, where there was paradoxical increment in the HIV mortality rates. Mexico, Venezuela, and Uruguay had an overall increased mortality pattern as well but with varying trends.

When the mortality rate was analyzed in relation to the ART coverage percentage, the countries with higher coverage rates (over 70%) had lower mortality rates, except for Uruguay with an increased mortality rate despite a reported 95% ART coverage. In general countries with lower coverage rates had increases in this mortality rates (Gonzalez et al. 2011).



Latin America and the Caribbean: Specific Characteristics of the HIV/AIDS Epidemic, Table 2 Coverage of diagnosis and antiretroviral therapy in selected countries of Latin America and the Caribbean between 2012 and 2013 (WHO 2013)

Country	People living with HIV (estimate)	prevalence of adult population	AIDS-related deaths
Argentina	^a	^a	1548
Bahamas	7700	3.2	542
Belize	3300	1.5	114
Bolivia	15,000	0.2	1181
Brazil	730,000	0.5	15,833
Chile	38,000	0.3	654
Colombia	140,000	0.5	
Costa Rica	7600	0.2	269
Cuba	16,000	0.2	191
Dominican Republic	46,000	0.7	1694
Ecuador	37,000	0.4	1629
El Salvador	21,000	0.6	603
Guatemala	53,000	0.6	2607
Guyana	7700	1.4	194
Haiti	140,000	2.0	6399
Honduras	24,000	0.5	1547
Jamaica	30,000	1.8	1270
Mexico	180,000	0.2	5563
Panama	16,000	0.6	501
Paraguay	16,000	0.4	344
Peru	65,000	0.3	2822
Suriname	3200	0.9	114
Uruguay	14,000	0.7	454
Venezuela	100,000	0.6	4403

^aInformation not reported in the reference

Opportunistic Infections

Tuberculosis and Multidrug-Resistant Tuberculosis

The association between HIV infection and tuberculosis is well known. People living with HIV are 29 times (CI: 26–31) more likely to develop tuberculosis compared to people without HIV who live in the same region. Of all deaths due to tuberculosis in the world during 2009, 24% were people with HIV coinfection (WHO 2011).

Patients with HIV develop tuberculosis in around 40% of the cases, especially in those who remain undiagnosed or have not been started yet

Latin America and the Caribbean: Specific Characteristics of the HIV/AIDS Epidemic, Table 3 Antiretroviral coverage based on World Health Organization 2013 guidelines in selected countries of Latin America and the Caribbean (UNAIDS 2014)

Country	% Coverage	Country	% Coverage
Argentina	69	Guatemala	37
Bahamas	30	Guyana	59
Barbados	70	Haiti	45
Belize	50	Honduras	44
Bolivia	23	Jamaica	32
Brazil	64	Mexico	51
Chile	67	Panama	49
Colombia	34	Paraguay	32
Costa Rica	64	Peru	49
Cuba	71	Trinidad and Tobago	55
Dominican Republic	52	Uruguay	46
Ecuador	37	Venezuela	46
El Salvador	54		

on ART. During the first 12 months after ART is initiated, the risk of developing TB may increase paradoxically, perhaps due to the ease of diagnosis after partial immune reconstitution. Nonetheless, many trials document the vital benefits of ART for the treatment of TB-HIV coinfecting persons.

An active search for HIV infection is encouraged by the WHO for all TB patients. In 2009, 43% of patients with TB were tested for HIV. Of these, 17% had HIV infection. Countries with high rates included Colombia (23% HIV-TB coinfection), Brazil (22%), Mexico (21%), and Ecuador (20%) (WHO 2014).

In Peru, among the newly diagnosed patients with tuberculosis, 1.5–4% are found to be coinfecting with HIV (Cerro and Gotuzzo, unpublished).

A study conducted in Peru showed that PLHIV were at ten times greater risk of developing multidrug-resistant (MDR) TB than HIV-negative patients (Campos et al. 2003). Although in a selected population, this raises concern for the importance of TB surveillance in HIV patients, both for their care and to reduce MDR-TB transmission to the general population.

Leishmania

An increase of visceral leishmaniasis (VL) has been seen in Latin America, especially among children and young adults. The area of spread coincides with the regions of higher HIV incidence. Therefore, coinfection of HIV and visceral leishmaniasis is believed to be responsible of the increased rates of VL in Latin America, notably in Brazil that harbors more than 90% of all the cases of visceral leishmaniasis in Latin America. The clinical picture between those with visceral leishmaniasis alone compared to those with HIV-visceral leishmaniasis coinfection is similar, both with fever and hepatosplenomegaly. However, there is a slight increase in the proportion of cases that present with diarrhea in the PLHIV group. Furthermore, HIV-visceral leishmaniasis coinfection also results in higher rates of relapse, with 10–56.5% of all cases. Mortality reports have ranged from 8.7% to 23.5% in this coinfecting group (Lindoso et al. 2014; WHO 2003).

Chagas Disease

Trypanosoma cruzi has surfaced as an opportunistic pathogen in people with HIV infection in Latin America, mainly Brazil, Argentina, and Colombia. Although, in this region, Chagas disease is a characteristic of rural areas, and HIV infection is more common in urban areas, the urbanization of the rural population is responsible for coinfection. Recently, oral transmission has been identified, which allows to understand the new transmission pattern.

It is estimated that between 20% and 40% of people with HIV and *T. cruzi* coinfection may experience reactivation, with levels of parasitemia compared to the ones seen in acute infection. Furthermore, death is more common in coinfecting persons and occurs earlier.

For HIV-Chagas coinfecting persons, two manifestations have been described: (1) acute myocarditis with anasarca and (2) encephalitis with multiple necrotizing foci. Tissue samples show multiple amastigotes. Many patients may have the tumor or pseudotumor form (brain “chagoma”), making it part of the differential diagnosis with toxoplasmosis (Pitella 2009). This phenomenon is reported also in Latin

Americans living abroad making the screening for coinfection necessary (Andrade et al. 2014). In a recent systematic review of 83 articles and 291 patients, the mortality due to Chagas meningoencephalitis without treatment was 100% (Almeida et al. 2011).

HIV-Associated Malignancies

A study published by the Caribbean, Central and South America Network for HIV Research (CCASAnet) in 2011 showed that in a cohort of 455 cancers from 428 patients, 406 of the reported cancers (82%) were AIDS defining, particularly Kaposi sarcoma and non-Hodgkin’s lymphoma. Of the non-AIDS-defining cancers, the most common were Hodgkin’s lymphoma and skin cancers. In this cohort, almost half of the cancers were diagnosed within 1 year after the HIV diagnosis (Fink et al. 2011).

The same study also found low rates of invasive cervical cancer. However, most of the patients included in the cohort were male, and many women were receiving regular cervical screening. The cases of invasive cervical cancer that were found were mostly in advanced stages, presumably in unscreened women (Andrade et al. 2014). Since this malignancy develops relatively faster in HIV-infected women, it has been suggested that countries change their screening programs with Papanicolaou smears every 6 months. Human papillomavirus (HPV) is also responsible for anal carcinoma, a prevalent malignancy among both MSM and women which makes screening for HPV.

Conclusions

Latin America and the Caribbean countries have had some stability in HIV transmission patterns in recent years, with the number of people living with HIV having plateaued. There has been a change in the pattern as well, with a rising proportion of heterosexual women and men, though the epidemic among MSM continues to drive the epidemic in many LAC nations.

An increasing number of PLHIV are now on ART in the LAC region, due to changes in public policies and efforts on expanding coverage. However, there are still no massive screening policies in place, ART coverage is still suboptimal, and there are no broad prevention strategies being promulgated, such as the diffusion of the use of condoms, especially in heterosexual men and women who were not considered as population at risk of being infected.

Challenges facing LAC include the need to expand the HIV continuity of care, testing, linkage, care with ART, and adherence/retention (Fink et al. 2011; García et al. 2014; De Boni et al. 2014). Strengthening health systems as well as improving public policies are vital, as is addressing the widespread stigma of HIV infection in LAC. Improved partnerships with political and religious forces in the region are needed.

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Leishmaniasis and HIV

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Definition

Leishmaniasis is a group of chronic parasitic diseases caused by a number of species of flagellate protozoa of the genus *Leishmania*. Up to 20 *Leishmania* species can cause disease in humans. The infection can be localized in the skin (cutaneous leishmaniasis, or CL, classifiable as localized cutaneous and diffuse cutaneous leishmaniasis), mucosae (mucocutaneous leishmaniasis, or ML), or disseminated in the reticuloendothelial system (visceral leishmaniasis, or VL).

Introduction

Leishmaniasis is usually a zoonosis transmitted by sandflies, which are endemic in and of Southern Europe, Asia, Africa, and the Americas. Transmission may follow an anthroponotic or zoonotic cycle that also varies by region. Sporadic interhuman transmission through needle exchange in intravenous drug users (IDUs) has also been reported, especially in Southern Europe

(Monge-Maillo et al. 2014). Infection may be asymptomatic or may manifest as a cutaneous disease that is pleomorphic in presentation, a mucocutaneous disease or the visceral form that may be lethal if untreated. The clinical expression of leishmaniasis depends on a complex interaction between the type of infecting species and the host's immune response; the result of this interaction may range from asymptomatic carriage to fatal disease (Pace 2014).

HIV-infected individuals and transplant recipients have an increased risk of developing the disease. The most common clinical presentation of leishmaniasis in HIV-infected individuals is a disseminated visceral disease syndrome, but the distribution varies geographically, reflecting differences in the predominant parasitic species. In Europe, visceral disease has been reported in 95% of cases (87% typical visceral, 8% atypical visceral) (Alvar et al. 2008). In contrast, in Brazil, mucocutaneous (43%) and cutaneous (20%) are the most common forms (Lindoso et al. 2014).

In Southern Europe, before the introduction of combined antiretroviral treatment (cART), the prevalence of VL among HIV-infected patients was 2–9%, 10,000 times the rate in the general population. Since cART was introduced in 1997, a marked decrease in the number of coinfecting cases in this region has been reported (from 1,440 cases during the period 1990–1998 to 299 cases during 2001–2006, though this reduction was not so marked in Portugal) (Monge-Maillo et al. 2014). The development of new diagnostic methods to identify accurately the level of parasitemia and the risk of relapse is one of the main challenges in improving the treatment of coinfecting patients.

Until recently, antimonial drugs were the mainstream choice for VL treatment, despite resistance having emerged, especially in the Indian subcontinent, and this class of drugs having been proven to be more toxic in immunosuppressed patients. Liposomal amphotericin B, miltefosine, and combined treatments have been investigated more recently as active agents for VL in HIV-infected individuals (Murray 2012). Treatment options for CL and ML are still suboptimal.

Biology, Epidemiology, and Transmission

Biology

Leishmaniasis is a group of vector-borne diseases that are transmitted by sandflies and caused by obligate intracellular protozoan parasites of the genus *Leishmania*, belonging to the family Trypanosomatidae, order Kinetoplastida. The genus *Leishmania* is named after Sir William Leishman, who discovered the flagellate protozoa.

All member of the genus *Leishmania* spend their life cycle in two hosts: the mammalian host and the insect vector, the female sandfly. In humans and other mammalian hosts, they multiply within macrophages, in which they occur exclusively in amastigote form, having an ovoid body containing a nucleus and kinetoplast. In the sandfly, they occur in the promastigote form, with a spindle-shaped body and a single flagellum arising from the anterior end.

The genus *Leishmania* includes a number of different varieties and subspecies, which differ in several features such as antigenic structure, isoenzymes, and other biochemical characteristics, growth properties, and host specificity. *Leishmania* species can also be classified on the basis of geographical distribution. Human infection is caused by about 21 of the 30 species that infect mammals. These include the *L. donovani* complex with two species: *L. donovani* and *L. Infantum* (also known as *L. chagasi* in the New World), both causing VL, although *L. infantum* can may also lead to CL; the *L. tropica* complex with three species: *L. tropica*, *L. major*, and *L. aethiopica*; the *L. mexicana* complex with three main species: *L. mexicana*, *L. amazonensis*, and *L. venezuelensis*; and the subgenus *Viannia* with four main species: *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (V.) panamensis*, and *L. (V.) peruviana*, all causing CL and/or ML.

The vector responsible for the transmission of the *L. donovani* and *L. tropica* complexes is the *Phlebotomus* species, and for *L. braziliensis* and *L. mexicana*, it is the sandfly of genus *Lutzomyia*.

Epidemiology and Transmission

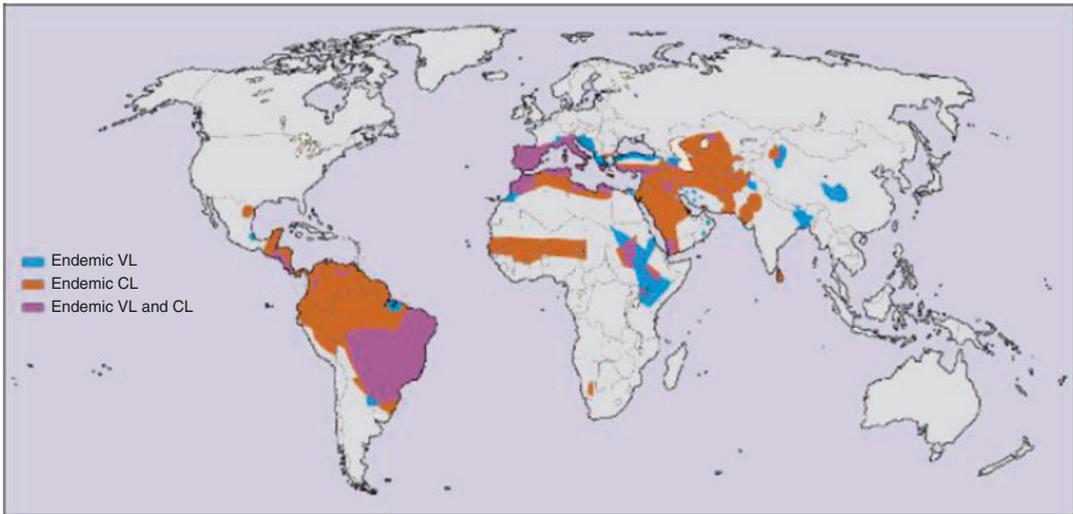
Global leishmaniasis annual incidence is 1.5 million new cases.

Globally, the most frequent form is cutaneous leishmaniasis, accounting for 0.7–1.3 million new cases annually. This form is present mainly in the Americas, Mediterranean countries, the Middle East, and Central Asia. Visceral leishmaniasis (kala-azar) is less frequent, with 200,000–400,000 new cases every year and is highly endemic in India and East Africa (Alvar et al. 2008).

HIV-*Leishmania* coinfection has been reported since the very start of the HIV epidemic in the 1980s, mainly in Southern European countries. The widespread adoption of cART has caused a notable reduction in incidence in developed countries, and nowadays the Mediterranean basin only adds 2,000–3,000 new diagnoses to the global burden (Monge-Maillo et al. 2014). In low-income areas such as the Indian subcontinent, Latin America, and East Africa, HIV-*Leishmania* coinfection still represents a major concern. Contrary to the situation in Europe and North America, incidence in these areas seems to be increasing. As an example of this, the proportion of HIV infections in new leishmaniasis diagnoses in Brazil increased from 0.7% in 2001 to 8.5% in 2012 (Lindoso et al. 2014). Similar increases have been observed in some areas of India, where HIV patients accounted for 0.88% of all *Leishmania* infections in 2000, increasing to 2.18% in 2006. Higher incidences are found in some areas of East Africa, like the Ethiopian region of Humera, where the prevalence of HIV between patients with leishmaniasis is as high as 31% (Masur et al. 2014). Figure 1 shows the distribution of different forms of leishmaniasis in different endemic regions (Davidson 2010).

There are some well-known risk factors for the acquisition of the infection, the most decisive being related to poverty (poor hygienic and domestic conditions, migration, etc.) and climate change (global warming and land degradation).

It has also been established that there is a new transmission cycle mediated by needle sharing among intravenous drug users, particularly in areas of Southern Europe and the Mediterranean



Leishmaniasis and HIV, Fig. 1 Geographical distribution of leishmaniasis

basin, like Spain, where 34–55% of used needles were found to contain *Leishmania*.

Clinical Presentation

All forms of leishmaniasis, particularly visceral leishmaniasis, behave as opportunistic infections in immunosuppressed HIV-infected patients, yet they are not included in the CDC classification of opportunistic infections. The predominant presentation depends on the epidemiology and the involved species; in Brazil, for example, mucocutaneous, visceral and cutaneous clinical presentations are seen in comparable proportions in HIV-infected individuals, but in southern Europe, cases are almost exclusively visceral.

cART has decreased the proportion of symptomatic cases in Europe, but its impact on the clinical presentation in resource-limited settings is much more restricted.

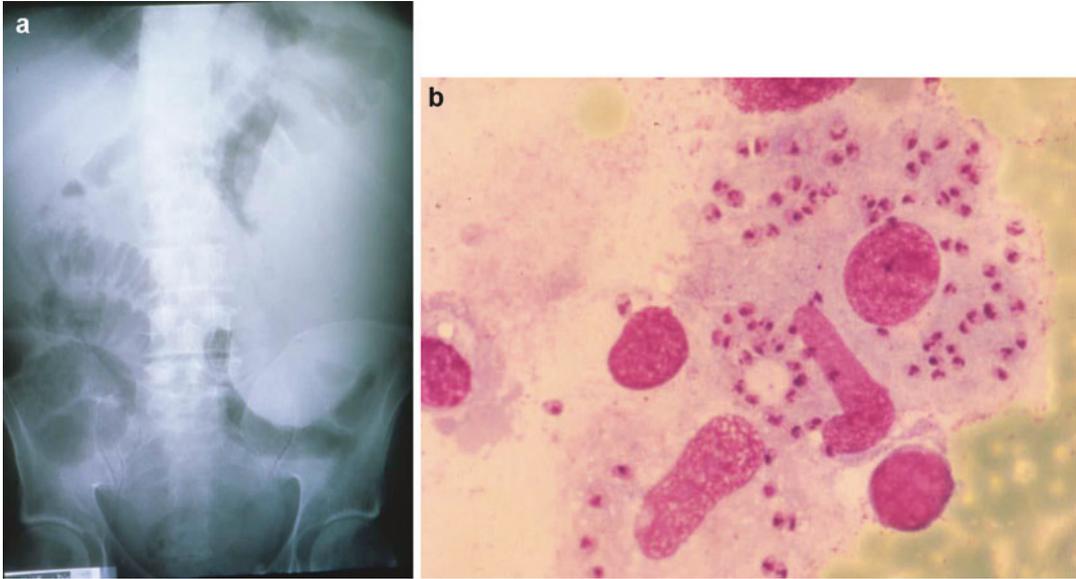
Visceral Leishmaniasis in HIV-infected Patients

The clinical presentation of visceral leishmaniasis in HIV-infected patients is very similar to that in HIV-seronegative individuals, but cases of progressive disease are more frequent. Patients have

typically less than 200 CD4 cells/ μ l and are infected with *L. infantum* (Europe), *L. donovani* (Africa and India), or *L. infantum/chagasi* (South America). Nevertheless, asymptomatic infections are not infrequent.

The incubation period can be as short as days following infection with the parasite, but most cases develop as result of the reactivation of an asymptomatic prior (even years-old) infection with *Leishmania* when immunosuppression progresses, most frequently in patients with less than 200 CD4 cells/ μ l, and most severe cases with visceral leishmaniasis have less than 100 CD4 cells/ μ l.

Clinical presentation frequently includes fever, splenomegaly and hepatomegaly, generalized lymphadenopathy, and profound weakness. Pancytopenia is prominent in routine lab tests. Frequently associated abnormalities are hypergammaglobulinemia (both due to HIV and to leishmania infections) and renal involvement. Acute and proliferative glomerulonephritis have been described, as well as proteinuria and abnormalities in globular filtration. Gastrointestinal symptoms, such as diarrhea, are more frequent in HIV-infected patients than in HIV-uninfected individuals, as a result of a profound parasite infiltration into the bowel. Atypical organ involvement seems to be more frequent in HIV-infected patients.



Leishmaniasis and HIV, Fig. 2 51-year-old male, heterosexual, diagnosed in 1994 with HIV stage C3. He had a history of *Pneumocystis jiroveci* pneumonia (PCP) and disseminated nocardiosis. His CD4 count was 28 cells/ μ L. The patient received antiretroviral treatment and secondary prophylaxis of opportunistic infections irregularly. In 1997, he was admitted for febrile syndrome, an enlarged

spleen of 12 cm below the costal margin and severe pancytopenia. The abdominal radiography showed a splenic silhouette that reached the left iliac crest (a). A bone marrow aspirate showed numerous leishmania amastigotes in the cytoplasm of a cell of the mononuclear phagocyte system (b) (May-Grünwald-Giemsa, x1000). The NNN medium culture was positive for *Leishmania donovani*

Leucopenia and thrombocytopenia are less frequent and less marked in patients receiving cART. Clinical relapses are more frequent in HIV-positive patients than in the HIV-uninfected population and frequency decreases with cART, but an undetectable viral load does not prevent relapse where immunological recovery has not been achieved. Patients relapsing have higher chances of further relapses, in part due to the delayed CD4 cell recovery in these patients.

Clinical presentation does not differ markedly according to CD4 cell count, although prognosis is poorer and mortality higher in patients with more pronounced immunosuppression.

Although the initial clinical presentation of visceral leishmaniasis is similar in HIV-infected and noninfected patients, there are major differences in outcomes. Global mortality is higher among HIV-positive patients compared to HIV-negative individuals, particularly if other concomitant opportunistic infections are present. Toxicity related to antileishmania treatment is also higher. Patients have extremely high relapse rates and the

clinical course is very frequently much prolonged. If patients are not receiving cART, relapse rates are as high as 90% within 12 months. Figure 2 shows some clinical and laboratory features of a case of visceral leishmaniasis.

Cutaneous Leishmaniasis in HIV-infected Patients

Localized cutaneous leishmaniasis is typically seen in patients with limited immunosuppression, and clinical presentation resembles that of HIV-uninfected individuals: papules, plaques, or ulcers in exposed body areas. The pathology of these cases is the same as that of HIV-negative patients. The incubation period does not seem to be affected.

Nevertheless, the parasite can disseminate and cause disseminated cutaneous leishmaniasis much more frequently than in HIV-negative patients, particularly in those with more pronounced immunodeficiency. This may or may not be associated with a concomitant visceral leishmaniasis. Indeed, every HIV-infected

patient with cutaneous leishmaniasis should be examined carefully to exclude visceral leishmaniasis. As for visceral leishmaniasis, recurrence and treatment failures are higher in HIV-positive patients.

Post-Kala-Azar dermal leishmaniasis follows the treatment of visceral leishmaniasis in a small proportion of cases due to *L. donovani*. Although it is rarely seen in the Americas or Europe, when reported – following cases due to *L. infantum* or *L. chagasi* – it has been in HIV-positive individuals. Cutaneous lesions may persist for long periods of time and consist of hyperpigmented or hypopigmented macules that progress to papules, nodules, and verrucous forms.

Mucocutaneous Leishmaniasis

ML is more frequently seen in South America and, as described for visceral and cutaneous leishmaniasis, the clinical course is frequently more aggressive and treatment failure more frequent.

Diagnosis

The techniques for diagnosing *Leishmania* infection in HIV patients have not changed significantly in recent years.

Microscopy

The demonstration of amastigotes in smears of tissue aspirates is the gold standard for the diagnosis of VL (Elmahallawy et al. 2014). Possible specimens include peripheral blood, bone marrow, splenic aspirate, and enlarged lymph nodes. Useful stains for the smears are Giemsa, Hematoxylin, and Eosin or Wright's stain. Stained smears are examined under an oil immersion objective. Amastigote parasites are seen within macrophages.

While splenic aspiration is the most sensitive method (98% sensitive), bone marrow puncture (50–85%, positive) is a safer procedure, as there is risk of hemorrhage in splenic puncture particularly in patients with an advanced stage of disease with soft enlarged spleens. Splenic aspiration is contraindicated in patients with prolonged prothrombin time or if platelet count is less than

40,000/mm³. Liver biopsy may often be diagnostic but also carries risk of hemorrhage.

Culture

Different tissue materials or blood are cultured on an NNN medium. This is a rabbit blood agar slope consisting of two parts of salt agar and one part of defibrinated rabbit blood. The material is inoculated in water of condensation, and the culture is incubated at 22–24 °C for 1–4 weeks. At the end of each week, a drop of culture fluid is examined for promastigotes. Other biphasic media, like Schneider's drosophila tissue culture medium with added fetal calf serum, can also be used.

Serology

Serological tests are not considered accurate methods for diagnosis of VL in HIV patients because of their limited sensitivity. The available evidence indicates that serological tests should not be used to rule out VL in HIV-infected patients.

The combined use of the direct agglutination test (DAT) and the rK39-immunochromatographic test (rK39-ICT), both rapid and easy to use methods, has been shown to have a sensitivity of 98% for VL diagnosis HIV-positive patients.

The detection of *Leishmania* antigen in urine using the latex agglutination test, commercialized as KAtex, initially appeared to be a promising, noninvasive tool for VL diagnosis and treatment follow-up. Its sensitivity in different studies in Europe, including both HIV-positive and HIV-negative patients, ranged from 69% to 100%, and a positive result after treatment was strongly associated with relapse, even though this has not been confirmed.

Molecular Biology

In recent years, peripheral blood-PCR analysis has been validated as a sensitive and specific tool to detect *Leishmania* parasites in coinfecting patients. Whereas classical diagnostic methods, such as bone marrow aspirate culture and microscopy, are still in use, diagnosis in more and more clinics is mainly based on the combination of the molecular detection of parasite DNA in peripheral blood by PCR and serology.

Bone marrow aspirates are still a source for parasite detection by PCR because of the increased sensitivity when compared with peripheral blood analysis. Despite the widespread use of molecular diagnosis techniques for leishmaniasis, there is, however, still a lack of consensus on the method for diagnosis of VL in HIV infected patients. Further development of common guidelines for diagnosis is needed.

It has been proposed to use real-time PCR as a suitable tool for monitoring the parasite load during follow-up of coinfecting patients and to predict the risk of relapses after treatment together with CD4+ cells, secondary prophylaxis, and previous history of VL relapse.

WHO guidelines for VL/HIV diagnosis have no specific regional considerations. The methods for diagnosis and follow-up (to determine cure and to predict relapses), therefore, vary considerably but are mainly based on a culture of buffy coat from peripheral blood, blood PCR, and bone marrow aspirate microscopy and culture and/or bone marrow PCR.

Differential Diagnosis (see Table 1)

Differential diagnosis is large and depends on the geographical localization, the clinical presentation (visceral, cutaneous, or mucosal leishmaniasis), and the degree of immunosuppression.

In HIV-patients with no or mild immunosuppression, differential diagnosis is the same as in HIV-uninfected patients.

The late stage of visceral leishmaniasis, most frequently seen in severely immunosuppressed patients, must be differentiated from hematological and lymphatic malignancies, from disseminated tuberculous and nontuberculous mycobacterial (infections and from progressive histoplasmosis. Acute visceral leishmaniasis has a much larger differential diagnosis including malaria, enteric fever, and several other conditions related to the specific epidemiological context.

Differential diagnosis of cutaneous and mucocutaneous leishmaniasis includes cutaneous tuberculosis, atypical mycobacterial infections, fungal infections such as histoplasmosis, paracoccidioidomycosis or sporotrichosis, leprosy, and neoplasms of the skin.

Anti-*Leishmania* Treatment

Management of leishmaniasis is complex and depends on the clinical syndrome and the causative organism. Only a few anti-leishmanial drugs are available, and their efficacy varies according to the species and region of infection. *L. donovani*, for example, does not respond to drugs in the same way in the Indian subcontinent and in East Africa. Some patients relapse within 6 months to

Leishmaniasis and HIV, Table 1 Differential diagnosis of leishmaniasis in HIV-infected patients^a

Visceral leishmaniasis	Cutaneous leishmaniasis	Mucosal leishmaniasis
Hematological malignancies ^b	Sporotrichosis	Disseminated histoplasmosis ^b
Lymphatic malignancies ^b	Chromomycosis	Paracoccidioidomycosis
Disseminated histoplasmosis ^b	Lobomycosis	Basal cell carcinoma
Disseminated mycobacterial infections	Leprosy	Midline granuloma
Brucellosis	Cutaneous tuberculosis	Nasal cavity lymphomas
Typhoid fever and enteric fevers	Cutaneous nontypical mycobacteria	
Malaria	Neoplasms of the skin	
Infectious mononucleosis	Chronic ulcerative lesions caused by typical bacteria	
Hemophagocytic syndromes		
Infectious endocarditis		
Hepatosplenic and acute schistosomiasis		

^aDifferential diagnosis largely depends on degree of immunosuppression, epidemiology, and geographical area. In patients without immunosuppression, differential diagnosis of leishmaniasis resembles that of seronegative individuals

^bParticularly in patients with severe immunosuppression

1 year irrespective of the regimen used, relapse being more common among untreated HIV coinfecting individuals. In recent years, intensified research efforts have led to significant changes in the therapeutic approach to VL. Despite this, treatment options for CL and ML are still poor.

Treatment of Visceral Leishmaniasis(VL) (see Table 2)

Until the 1990s, VL therapy was based on pentavalent antimonials, with amphotericin B deoxycholate and pentamidine as second-line drugs. In recent years, miltefosine, paromomycin, and, above all,

Leishmaniasis and HIV, Table 2 Options for treatment and secondary prophylaxis for visceral leishmaniasis in HIV-infected adults

Drug	Posology	Remarks
First episode ^a		
<i>First Line</i>		
Liposomal Amphotericin B	4 mg/kg/day days 1–5, 10, 17, 24, 31, and 38 (to achieve a total cumulative dose of 20 to 60 mg/kg body weight)	Monitoring for dose-dependent nephrotoxicity. Premedication with diphenhydramine, paracetamol, or limited doses of corticoids reduces the rate of infusional adverse events
<i>Alternative regimens</i>		
Amphotericin B lipid complex	3 mg/kg/day for 10 days iv	
Amphotericin B deoxycholate	0.7 mg/kg/day for 28 days (to achieve a total dose of 1.5 to 2.0 g)	
Miltefosine	150 mg/day (50 mg/8h) po 28 days po	
Liposomal Amphotericin B + Miltefosine	30 mg/kg body weight liposomal amphotericin B divided as 6 equal dose infusions iv, combined with 14 days of 50 mg/12h miltefosine po	Only observational data available in a study from India
Meglumine antimoniate	20 mg/kg/day for 28 days or im	More toxicity observed in HIV-infected individuals. Avoid if possible
Relapses		
Same regimens as for first episode	Higher doses and longer duration of treatment may be needed	Consider combined treatments, although level of evidence is low
Secondary prophylaxis		Maintain secondary prophylaxis until HIV-RNA is suppressed and CD4 T-cell count is above 200–350 cells/μL during 3–6 months. Combined treatments may also be considered
<i>First line</i>		
Liposomal amphotericin B	4 mg/kg every 21 days iv	
Amphotericin lipid complex	3 mg/kg every 21 days iv	
<i>Alternative regimens</i>		
Miltefosine	100 mg 2 times weekly po	
Pentavalent antimony	20 mg/kg every 28 days iv or im	
Pentamidine ^b	6 mg/kg/14–28 days iv	

^aParomomycin (an aminoglycoside) has been shown to be used successfully in a small number of HIV-negative visceral leishmaniasis patients in India. No efficacy data are available in HIV-infected patients

^bPentamidine is no longer recommended to treat primary visceral leishmaniasis. It is only an alternative regimen for secondary prophylaxis when other options are not available



liposomal amphotericin B have also been approved for VL treatment. These drugs largely increased the therapeutic options, but their efficacy varies geographically and not all products have been evaluated to the same degree in every region. In general terms, the best therapeutic option seems to be liposomal amphotericin B. Despite the level of evidence being lower, combination regimens have proven useful in many clinical conditions and may be the future of VL treatment, although more data are needed. In 2010 WHO published evidence-based recommendations for every geographical region, although the price and availability of each single drug may represent a challenge for VL treatment, especially in low-income countries. In 2016, the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH) updated the diagnosis and treatment guidelines (Aronson et al. 2016).

Immunocompromised patients have lower cure rates, higher mortality, and more relapses compared with HIV-negative patients. The side effects of anti-leishmanial drugs are more frequent and severe than in immunocompetent patients, and a full course of antimonials is generally considered too toxic for use in coinfecting patients. Based on a few clinical trials on efficacy and safety of anti-leishmanial treatment in HIV-infected individuals, liposomal amphotericin B is considered the drug of choice and is given in a total dose of 40 mg/kg (4 mg/kg on days 1–5, 10, 17, 24, 31 and 38) (Aronson et al. 2016; Iribarren et al. 2016). Despite this currently being the best treatment option, mortality and parasitological failure rates are still high at 7–12%, and up to 32%, respectively.

Amphotericin B deoxycholate (0.7 mg/day for 28 days) and amphotericin B lipid complex (3 mg/kg/day iv for 10 days) can be an alternative (Aronson et al. 2016; Iribarren et al. 2016). More recent data from an observational study in India have also proven that combined treatment with liposomal amphotericin B (L-amB) and miltefosine (30 mg/kg body weight intravenous L-amB divided as 6 equal dose infusions combined with 14 days of 100 mg/day oral miltefosine) may be useful as a first option for the treatment of VL in HIV-coinfecting patients

(Mahajan et al. 2015). Second-line treatment options for visceral leishmaniasis in HIV-coinfecting patients also include miltefosine and paromomycin. In the treatment of relapse, new courses of L-amB (at conventional or higher doses) or combined treatment can be administered. Limited data are available on the use of miltefosine for the treatment of VL relapses; in an observational study, miltefosine induced (an often transient) remission in 64% of patients (Marques et al. 2008).

Many VL patients are very ill when they are diagnosed. Dehydration, renal, and/or hepatic dysfunction, anemia, hypoproteinemia with ascites and edema, subsequent cardiac decompensation, and concurrent infections are seen as complications of VL. Supportive treatment is a milestone for treatment of VL, and all patients should be properly hydrated, have their hydroelectrolytes balanced, and should be given nutritional supplements. Many may need blood transfusions and treatment for concomitant infections such as tuberculosis, pneumonia, and diarrhea (Boelaert and Shundar 2014). Patients responding to treatment become afebrile in 4–5 days, and the other clinical symptoms and biological parameters slowly normalize. Complete regression of splenomegaly may take several months. An initial cure can be declared if there is clinical improvement at the end of treatment. If the patient is clinically well after 6 months of treatment, this can be taken as an indicator of definite cure.

Antiretroviral treatment has improved survival in HIV/*Leishmania* coinfection and prevents the development of clinical VL in asymptotically coinfecting patients. cART should be started as soon as possible (within 2 weeks) after starting specific *Leishmania* treatment and should not be stopped if already being taken. As noted below, after cART initiation, an immune reconstitution syndrome may be observed. Some preliminary *in vitro* data have demonstrated certain anti-leishmanial activity of the HIV protease inhibitors. Based on this, some authors postulate that the initial antiretroviral treatment in coinfecting patients should contain an HIV protease inhibitor (van Griensven et al. 2013), since it could improve

the initial response to *Leishmania* treatment and help in secondary prophylaxis to prevent relapse of visceral leishmaniasis. The use of HIV protease inhibitors for visceral leishmaniasis should, however, be further investigated in a clinical context.

Treatment of Cutaneous and Mucocutaneous Leishmaniasis

Few data are available on the efficacy of treatment for cutaneous, mucocutaneous, or diffuse cutaneous leishmaniasis in HIV coinfecting patients. On the basis of data in HIV-negative patients with cutaneous leishmaniasis and case reports in HIV coinfecting patients, first-line treatments include liposomal amphotericin B, and pentavalent antimony, by IV or IM route, for 3–4 weeks depending on the form of the disease and the clinical response. Nevertheless, pentavalent antimony was demonstrated to increase viral transcription and HIV replication in cultures of human peripheral blood mononuclear cells, raising concerns about its use in coinfecting patients. A first-line parenteral treatment should be used for mucocutaneous and disseminated cutaneous disease and for localized cutaneous disease caused by *L. braziliensis*, the species most likely to cause mucocutaneous disease. Potential second-line alternatives for cutaneous leishmaniasis include miltefosine, topical paromomycin, intralesional pentavalent antimony, and local heat therapy. However, the effectiveness of these modalities is dependent on the infecting species of *Leishmania*.

Secondary Prophylaxis/Maintenance Therapy

The high frequency of relapses is the most salient feature in the treatment of HIV/*Leishmania* coinfection. In a patient not on antiretroviral therapy, VL almost always relapses within 1 year, but this can be partially prevented with secondary prophylaxis regimens.

Numerous schemes have been proposed, although a definitive recommendation cannot be made because of lack of controlled data: monthly injections of antimonial, twice-monthly injections of L-amB, or of pentamidine, oral miltefosine twice weekly, daily allopurinol or itraconazole. Most data about secondary prophylaxis/maintenance therapy come from the

Mediterranean region. A randomized trial compared amphotericin lipid complex (3 mg/kg every 21 days) with no prophylaxis; this trial reported relapse rates of 50% versus 78%, respectively, after 1 year of follow-up. In retrospective studies, monthly pentavalent antimony or lipid formulations of amphotericin every 2–4 weeks were also associated with decreased relapse rates. Daily allopurinol, in a dose of 300 mg three times daily, used for maintenance therapy is less effective than monthly pentavalent antimony and is not recommended. Although no published data on efficacy are available, maintenance therapy might be offered in immunocompromised patients with cutaneous leishmaniasis with multiple relapses after adequate treatment (Masur et al. 2014).

Although data are insufficient to provide a strong evidence-based recommendation, discontinuation of secondary prophylaxis after successful treatment of leishmaniasis might be considered after a sustained (i.e., more than 3–6 months) viral suppression and an increase in the CD4⁺ count to levels greater than 200 or 350 cells/ μ L after initiation of cART (Masur et al. 2014). In coinfecting patients suffering multiple relapses, splenectomy may restore hematological parameters and reduce the need for blood transfusions, but it does not protect from relapses (Fig. 3).

Antiretroviral Therapy Initiation & IRIS

cART in HIV-*Leishmania* coinfecting patients is known to improve survival and decrease relapses after anti-*Leishmania* treatment cessation. There is no evidence-based recommendation about when to start treatment, but international guidelines recommend to start or to optimize HIV treatment as soon as patient can tolerate it (Masur et al. 2014; Iribarren et al. 2016).

Unmasking immune-reconstitution inflammatory syndrome (IRIS) is uncommon in the setting of HIV-*Leishmania* coinfection, although it has been reported in clinical cases, appearing between 2 weeks and 4–8 months after ART initiation. The most well-described form of IRIS is the post-Kala-azar dermal leishmaniasis (PKDL), but cases of disseminated visceral leishmaniasis and disseminated cutaneous leishmaniasis in the context of



Leishmaniasis and HIV, Fig. 3 Differential diagnosis of cutaneous and mucosal leishmaniasis. **(a)** Cutaneous leishmaniasis in an HIV negative patient. Infection acquired in an endemic area in the Amazon region. **(b)** Cutaneous ulcer due to disseminated histoplasmosis in a severely immunosuppressed HIV-infected patient. Histoplasmosis

in an important differential diagnosis. **(c)** Mucosal leishmaniasis in an HIV-negative patient. Infection was acquired in an endemic region in Brazil. **(d)** Mucosal involvement in a severely immunosuppressed HIV-infected patient due to disseminated histoplasmosis. Histoplasmosis in an important differential diagnosis

cART initiation have also been reported. Evidence supporting any specific clinical management for this condition is not available (Zijlstra 2014).

Prevention

Today no vaccine is yet available for use in humans, but candidate vaccines are in development for VL and CL (Pace 2014).

Primary prevention of leishmanial infection relies on reservoir host control in areas with zoonotic transmission; vector control activities, such as indoor residual spraying and/or insecticide-treated nets; and measures to decrease the transmission of infectious agents in IDUs, such as needle-exchange programs. The prevention of exposure to parasites of the *Leishmania* species should be based on canine health surveillance in regions where the disease is prevalent such as in

the Mediterranean basin, where *L. infantum* is predominant, and on avoiding exposure to dogs especially in the case of immunosuppressed patients. Measures to decrease the transmission of infectious agents in IDUs, such as the abovementioned needle-exchange programs, are also encouraged.

Primary prophylaxis is not indicated in HIV-infected *Leishmania*-seronegative individuals (Iribarren et al. 2016).

Conclusion

Although the introduction of cART has led to a decline in Leishmaniasis incidence in the Mediterranean region, the disease is still prevalent in other endemic areas. Efforts to improve

diagnostic tools (especially in monitoring responses to treatment and so reducing risk of relapses) and more clinical trials on treatment focusing on combination therapy in HIV-infected individuals in different endemic areas are needed in order to improve the management of this neglected parasitic disease.

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Long-Acting Nanoformulated Antiretroviral Therapy

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Definition

Antiretroviral therapy remains the gold standard for the treatment of human immunodeficiency virus infection. While having dramatic effects on disease morbidity and mortality, it demands life-long adherence. Secondary toxicities, viral mutation, comorbid disease, constitutional drug-associated symptoms, adherence of complex regimens “pill burdens,” and drug-drug interactions are further complicating limitations. Nanoformulated long-acting antiretroviral therapy with half-lives measured in week(s) to month (s) requires infrequent administration but with equivalent or superior therapeutic efficacy compared to standard drug regimens and has the potential to revolutionize current antiretroviral therapy. Cell-targeted nanoformulations provide yet another boost by improving drug delivery to

reservoirs and affecting opportunities for viral eradication.

Introduction

While antiretroviral therapy (ART) has significantly improved human immunodeficiency (HIV) infection-associated morbidity and mortality, it has failed to eradicate virus and is not a disease cure. While infected people can readily achieve virologic control and long productive lives as a result of ART interruption of the cycles of viral replication, ART does not affect latent or restricted infections. Such infections predominantly target naïve and long-lived memory CD4⁺ T cells and mononuclear phagocytes (MP; monocytes, tissue macrophages, microglia, and dendritic cells). Indeed, HIV infection is incurable due to its persistence in the central nervous system (CNS), lymphoid tissue (spleen, lymph nodes, and gut-associated lymphoid tissue (GALT)), bone marrow, and the genital tract. Integrated provirus contained within host cellular DNA currently is not readily removed and circumvents any effective host innate and adaptive immune responses. A number of recent studies have focused on therapies to affect viral latency by stimulating productive infection and immune clearance or by directing excising viral DNA. Limitations abound in the specificity and sensitivity of such approaches. For now, lifelong ART remains the gold standard for the treatment of HIV infection with the goals including maximally and durably suppression plasma HIV viral load, restoration and preservation of immunologic function, reduction of HIV-associated morbidity and mortality, and prevention of HIV transmission. Complicating such goals rests in medication compliance and its parallel development of viral mutations and resistance of ART. Natural elimination of chronically infected cells over time is also a limitation secondary to the long-lived nature of latently infected cells, and low level spreading viral infection that occurs as a consequence of limited antiretroviral drug (ARV) penetrance in reservoirs of infection poses yet an additional hurdle for any strategy seeking to

eliminate virus in its human host. Secondary toxicities also pose limitations. To the ends of increased ARV penetrance into cellular and anatomical reservoirs of HIV infection and reduction of longer-term viral persistence, improved ART compliance and pharmacokinetics and the ability to deliver ARV to subcellular action sites would not only improve therapeutic endpoints but also improve ARV delivery and positively affect viral eradication strategies. To such ends, long-acting nanoformulated antiretroviral therapy (nanoART) was developed. Long-acting nanoART has shown early success, in models of viral infection, to improve ARV biodistribution to anatomical reservoirs including those tissues with restricted drug access. While cellular reservoirs including, but not limited to, latently infected resting memory CD4⁺ T cells and long-lived MP are not eliminated, pharmacodynamics studies demonstrate improved viral clearance. With a half-life of restricted resting memory CD4⁺ T cells of 44 months and the unfortunate conclusion that over 60 years of ART would be required for the eradication (Pierson et al. 2000), long-acting nanoART provides the means to limit ongoing viral replication with the potential to improve both compliance and viral elimination strategies.

Nanotechnology, Nanomedicine, and Nanoformulation

The merger of improved ARVs with nanomedicine for drug delivery is a potential formidable combination. Newer integrase and non-nucleoside reverse transcriptase inhibitors (NNRTI) have shown greater therapeutic indices and drug half-lives. When nanotechnologies are added to allow for improved reservoir targeting, drugs can remain in protected subcellular compartments, and the drug's half-lives extend even further. Nanotechnology is the engineering and manufacturing of materials at the atomic and molecular scale. Nanomedicine, according to the National Institutes of Health (NIH), is the application of nanotechnology to disease treatment, diagnosis, monitoring, and control of biological system. Rational design of nanoformulations for

the delivery of different therapeutic agents including small molecule drugs, peptides or proteins, and genes is the most active research area in nanomedicine. Many reviews have been published to report the recent progress in the management of HIV infection that was achieved through the application of nanoformulation contributed by experienced and diligent scientists across the world (Edagwa et al. 2014). The particle sizes of these nanoformulations range from 1 to 1,000 nm, which give them unique physical, chemical, and biological properties that are distinct from those of bulk materials or single molecules. For example, nanosized particles can provide higher bioavailability of drugs because of the increased surface area followed by an increased dissolution rate of the drug compounds according to the Noyes-Whitney equation. Importantly, nanoparticles with smaller size and proper composition have the potential to cross the blood-brain barrier (BBB), delivering antiretroviral drugs to the brain, the anatomical reservoir of HIV.

Nanoformulations that have been used for the delivery of ARV can be roughly divided into four categories: drug suspension (nanosuspension), polymer-based nanoformulation, lipid-based nanoformulation, and inorganic nanoparticles.

Nanosuspensions, also known as drug suspension or nanocrystals, are submicron colloidal dispersions of pure particles of drug, which are stabilized by surfactants. It is the most promising type of nanoformulation that can be used for the development of long-acting nanoART. Compared to other nanoformulations, the key features of nanosuspensions are the minimal use of excipients and the industrial feasibility. The former advantage implies both high drug content and diminished excipient-related toxicity. For other nanoformulations, low drug loading, potential excipient-related toxicity, and manufacturing difficulties have severely constrained the clinical application. For example, liposome, the first generation of nanoformulation and one of the most successful drug delivery systems, was first described by Bangham and his co-workers in 1961, and the first liposomal pharmaceutical product, Doxil[®], received clinical approval in 1995. It took more than 30 years for the liposomal

pharmaceutical products to appear on the market. But it was less than 10 years for the first nanosuspension product, Rapamune[®], to be available on the market after the first patent application of nanosuspension filed in the beginning of the 1990s. Besides those two essential benefits that have been mentioned above, as one type of nanoformulation, the sizes of nanosuspensions are usually between 200 and 600 nm, which lead to enhanced dissolution, absorption, and bioavailability of drugs. This type of nanoformulation has already been widely used for water-insoluble drugs. But some hydrophilic drugs which undergo chemical modification to improve their hydrophobicity can also be manufactured using this technique. Besides that, scientists recently developed novel solid-in-oil nanosuspensions for hydrophilic drugs. Another prominent advantage of nanosuspension is the reduced injection volume as a result of nearly 100% drug loading, which is essential for intramuscular (IM) and subcutaneous (SC) administration, namely, depot injections, that allows the nanosuspension to release its active therapeutic agents in a consistent way over a long period of time to further improve the sustained release profile of the drugs. However, it can also be administrated through other routes like oral, topical, intravenous, ocular, and pulmonary routes.

Nanosuspension can be prepared by two basic methods, bottom-up technologies and top-down technologies. Bottom-up technology is an assembling method to form nanoparticles by precipitation. Top-down technology involves the particles size reduction of large particles, which comprises high-pressure homogenization and bead/pearl milling methods. However, the combination techniques, pretreatment followed by a size reduction step, are also being employed. The ingredients used in the formulation of nanosuspension include crystalline-active therapeutic agents along with stabilizers (surfactants, polymers), tonicity agents (salts, sugars), cryoprotectants, and solvents.

Polymer-based nanoformulations use different polymers as the carriers for therapeutic agents. Nano-conjugates, nanoparticles, micelles, dendrimers, and polyplexes are important classes of polymer-based nanoformulations. The most widely studied polymers include natural polymer

such as carrageenan, chitosan and gelatin, and synthetic polymer including polyethylene glycol (PEG), poly(ethylene oxide) (PEO), polypropylene oxide (PPO), polycaprolactone (PCL), etc. The therapeutic agents can be conjugated to the polymers or entrapped by the polymers to improve the chemical, enzymatic, and metabolic stability of drugs.

Nanoparticles (Woodrow et al. 2009) with a size of less than 200 nm were developed using FDA-approved biodegradable and biocompatible polymers poly(lactic-co-glycolic acid) (PLGA). Over 1,000 siRNA molecules targeted against the gene encoding for mitogen-activated protein kinase (MAPK1) were encapsulated per particle using spermidine as a counterion. When applied topically to the vaginal mucosa, this nanoformulation led to efficient and sustained gene silencing for at least 14 days with less irritation and inflammation compared to siRNA lipoplexes. This study clearly demonstrated sustained release potential of PLGA nanoparticles that can be investigated for the control of sexually transmitted infections and prevention of HIV transmission.

Liposome and solid lipid nanoparticles (SLN) are two extensively explored classes of lipid-based nanoformulations. Numerous studies have used lipid-based nanoformulations containing lipids to enhance the oral bioavailability of some poorly water-soluble, highly lipophilic drugs. But these systems can also be easily modified for topical, pulmonary, or parenteral delivery. In fact, parenterally administered liposomes are naturally cleared by mononuclear phagocytic system (MPS), which can be employed for macrophage targeting, the cellular reservoirs of HIV, and then lymphatic systems such as lymph node, anatomical reservoirs of HIV. And SLN has been extensively studied because of its BBB crossing ability for targeting CNS besides lymphatic tissues, which is its prominent advantage among drug carriers. Both natural lipids obtained from plant or animal sources and synthetic phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), are used for the preparation of lipid-based nanoformulations.

SLN (Chattopadhyay et al. 2008) loaded with atazanavir (ATV) was formulated by a thin film

hydration technique. The mean particle size of a freshly prepared SLN was about 167 nm with high encapsulation efficiency. Uptake studies using a well-characterized human brain microvessel endothelial cell line hCMEC/D3 and [³H]-ATV demonstrated that there was a significantly higher uptake of ATV in hCMEC/D3 cells when it was delivered by SLN and no toxicity was observed up to a concentration corresponding to 200 nM of ATV. This study suggested that SLN could be a promising nanoformulation to enhance brain uptake of ARV.

Inorganic nanoparticles can be tailored to act as carriers for therapeutic or imaging agents. Silver, gold, magnetic, silica, and calcium nanoparticles have been evaluated for the treatment and prevention of HIV. They also can be used in the pathologic research of HIV infection. It was reported (Bade et al. 2015) that brain region-specific HIV-1-induced neuropathology in chronically HIV-1-infected NOD/scid-IL-2R γ ^{null} (NSG)-humanized mice can be detected by manganese (Mn²⁺)-enhanced magnetic resonance imaging (MEMRI).

Synthesis and Characterization of NanoART

A variety of nanosuspensions (Nowacek et al. 2011) were prepared for crystalline indinavir (IDV), ritonavir (RTV), ATV, and efavirenz (EFV) by wet milling. The influence of the physical characteristics of these formulations, including particle size, surfactant coating, and surface charge and shape, on the cell uptake, release, and antiretroviral efficacy was evaluated in a monocyte-derived macrophage (MDM) based *in vitro* testing system. It was concluded that drug type, surfactant coating, and shape demonstrated profound impact on particle uptake, drug release, and antiretroviral activity, while particle charge and size only had minor effects. For example, it was shown that long-rod-shaped nanoparticles were taken up more rapidly than short rods, and spherical particles were taken up even slowly than short rods.

Several different nanoformulations (Balkundi et al. 2011) were manufactured for crystalline

IDV, RTV, ATV, and EFV by three different methods, namely, wet milling, homogenization, and sonication using a variety of excipients, and the properties of these formulations were compared by using an established MDM scoring indicator system. The results showed that the manufacturing methods affected the physicochemical properties of the nanoformulations. The IDV nanosuspensions were the most diverse with sizes of 252 and 418 nm and charges of -40.6 and -15.1 mV, manufactured by wet milling and homogenization, respectively, and the IDV PLGA nanoparticles prepared by sonication had a size of 367 nm and a charge of -9.6 mV. Different preparation methods and excipients also affected the biological, immune, virological, and toxicological properties of these formulations demonstrated by mixed results from drug uptake, retention, release, and antiretroviral activity studies. For example, in *in vitro* cell uptake study, ATV concentration in cells after 8 h incubation was three- to fourfold lower following PLGA nanoparticles treatment than that for homogenized or milled particles. However, it is shown that the most significant difference that affects cell targeting and antiretroviral activity of nanoparticles was the drug itself. Based on those results, it was proposed that the clinical utility of nanosuspension might depend on the choice of drug and formulation composition.

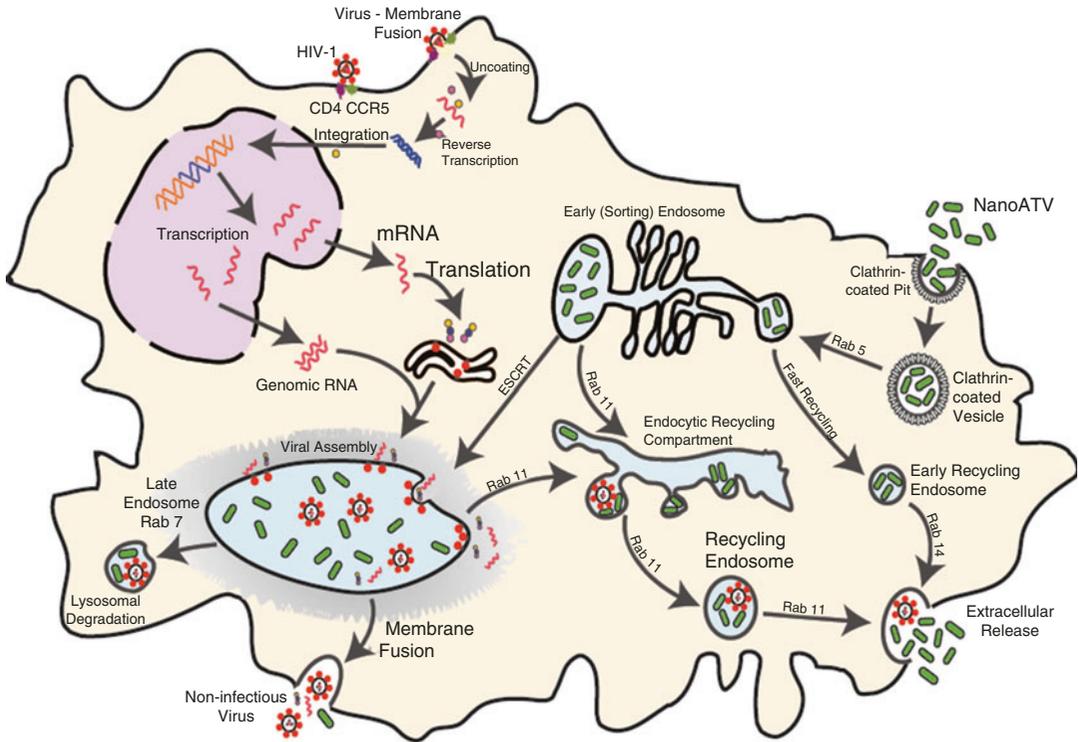
Subcellular ARV Delivery by NanoART

Intracellular trafficking (Kadiu et al. 2011) of RTV nanosuspension from the point of initial uptake to final release in MDM was investigated. It was now known that most nanoparticles taken up by MDM through clathrin-dependent endocytosis were sorted into a recycling pathway, from early endosome to recycling endosome, while bypassed lysosomal degradation, and drugs finally were released intactly from MDM without reduction in antiretroviral efficacy. siRNAs against Rab8, Rab11, Rab14, and brefeldin-A (BFA), a disruptor of recycling and secretory activities, were used to disrupt this pathway. Compared to untreated, siRNA scramble- and

Rab8 siRNA-treated MDMs, Rab11 and Rab14 siRNA-treated cells retained more drugs and released fewer drugs into the media. MDM treated with BFA showed similar impaired drug release, resulting in aggregation of nanoparticles in the perinuclear region. Proteomic and biological analysis suggested that particle recycling was primarily Rab11 regulated. Interestingly, it was shown that intact nanoparticles could be released from MDM with same size and shape as the original particles and maintained the antiretroviral activities. These results suggested the potential and advantages of targeting macrophage for cell-based drug delivery.

A further study (Guo et al. 2014) demonstrated that ATV nanosuspension (nanoATV) enhanced drug antiretroviral efficacy by improving the establishment of the subcellular drug depots and altering drugs distribution toward sites of active viral replication (Fig. 1). Late and recycling endosome compartments were pulled down by immunoaffinity chromatography with Rab-specific antibodies conjugated to magnetic beads, and ATV nanoparticles were seen in these subcellular compartments. It was discovered that nanoATV persisted in Rab7⁺ late endosomal compartments of HIV-1-infected MDMs, the site of active viral assembly, while most of nanoparticles are stored in Rab11⁺ recycling endosomes of uninfected MDMs. These results were cross validated by immunofluorescent staining of these compartments for confocal microscopy. Since infectious HIV-1 released from MDM are assembled in late endosomes, such colocalization between HIV and nanoATV in subcellular compartments might provide additional benefits in restricting viral replication. Thus it was reasoned that the action of nanoART could be amplified by directing nanoART to the late endosomal sites operative for viral assembly.

Quantitative SWATH-MS proteomics (Arainga et al. 2015) was applied to investigate cellular proteins that are deregulated by native ATV or nanoATV with or without HIV-1 infection in MDMs. The results showed that HIV-1 and nanoATV engaged endolysosomal trafficking for assembly and depot formation, respectively. Compared to virus, nanoATV oppositely regulated



Long-Acting Nanoformulated Antiretroviral Therapy, Fig. 1 Intracellular pathways of HIV-1 and nanoATV. NanoATV enters MDMs via clathrin-coated pits and is then transported to the early endosome. Then most of the nanoparticles are transported to the Rab11⁺ recycling endosome

in uninfected MDMs, while most of the nanoparticles persisted in Rab7⁺ late endosomal compartments in HIV-1-infected MDMs, the site of active viral assembly (Modified with permission from Guo et al. (2014))

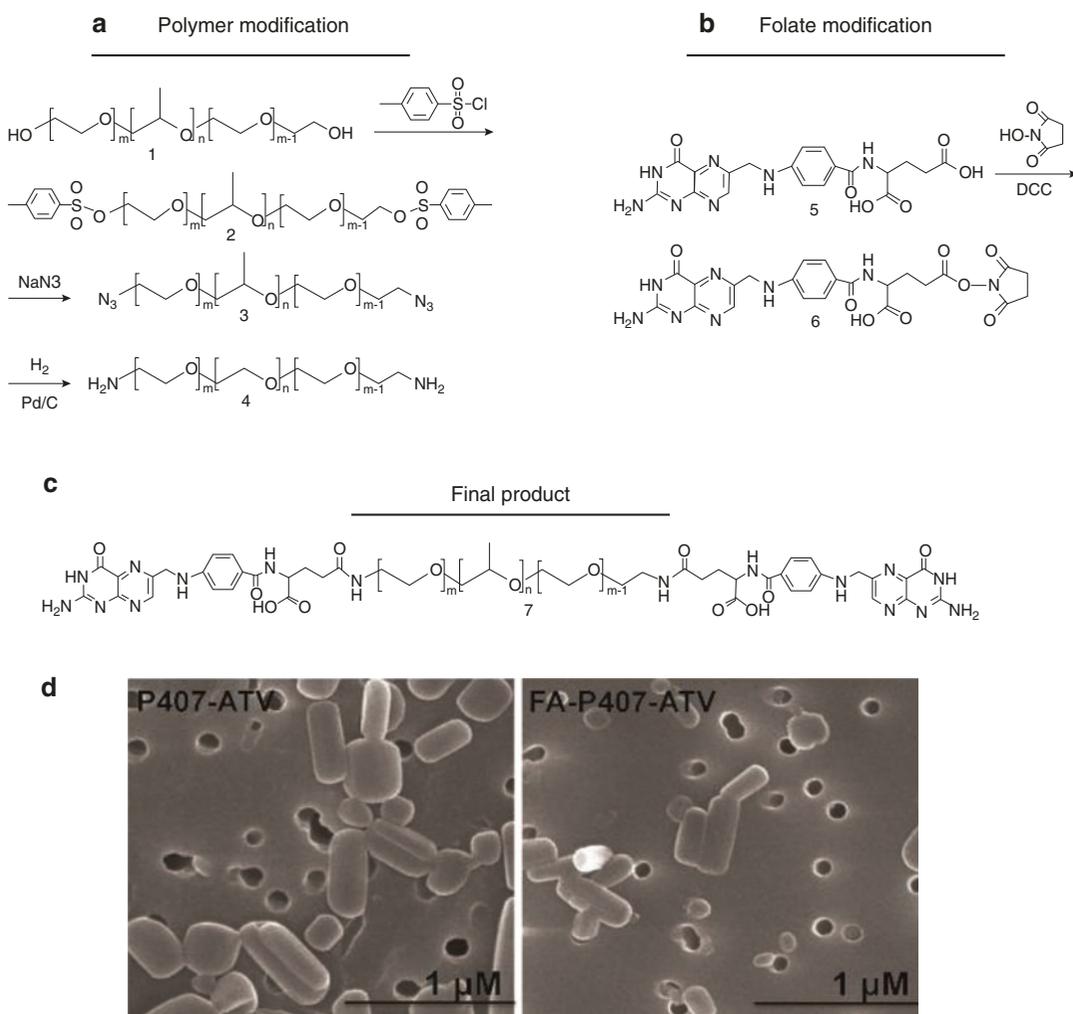
endolysosomal proteins. For example, in nanoATV-treated HIV-1-infected MDMs, Rab5 and Rab7 were downregulated, while upregulation of these proteins was observed in HIV-1-infected MDMs. DAVID and KEGG bioinformatics were used to analyze the proteomic data and the relationships between secretory, mobility, and phagocytic cell functions, and virus and particle trafficking was shown. In this study, a previously unknown mechanism for how long-acting nanoART provides a strategic advantage to combat viral infection was discovered.

Cell-Targeted NanoART

Macrophages, one of the cellular reservoirs of HIV, serve to disseminate virus and act as host cells for HIV infection and ongoing viral

replication. By definition, macrophage can be harnessed as a cell carrier for antiretroviral drugs, namely, cell-based drug delivery system. Because of its migratory function, macrophage can deliver drugs to areas of sustained viral growth and inflammation, which will otherwise be inaccessible due to either physical or biochemical barriers. Surface modification of nanoformulation can be employed to prepare cell-targeted nanoART for macrophage targeting, using ligands that will specifically bind to some surface receptors of macrophages and trigger the internalization of nanoparticles through phagocytosis or endocytosis. This strategy has been extensively studied in cancer treatment or other disease by targeting certain overexpressed receptors on the surface of target cells.

Based on published literature, it was proposed (Puligujja et al. 2013) that since activated



Long-Acting Nanoformulated Antiretroviral Therapy, Fig. 2 Synthesis of folate-conjugated poloxamer 407 (FA-P407). Schematic representation of the modifications for (a) poloxamer 407 and (b) folate and subsequent

reaction to yield (c) FA-P407. (d) Scanning electron micrographs of FA-P407-ATV and P407-ATV (Modified with permission from Puligujja et al. (2013))

macrophages express folate receptor 2 (FOLR2), its ligand folate-coated nanoART could facilitate macrophage uptake and increase cell retention of antiretroviral drugs. Folate-modified poloxamer 407 (FA-P407) was synthesized (Fig. 2), and free-base ATV nanosuspensions were prepared by high-pressure homogenization with or without using FA-P407. When MDM was treated with 100 μM FA-P407-ATV or P407-ATV, twofold greater cell uptake of nanoATV coated with 20% or 40% FA-P407 by MDM was observed, compared to nontargeted nanoATV. The antiretroviral

efficacy of targeted nanoATV was tested by first treating MDM with 100 μM FA-P407-ATV or P407-ATV for 8 h and then challenging treated MDM with HIV-1_{ADA} at 1, 5, 10, and 15 days later. FA-P407-ATV exhibited better antiretroviral activity than P407-ATV as HIV-1 replication was reduced by 87%, 80%, 82%, and 81% with FA-P407-ATV compared to 80%, 70%, 68%, and 64% with P407-ATV at days 1, 5, 10, and 15, respectively.

The pharmacokinetics, biodistribution, and pharmacodynamics of nanoART in vivo were

evaluated using Balb/cJ mice and HIV-1-infected hu-PBL-reconstituted NSG mice (Puligujja et al. 2015). The mice were injected with 50 or 100 mg/kg FA-coated or FA-uncoated nanoART, and the plasma and tissue ATV concentrations following FA-nanoART treatment were significantly higher than uncoated nanoART and were dose-dependent. The immune restoration after treatment represented by CD4:CD8 ratio was measured by flow cytometry. Compared to untreated controls, CD4:CD8 ratios were increased in tissues of HIV-1-infected mice. The antiretroviral activities of nanoART were also measured in the levels of HIV-1p24 antigen and HIV-1gag RNA, and the findings suggested that nanoART could effectively suppress viral replication. Interestingly, it was shown that the interaction between nanoART and macrophage increased the folate receptor expression, which further supported the rationale of using nanoART.

Limitations

Long-acting nanoART administered intramuscularly or subcutaneously has several disadvantages including limited injection volumes, painful nature of administration, considerable delay in onset of action compared to intravenous injection, and inability to withdraw drugs from circulation in the event of toxicity once the therapy starts. Additional limitations associate with the chemical and pharmacologic properties required for drugs, such as longer intrinsic half-life, higher protein binding, and satisfactory hydrophobicity capacities.

Ongoing and Future Perspective

GlaxoSmithKline is the leading pharmaceutical company for the development of long-acting nanoART for HIV treatment and prevention (Spreen et al. 2013). They developed a long-acting injectable nanosuspension that had 200 nm median particle size for cabotegravir (GSK1265744), an integrase inhibitor, by using wet milling technology. They reported that in the

phase I study of this nanoformulation, following single IM or SC injection, the mean plasma level concentration of cabotegravir remained well above the PA-IC₉₀ for about or longer than 24 weeks for dose of at least 200 mg. And cabotegravir was still detectable in plasma up to 48 weeks. A phase IIa clinical trial for the evaluation of the safety, tolerability, and acceptability of GSK1265744 long-acting injectable formulation (744 LA) in adult male subjects is ongoing. Long-acting injectable nanosuspension, called TMC278 LA, for rilpivirine (RPV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), was also developed (Baert 2009). The free base of RPV is a stable crystalline polymorphic drug substance with very low water solubility. Its nanosuspensions can be easily manufactured by wet milling technology. The average particle sizes of nanosuspensions were 200, 400, and 800 nm, and all of them were stable over 6 months. Following a single-dose administration, the plasma concentration profiles showed sustained release of RPV over 3 months in dogs and 3 weeks in mice. Compared to 400 and 800 nm nanosuspensions, 200 nm nanosuspension achieved higher and more stable plasma concentration profiles, acted as long-acting injectable nanoformulation. In a further preclinical investigation (Hoeben et al. 2010), 200 nm nanosuspension of TMC278 was administered in rats and dogs as single IM or SC injection. RPV showed sustained and dose-dependent release over 2 months in rats and over 6 months in dogs. In the IM injection groups, the RPV concentrations in the lymph nodes exceeded the plasma concentrations by over 100-fold 1 month after administration and then decreased to three- to sixfold of the plasma concentrations 3 months after administration. It was suggested that such observation was due to the nanoparticle uptake by macrophages and then the establishment of secondary depots in lymph nodes. In the phase I study of TMC278 LA, single 400 and 600 mg doses, administered subcutaneously around the umbilicus or intramuscularly in the gluteus maximus muscle, gave prolonged RPV plasma exposure of approximately 20 ng/mL after 8 weeks without serious adverse events. The

combination use of 744 LA and TMC278 LA was also clinically evaluated. In the phase I clinical trial, pharmacokinetics, safety, and tolerability for the combination use of these two long-acting nanoART were assessed in healthy subjects. 744 plasma concentrations exceeded the protein-adjusted IC_{90} , and RPV plasma concentrations were comparable to steady-state oral RPV formulation (25 mg/day), with grade 1 injection site reactions being most commonly reported and without grade 3 or 4 adverse events. A phase IIb clinical trial of the combination use of 744 LA and TMC278 LA is ongoing.

By the end of 2013, 35 million people are living with HIV, while 1.5 million died due to AIDS-related symptoms globally. The introduction of ART successfully shifted HIV infection from a deadly disease to a chronic illness. Poor adherence is among the major issues facing life-long ART. It was estimated that the average rates of nonadherence to ART range from 50% to 70%. Poor adherence does not just lead to the loss of virologic control; it may also contribute to the development of drug-resistant strains of HIV. Drug-resistant strains of HIV can also be transmitted, leaving newly infected or treatment-naïve patients with fewer treatment options. One means to address this issue is through long-acting nanoART. These require only infrequent dosing and may improve long-term therapeutic responses by avoiding missing doses or treatment fatigue. In the last decades, institutions and companies have devoted to the development of long-acting nanoART with the following characteristics: comparable antiviral efficacy with existing oral ART, high drug-loading capacities and suitable pharmacokinetic characteristics allowing infrequent dosing at a practical injection volume, no or minimal incremental toxicity related to the method of administration, existence of corresponding oral formulations facilitating treatment initiation and discontinuation, feasibility of industrial production, and desirable stability for the distribution and storage of drug products (Spreen et al. 2013). Several qualified nanoformulations are already under evaluation in clinical trials (Spreen et al. 2013; Baert 2009; Hoeben et al. 2010).

Overall, it was shown that targeted delivery of nanoART to macrophages potentially improves antiretroviral efficacy, since macrophages can store nanoART for longer periods of time and disseminate the cargos to HIV sanctuary sites because of its migratory nature. It is possible by maintaining the optimal antiretroviral drug concentration in HIV cellular and anatomical reservoirs (CNS, GALT, lymphoid tissues), and macrophage-targeted nanoART will achieve the goal of HIV eradication. The development of next-generation cell-targeted long-acting nanoART should work as follows: with the advancement of technologies in cell membrane isolation and mass spectrum-based proteomics, more cell surface markers of specific type of cells, such as macrophages, can be identified. Some of them involved in phagocytosis or endocytosis function can be served as potential targets for the development of innovative delivery system. Ligands with high affinity and specificity to the chosen receptors can be identified by using a variety of techniques including, but not limited to, phage display, enzyme-linked immunosorbent assay (ELISA)-like high-throughput screening (HTS) assay, or computational design. The final stage is the manufacture of the decorated nanoART with the newly identified ligands and the *in vitro* and *in vivo* evaluation of this nanoART.

Conclusion

In summary, the long-acting nanoART possessed tremendous potential in the management of HIV infection as described here. Some of them already advanced to clinical trials with hope of success. And the next-generation cell-targeted long-acting nanoART with improved antiretroviral efficacy and diminished toxicity is on its way.

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Long-Term Nonprogressors and Elite Controllers

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Definitions

Long-term nonprogressor (LTNP): a patient able to maintain a high CD4 T-cell count for unusually long periods of time in the absence of antiretroviral therapy (ART). This is an immunologic definition.

Elite controller (EC): a patient characterized by undetectable plasma viral loads for extended periods of time in the absence of ART. This is a virologic definition.

Posttreatment controller (PTC): a formerly viremic patient having received ART and in whom the viral load remains undetectable during a prolonged period after ART discontinuation.

In the absence of antiretroviral therapy (ART), HIV-1 infection in most patients is characterized by ongoing viral replication, progressive CD4 cell depletion, the development of AIDS, and death. Ever since the start of the AIDS pandemic, it has become clear that a small proportion of HIV-infected patients experience a mild course of infection. Patients with prolonged, AIDS-free survival in the absence of ART were termed long-term nonprogressors (LTNPs) (Gurdasani et al. 2014; Okulicz and Lambotte 2011). Once tests for viral load (VL) had become available, it became clear that (i) LTNPs were heterogeneous

and (ii) a subgroup was spontaneously able to control viral replication. At present, LTNPs are now usually defined as patients able to maintain a high CD4 T-cell count for unusually long periods of time in the absence of antiretroviral therapy. It should be noted that the VL is not taken into account in this definition. In contrast, elite controllers (ECs) are an uncommon subset of HIV-1-infected individuals with the ability to spontaneously control HIV loads for prolonged periods of time (Gurdasani et al. 2014; Okulicz and Lambotte 2011; Sáez-Cirión et al. 2012; Walker and Yu 2013). Again, it should be noted that the CD4 T-cell count is not taken into account in the definition of an EC. Although some patients are both LTNPs and ECs, the overlap is limited. Elite controllers have been extensively studied with a view to elucidating the mechanisms of spontaneous HIV control and thus facilitating the development of novel anti-HIV therapeutic approaches (including vaccines). However, there are probably several different mechanisms leading to virus control, and long-term outcomes in ECs have yet to be characterized. This entry will focus on the identification of ECs and the latter's epidemiological profile, clinical characteristics, and outcomes. It also gives an overview of the HIV control mechanisms that may be operating in these patients.

Recent observations have indicated that the initiation of a long-term course of treatment during the primary infection is associated with prolonged control of HIV viremia after ART withdrawal (Walker and Yu 2013). Accordingly, the few patients with lasting HIV control after ART withdrawal were referred to as posttreatment controllers (PTCs). These encouraging observations suggest that host responses can be manipulated and may lead to a functional cure.

The Differences Between Long-Term Nonprogressors and Elite Controllers

The first LTNPs were described in 1990–1995. Some were referred to as slow progressors. These patients are defined according to an immunologic parameter (the CD4 T-cell count). Various studies have shown that 2–15% of patients with

HIV can be classified as LTNPs (Okulicz and Lambotte 2011; Sáez-Cirión et al. 2012). Much research has since focused on determining the factors associated with nonprogression (Sáez-Cirión et al. 2012; Walker and Yu 2013). The presence of attenuated viruses such as the Nef-mutated viruses found in the Sydney cohort may explain some cases. Genetic factors (such as CCR5- Δ 32 polymorphisms and high frequency of B27 and B57 HLA class I alleles) have also been reported. In different groups of LTNPs, the immune response involves high titers of neutralizing antibodies, multifunctional, HIV-specific CD4 and CD8 T-cell responses, and the enhanced production of soluble antiviral factors. However, the data are heterogeneous, and a clear consensus on the most relevant mechanisms has not been reached.

This heterogeneity may be due (at least in part) to the fact that the exact definition of an LTNP (the CD4 T-cell count, the follow-up time, and the HIV load) differed from one study to another. These definitions were recently reviewed, and a common definition of an LTNP has been suggested: an asymptomatic individual infected for 8 or more years and with a CD4 T-cell count above 500 cells/ml in the absence of ART (Gurdasani et al. 2014). In fact, a large number of the individuals in LTNP cohorts eventually show a drop in their CD4 T-cell counts and progress to immunosuppression (Okulicz and Lambotte 2011). At present, most viremic LTNPs have received ART at some point. This explains probably the relatively low prevalence of LTNPs in recent studies (from 0.4% to 3.3%) (Okulicz and Lambotte 2011; Grabar et al. 2009).

A very small proportion of LTNPs are able to maintain low immune activation and asymptomatic infection despite an extremely high VL, a phenotype that resembles the nonpathogenic simian immunodeficiency virus infection in African nonhuman primates (Sáez-Cirión et al. 2012).

However, the LTNPs with high CD4 T-cell counts who remain asymptomatic are usually those with the lowest VLs. The availability of routine VL testing has made it possible to identify ECs (the definition of which is based on virologic criteria, in contrast to LTNPs). Since prolonged

virologic control (i.e., low VLs) is associated with elevated CD4 T-cell counts, there is nevertheless a limited degree of overlap between ECs and LTNPs. Two recent cohort studies have found between 4% and 12% of LTNPs meeting the criteria for ECs (Okulicz and Lambotte 2011; Grabar et al. 2009). This limited overlap strongly suggests that the determinants of viral control and CD4 T-cell homeostasis leading to slow progression phenotypes are distinct (Gurdasani et al. 2014). For instance, specific HLA class I alleles might be associated with the delayed loss of CD4 T cells but would not affect levels of HIV replication (Walker and Yu 2013) ► [Non pathogenic SIV infection of sooty Mangabeys](#).

Definitions of Elite Controllers

Elite controllers are typically defined by a series of VL test results below the limit of detection (in the absence of ART) (Gurdasani et al. 2014; Okulicz and Lambotte 2011; Sáez-Cirión et al. 2012; Walker and Yu 2013). These ECs have also been referred to as “elite suppressors,” “HIV controllers,” and “aviremic controllers” in the literature. A recent article reviewed all the definitions of extreme phenotypes related to HIV control and progression (Gurdasani et al. 2014). The definition of a “controller” has varied from one study to another, which may explain some of the differences in the patients’ immunologic and virologic characteristics. Three main parameters must be taken into account: (i) the period of sustained virologic suppression in the absence of ART, (ii) the VL threshold considered, and (iii) the allowance for some episodes of detectable VLs. These “blips” can be due to concomitant illnesses, vaccinations, variations in VL testing, or other unidentified factors. Most studies have used one of the three following definitions for ECs and/or controllers. Firstly, ECs are usually defined as having three or more VL determinations below the limit of assay detection (generally <50 copies/mL) over a period of at least 12 months in the absence of ART (Gurdasani et al. 2014; Walker and Yu 2013). Secondly, a stringent definition of an HIV controller is based on long follow-up data:

known HIV-1 infection for more than 10 years, with more than 90% of the VLs below the limit of detection (Gurdasani et al. 2014; Okulicz and Lambotte 2011; Sáez-Cirión and Pancino 2013). Patients fulfilling this criterion also fulfill the criterion for definition as an EC. The third definition is wider: HIV-infected individuals with at least three measurements of plasma HIV RNA (<2,000 copies/ml) over a period of at least 12 months in the absence of ART. This definition includes a separate group of patients with persistently low but detectable VLs in standard clinical assays (50–2,000 RNA copies/mL), termed viremic controllers. In the present review, ECs are defined according to the first and second definitions (Gurdasani et al. 2014; Walker and Yu 2013) ► [Viremic non progressors](#).

The Prevalence of Elite Controllers

Several studies have estimated the prevalence of ECs in the HIV-1-infected population. Transient control of viremia is not uncommon. For example, 6.7% of the patients in one study had two consecutive HIV RNA values below 500 copies/mL (Okulicz and Lambotte 2011). Prolonged control of viremia, however, is rare and generally occurs in less than 1% of patients. In several cohorts in France and in the USA, the reported prevalences range from 0.2% to 0.6% (Gurdasani et al. 2014; Okulicz and Lambotte 2011; Sáez-Cirión and Pancino 2013; Walker and Yu 2013).

Demographic Characteristics of Elite Controllers

The median age at HIV diagnosis is between 25 and 30, and the median age at enrolment is between 45 and 50 (Sáez-Cirión et al. 2012). These values highlight the long duration of HIV infection in ECs, with persistent, undetectable viremia and no disease progression for a median of 15–17 years of infection (Sáez-Cirión et al. 2012). Both male and female ECs are observed, but females seem to be more frequent in several cohorts (relative to age-matched general

populations of HIV-1-infected individuals). Studies of demographic characteristics have failed to reveal a preferential association with ethnic origin or the mode of HIV infection. Although there are few data on coinfection by other pathogens in ECs, at least some of these patients appear to be able to control or even clear hepatitis C infections (Okulicz and Lambotte 2011; Sáez-Cirión et al. 2012).

Similarly, there is little information on the natural history of HIV control post-seroconversion. However, several studies have shown that control occurs rapidly after seroconversion in most of these individuals. However, about a quarter of ECs require more than 3 years achieving control (Madec et al. 2013). The mechanisms influencing rapid control and delayed control are probably not the same.

The Mechanisms of HIV Control

The Virus

Although a range of HIV subtypes have been observed in ECs, subtype B appears to be over-represented. This may be due to the prevalence of subtype B in HIV-infected populations in the USA and Europe at the time when most ECs became infected with HIV.

Patients who become controllers may harbor viruses with reduced replication capacity during the early stages of infection; this is likely associated with transmitted or acquired cytotoxic T-cell escape mutations or transmitted drug resistance mutations (Walker and Yu 2013). However, the presence of defective HIV virus is not a common feature in ECs, since several studies have shown that replication-competent viruses can be recovered from CD4 T cells (Walker and Yu 2013; Sáez-Cirión and Pancino 2013). In a recent study, transmission of viruses from ECs to humanized mice confirmed the pathogenicity of these strains (Salgado et al. 2014).

In many ECs, ultrasensitive single-copy VL (usVL) assays can detect extremely low levels of viral replication in plasma. If usVL assays are repeated over time, the proportion of ECs with a detectable plasma usVL tend toward 100%, and

the level of viremia is often similar to that observed in ART-suppressed patients (Walker and Yu 2013; Sáez-Cirión and Pancino 2013). Although the origin of HIV RNA in the plasma of patients on ART remains elusive, continuous viral replication is present in ECs (albeit at extremely low levels). Indeed, the analysis of Gag and Nef sequences from the plasma of HLA-B*57- and/or HLA-B*27-positive ECs revealed a temporal evolution of the viral sequences over time (i.e., testifying to active viral replication) (Walker and Yu 2013; Sáez-Cirión and Pancino 2013; Bailey et al. 2006). Paradoxically, the virus is able to replicate but does so at low levels only.

Several explanations for this paradox have been put forward and are described in this entry. The immune system, especially the HIV-specific T-cell response, is involved. The fitness cost associated with some escape mutations (driven by the T-cell response) may limit viral replication. Indeed, various studies have suggested that B*57- and B*27-restricted CD8 T-cell responses target epitopes located within structurally important sequences of the virus, where variations would come at a cost to viral fitness (Walker and Yu 2013; Sáez-Cirión and Pancino 2013; Bailey et al. 2006). Viral restriction factors seem able also to limit viral replication (see below). These mechanisms could contribute to the small size of the HIV-1 reservoirs in ECs. ECs have much smaller amounts of HIV DNA in their peripheral blood mononuclear cells (PBMCs), when compared with other groups of HIV-infected patients (and especially patients on ART).

These data suggest that early, intense immune pressure affecting viral fitness (rather than the characteristics of the infecting virus per se) is a key factor in viral control in ECs. Other host factors also influence the control of viral replication. The observations of HIV-1 controller/chronic progressor transmission pairs (Walker and Yu 2013) provided strong evidence that host factors primarily explain elite control of HIV-1 replication.

The Genetic Characteristics of ECs

As in some LTNP cohorts, there is an overrepresentation of protective HLA alleles (HLA B57

and B27, which contain the Bw4 motif) in ECs (Walker and Yu 2013; Sáez-Cirión and Pancino 2013). These alleles are present in 45–86% of the patients, depending on the cohort in question. HLA B5701 is enriched in North American and European controller cohorts, whereas the HLA B5703 allele is enriched in African populations. Genome-wide association studies have confirmed this relationship; the single-nucleotide polymorphisms with the most significant associations were located close to the HLA-B and HLA-C genes (International HIV Controllers Study et al. 2010). The strong association with certain HLA class I alleles, the impact of the amino acid composition of the class I-binding groove on viral peptide-binding capacity, and HIV-1 control all point to the role of the immune response (and especially a specific cytotoxic T-cell (CTL) response) in ECs (Walker and Yu 2013; International HIV Controllers Study et al. 2010).

Other genetic associations have been evidenced in ECs. Killer-cell immunoglobulin-like receptors (KIRs) are expressed on natural killer (NK) cells and bind to HLA class I molecules. The KIR genes are polymorphic. Some genotypes of KIR3DL1 (KIR3DL1**h*/**y*) and KIR3DS1 are associated with better control of HIV-1 when present in patients harboring HLA-B alleles with Bw4 epitope specificity (namely, HLA-B*57/27/58) (Walker and Yu 2013). These data suggest that the mechanisms of protection in ECs bearing these alleles are due not only to CTL responses but also to NK cell responses.

However, it should be borne in mind that the HLA genetic associations as a whole only account for 19% of the variance in the host's viral control (Walker and Yu 2013).

Immune Responses

Adaptive Responses

CD8 T Cells The significant link between MHC class I loci and EC status indicates that an adaptive, MHC class I-restricted CD8 T-cell-mediated control is involved ► [HIV and SIV, CD8 T cell responses to](#).

A large proportion of ECs have strong HIV-specific CD8 T-cell responses (Walker and Yu 2013; Sáez-Cirión and Pancino 2013). The great magnitude of the HIV-specific CD8 T-cell response in ECs contrasts with the low response observed in patients receiving ART but who also have an undetectable VL. The detection of strong, HIV-specific CD8 T-cell responses in ECs could be related to the persistence of low-level viral replication described above. The frequency of HIV-specific CD8 T cells in these ECs is similar to that observed in viremic progressors.

However, the cells in ECs are qualitatively different from the functionally impaired, exhausted cells in viremic individuals. HIV-specific CD8 T cells in ECs have several specific properties, some of which may be surrogate markers of efficacy for HIV vaccines (Walker and Yu 2013; Sáez-Cirión and Pancino 2013; Migueles et al. 2008).

1. *Functions.* ECs have high numbers of multifunctional HIV-specific CD8 T cells, as defined by the ability to exert multiple effector functions (including the secretion of several cytokines, such as IFN- γ , TNF- α , MIP-1 β , CD107a, and interleukin (IL)-2) following antigenic stimulation. A major, unique characteristic of HIV-specific CD8 T cells in ECs is their ability to proliferate in response to antigen, rapidly upregulate granzyme and perforin, degranulate, and thus suppress HIV-1 infection of autologous CD4 T cells (Sáez-Cirión and Pancino 2013; Sáez-Cirión et al. 2007; Migueles et al. 2008). This ability to rapidly kill HIV-infected CD4 T cells in vitro is associated with high expression levels of the T-box transcription factor T-bet. There is a positive correlation between the frequency of Gag-specific CD8 T cells and the ability of CD8 T cells to block viral replication. The rapid elimination of infected cell targets by CD8 T cells is consistent with the higher numbers of differentiated effector CD8 T cells in ECs than in HIV-1 progressors. In ECs, HIV-specific CD8 T cells are activated. The frequency of CD38 + DR+ activated HIV-specific CD8 T cells is lower than in viremic progressors but higher than in healthy donors.

2. *Targets.* Strong responses to HIV Gag and (to a lesser extent) HIV Nef are predominant. This is important because Gag is one of the first viral antigens to be produced in infected cells. These data emphasize the involvement of an immune response targeting the first steps in the viral cycle.
3. *The T-cell receptor (TCR).* Gag-specific CTLs from ECs can mediate effective viral control because of their higher functional avidity (i.e., the ability to react to lower antigen concentrations and to recognize a wider range of epitope variants) relative to HIV progressors (International HIV Controllers Study et al. 2010). This point has been well evidenced in Gag-specific CD8 T cells in ECs bearing B27 and B57 HLA alleles. High avidity is associated with differences in the selection of epitope-specific TCR clonotypes during infection. When compared with TCR clonotypes in progressors, the high-avidity TCR clonotypes in ECs were better able to cross recognize naturally occurring viral variants and upregulate perforin and granzyme B (Walker and Yu 2013).

The selection and survival of these favorable TCR clonotypes might be due to several factors. The genetic background is likely to be especially important. Indeed, the peculiar amino acid composition of the protective HLA-B peptide-binding groove may favor optimal presentation of the viral peptide. HIV-specific CD8 T cells appear to cooperate more effectively with functional dendritic cells (Walker and Yu 2013). Indeed, myeloid dendritic cells (responsible for priming the T-specific response) have enhanced antigen-presenting capacities in ECs and do not produce the same set of cytokines as cells from progressors (Walker and Yu 2013). The low expression of negative immunomodulatory receptors (such as programmed death-1 (PD-1) and TIM3) may also favor the establishment and persistence of functional Gag-specific CD8 T cells (Walker and Yu 2013). The greater survival of HIV-specific CD8 T cells in ECs is also favored by the presence of functional central memory CD4 T cells (see below). The specific transcriptional profile of HIV-specific CD8 T cells of ECs also promotes

better survival. Upregulation of the genes involved in the heat shock response may regulate T-cell survival. In contrast, interferon-stimulated genes do not seem to be upregulated, as is also the case in models of nonpathogenic SIV infection in LTNPs and ECs (Peretz et al. 2012).

However, ECs are a heterogeneous population: high HIV-specific CD8 T-cell blood counts are not detected in about 50% of these individuals, and 20–60% of ECs lack protective B27 and B57 HLA alleles. However, two lines of evidence suggest that a potent HIV-specific CD8 T-cell response nevertheless has a role in some of these ECs. Firstly, multifunctional CD8 T-cell responses to HIV Gag are more intense in the rectal mucosa of ECs and are more closely correlated with controller status than peripheral blood responses are (Shacklett and Ferre 2011). Indeed, in some ECs, the majority of HIV-specific CD8 T cells appear to reside in the mucosa. Secondly, HIV-specific CD8 T cells with a virus-suppressing ability can be expanded upon in vitro stimulation with Gag peptides; this is consistent with the presence of HIV-specific CD8 T cells with a memory phenotype (Walker and Yu 2013) ► [HIV and SIV, CD4 T cell responses to](#).

CD4 T Cells The confirmed presence of functional HIV-specific CD8 T cells strongly suggests that HIV-specific CD4 T cells are likely to be present (Walker and Yu 2013; Sáez-Cirión and Pancino 2013; Porichis and Kaufmann 2011). This has been clearly demonstrated by the detection of multifunctional, Gag-specific CD4 T cells capable of producing many different cytokines in a high proportion of ECs. CD4 T cells in ECs share some characteristics with those found in patients on long-term ART. In the majority of ECs, the transcriptional profiles of CD4 T cells are similar to those in ART-treated patients but differ from those of healthy donors (except for a small subset). In both ECs and ART-treated patients, levels of DR+ activated CD4 T cells are higher than in healthy donors but are lower than in viremic subjects. It is known that CD4 T cells from both ECs and ART-treated patients can produce IL-2. Hence, these characteristics may merely reflect the achievement of viral control.

However, an increasing number of qualitative differences between HIV-specific CD4 T cells in controllers and progressors have been identified, and some of these differences are not fully corrected by viral control after the initiation of ART. This suggests that HIV-specific CD4 T cells from ECs have specific properties of relevance to HIV control and do not solely reflect low viremia. In ECs, central memory CD4 T (T_{CM}) cells are still present in the blood; they display high avidity for immunodominant Gag peptides and are capable of maintaining robust responses, despite the presence of low levels of viral antigen. Although the links between specific HLA Class II alleles and the characteristics of HIV-specific CD4 T-cell responses have yet to be investigated, the results of some studies suggest that ECs carrying the class II alleles HLA-DRB1*13 and/or HLA-DQB1*06 have the most robust HIV-specific CD4 T-cell responses (Shacklett and Ferre 2011).

It is known that there is attrition of the HIV-specific CD4 T-cell population in the gut mucosa in ART-treated subjects. This contrasts with the maintenance of a multifunctional response in ECs (Shacklett and Ferre 2011). Th17 cells (an IL-17-producing subset of CD4 T cells) have a major impact on the innate immune response and the regulation of inflammation in the mucosa. In HIV infection, depletion of these cells is associated with the dissemination of microbial products from the infected gut, increased systemic immune activation, and disease progression. In ECs, the blood Th17 count appears to be maintained (Hartigan-O'Connor et al. 2011).

In viremic patients, CD4 T-cell exhaustion is one of the mechanisms that lead to nonfunctional HIV-specific CD4 T-cell responses. This T-cell exhaustion is promoted by the expression of inhibitory receptors on CD4 T cells and the activation of inhibitory pathways. The CD4 T cells in ECs express lower levels of the negative immunoregulatory molecules PD-1 and cytotoxic T-cell-associated antigen 4 than the corresponding cells in ART-treated patients. This is consistent with the greater observed functionality of CD4 T cells in ECs. Regulatory T cells (Tregs) have also been linked to the suppression of antiviral

responses. Several researchers have reported that the Treg count in ECs is similar to or lower than that observed in uninfected individuals (Hartigan-O'Connor et al. 2011). This may contribute to the maintenance of high-quality, HIV-1-specific CD8 and CD4 T-cell responses in ECs. In addition, in contrast to the situation in ART-treated patients, HIV-specific CD4 T cells from ECs produce low amounts of IL-10 (a negative immunoregulatory cytokine). Another specific characteristic of CD4 T cells from ECs relates to their ability to maintain the production of IL-21. Several groups have shown that IL-21 secretion by HIV-specific CD4 T cells is higher in ECs than in individuals on ART, with the lowest levels detected in viremic subjects (Porichis and Kaufmann 2011). Interleukin-21 increases the expression of perforin, granzymes A and B, and the degranulation marker CD107 in HIV-specific CTLs – suggesting that HIV-specific IL-21⁺ CD4 T cells might help to control viral replication in humans. Interleukin-21 is produced by T follicular helper cells and has also an important role in B-cell differentiation (see below).

The survival of CD4 T cells is better in ECs than in other HIV-infected patients (Sáez-Cirión and Pancino 2013; Peretz et al. 2012; Porichis and Kaufmann 2011). T_{CM} cells and effector memory (T_{EM}) CD4 T cells from ECs are less susceptible to Fas- and TRAIL-mediated apoptosis and persist longer after multiple rounds of TCR triggering than cells from ART-treated patients. The survival of T_{CM} cells in ECs might be a consequence of inactivation of the FOXO3a pathway. CD4 T-cell homeostasis is also maintained because of optimal thymic output, homeostatic proliferation, and preserved lymphopoiesis (Walker and Yu 2013; Sáez-Cirión and Pancino 2013; Porichis and Kaufmann 2011).

B cells The extent of the humoral response's contribution to viral control in ECs is not clear because these individuals display a heterogeneous humoral immune response. Weakly reactive/partial Western blots have been reported for some ECs (Sáez-Cirión and Pancino 2013). The relatively low level of neutralizing antibodies in ECs emphasizes the positive correlation between viral

replication and the production of these antibodies. In contrast, antibody-dependent cell cytotoxicity (ADCC) and the antibody-dependent cell viral inhibition response (which are reportedly higher in ECs than in viremic patients) may have a role in virus control (Walker and Yu 2013; Sáez-Cirión and Pancino 2013). The higher ADCC levels found in B57-negative ECs require further investigation.

Innate Immunity

When HIV infects the host, an adaptive immune response is not present. During early infection, innate immune mechanisms may limit viral replication and enable the development of a strong, effective, HIV-specific CD4 and CD8 T-cell immune response. This hypothesis is supported by clinical data on the few early controllers described in European cohorts of primary infection. About 50% of patients who go on to become ECs will already have undetectable viremia when diagnosed during the primary infection (Madec et al. 2013). Both innate immune cells and cellular restriction factors might have a role in the context. Other evidences for a strong innate immune response relate to the fact that ECs appear to be able to spontaneously control other viral infections (such as hepatitis C virus) (Okulicz and Lambotte 2011).

Innate Immune Cells The data are limited: NK cells do not appear to have a major role in controlling HIV *ex vivo* – at least when ECs are studied years after the primary infection (Sáez-Cirión and Pancino 2013). In contrast, ECs maintain fully functional $\gamma\delta$ T-cell levels. This may well be one of the specific characteristics of ECs (Walker and Yu 2013). Dendritic cells also appear to be highly functional in ECs – in contrast to other groups of HIV-infected patients. The functional cooperation of myeloid dendritic cells with T cells has been described above. Plasmacytoid dendritic cells are present in ECs and are able to produce (i) normal amounts of IFN- α when exposed to HIV in contrast to viremic patients and (ii) higher amounts than healthy donors when exposed to influenza virus (Walker and Yu 2013).

Restriction Factors Innate immunity also relies on a number of intrinsic cellular antiviral defense mechanisms. In the setting of HIV infection, several proteins are involved in the restriction of HIV replication (including TRIM5a, APOBEC3G, tetherin, and SAMHD1). However, these proteins do not appear to have a special role in ECs. Some studies have failed to show reduced CD4 T-cell susceptibility to HIV. However, more recent studies of stringent HIV infection conditions clearly demonstrate reduced permissiveness to HIV-1 replication in CD4 T cells and monocytes/macrophages (Walker and Yu 2013; Sáez-Cirión and Pancino 2013). The mechanisms involved appear to be independent of the abovementioned restriction factors and might be related to the CDK inhibitor p21 pathway. Interestingly, the decreased susceptibility of CD4 T cells correlates with the level of viral DNA that these CD4 T cells carry *in vivo* – suggesting that the susceptibility may contribute to the decrease in the viral reservoir in ECs. After *ex vivo* superinfection of CD4 T cells from ECs, the low observed integration of HIV DNA indicates the presence of restriction factors that act on the HIV reservoir (Walker and Yu 2013). This finding implies that intrinsic cell resistance to infection may have an important role in controlling the size of the viral reservoir in ECs early in primary infection. Currently unidentified restriction factors may also have a role in limiting viral replication within latently infected CD4 T cells in ECs.

Long-Term Virologic Control and Clinical Outcomes

Elite controllers are heterogeneous. Viral “escape” appears to be rare in ECs and should prompt researchers to look for a superinfection (Okulicz and Lambotte 2011). In contrast, a slow, moderate decrease over time in the CD4 T-cell count is observed in most ECs. A few cases of severe CD4 T-cell depletion among ECs (below 350 cells/mm³ and even below 200 cells/mm³ in very rare cases) have been reported. AIDS events remain rare but have been described in a few ECS. The events are related to CD4 T-cell

criteria or to the development of hallmark features of AIDS (Okulicz and Lambotte 2011). Antiretroviral therapy is therefore prescribed in some ECs, although literature data are scarce. In a recent collaborative study of 34 ECs who had started combination ART, nearly all the patients recovered their CD4 T-cell counts. However, their gains were smaller than in patients with uncontrolled viremia at the start of therapy (Boufassa et al. 2014).

Several factors are involved in the decrease in the CD4 T-cell count ► **Chronic immune activation in HIV** (Sáez-Cirión and Pancino 2013). Low-grade HIV replication (with detectable “blips”) is associated with the CD4 T-cell decrease. Immune activation and chronic inflammation are probably also important factors. Despite controlled viremia in ECs, activation of blood CD4 and CD8 T-cell compartments is generally much the same as (or sometimes even greater than) those observed in ART-exposed patients. This activation is correlated with a decline in CD4 T-cell counts in these individuals. Blood levels of IP10 (a chemokine that mainly depends on the interferon pathways) are also higher in ECs than in healthy donors and are inversely correlated with the CD4 T-cell count. Hence, immune activation may be triggered by low-level residual HIV replication, an insufficient regulatory T-cell response and/or the translocation of bacterial products from the gut mucosa. Relative to noninfected individuals, ECs have higher blood levels of LPS and markers of monocyte activation (such as sCD14 and sCD163). These data recently prompted the performance of a pilot study in which ART was seen to be associated with a significant reduction in immune system activation in ECs (Walker and Yu 2013).

Immune activation and chronic inflammation might also cause the exhaustion of lymphopoiesis and impair the replenishment of the lost CD4 T cells. Alterations of the hematopoietic progenitor cells have been reported in ECs with decreasing CD4 T-cell counts (Sáez-Cirión and Pancino 2013).

The long-term consequences of this abnormal activation of the immune system in ECs have not been determined, although they possibly include

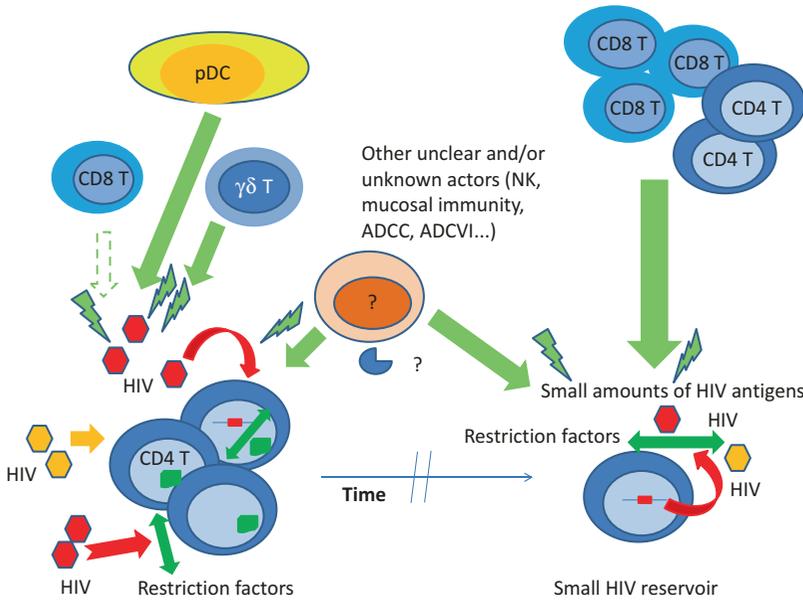
the loss of CD4 T cells and an increase in the cardiovascular risk. Given that ART reduces immune activation, ART might be useful in ECs. Indeed, the use of ART in ECs displaying a decrease in CD4 T-cell counts is now recommended in the official guidelines. The broader prescription of ART in ECs (i.e., those without a decrease in CD4 T-cell counts) remains subject to much debate ► **Post-treatment controllers**. The long-term clinical follow-up of ART-naïve ECs will be of paramount importance in identifying the long-term impact of HIV infection.

Posttreatment Controllers

In two recent studies (Walker and Yu 2013), viremic patients treated soon after the primary infection achieved an undetectable VL. The patients received ART for a median of 3 years and maintained an undetectable VL after treatment discontinuation (for a median period of 6 years). These PTCs do not seem to be ECs: they do not have a high frequency of HLA protective alleles, their CD8 T cells do not suppress HIV in CD4-CD8 T-cell cocultures, and their HIV-specific CD8 T-cell counts are low. However, PTCs have very low numbers of latently infected CD4 T cells (like ECs).

Conclusion

Although considerable progress has been made in understanding the factors and mechanisms associated with the spontaneous control of HIV-1 infection in ECs, many questions remain unanswered. Although ECs have specific transcriptomic, genomic, virologic, and immunologic features, they constitute a heterogeneous population. It is likely that spontaneous control of infection results from the concomitant operation of several of the mechanisms described above (Fig. 1). Although it is possible that several subgroups of HIC exist (depending on which mechanisms are involved in viral control), it is more likely that all the various mechanisms are interlinked and are regulated by factors such as residual viremia and immune activation. During



Long-Term Nonprogressors and Elite Controllers, Fig. 1 In primary infection, HIV infects only a limited number of CD4 T cells leading to a small HIV reservoir. Several factors contribute to control the extent of viral replication: HIV strains with reduced fitness (in orange), cell restriction factors limiting the infection of the target cells and the capacity of HIV to integrate, the innate immunity actors as $\gamma\delta$ T cells, plasmacytoid dendritic cells (pDC), and also the rapidly developing HIV-specific CD8 T cells. The limitation of viral replication allows the

development of functional HIV-specific CD4 and CD8 T-cell responses. In chronic infection, HLA class I-restricted HIV-specific CD8 and HIV-specific CD4 T cells are central components of HIV immune control. The CD8 selection pressure leads to reduced fitness of some HIV strains (in orange). Cell restriction factors still play a role in limiting viral replication in the long term. The role of many factors remains to be determined: NK cells, mucosal immunity (Th17 innate cells, cytokines), and humoral immunity especially ADCC and ADCVI

primary infection, cell restriction factors, innate immune components, and HIV strains with reduced fitness may all contribute to the small size of the HIV reservoir and the limited extent of viral replication. In PTCs, ART may have achieved the same result. A small viral reservoir may not only help to attenuate the dynamics of viral replication but may also favor the development and maturation of an optimal cellular immune response. In chronic infection, the combination of cell restriction factors and the pressure of functional HIV-specific CD8 and CD4 T-cell responses favor the achievement of viral control. However, residual viral replication is still present in ECs, most of whom show signs of mucosal damage, chronic immune activation, and inflammation. Although the maintenance of an optimally tuned immune response against HIV may have a cost, the latter is far outweighed by the benefits in the majority of ECs.

Studies of ECs have provided valuable information on vaccine strategies: limiting cell activation and obtaining high-avidity, anti-Gag HIV-specific CD4 and CD8 T cells appear to be important. The role of other factors (such as ADCC and the modulation of intrinsic restriction factors) requires further detailed investigation. Observations of PTCs are important because they show that the natural history of HIV infection can be modified by therapeutic intervention. Nevertheless, the underlying mechanisms remain to be characterized.

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Lung Cancer

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Definition

The term lung cancer is usually used to describe any of several types of primary tumors arising in the lung; common histological types include adenocarcinoma, squamous cell carcinoma, large cell carcinoma, bronchoalveolar cancer, and small-cell lung cancer. Taken together, lung cancer is the most common non-AIDS-defining malignancy in HIV-infected individuals. Lung cancer risk appears to be two to five times greater in the HIV-infected persons compared to the general population, and most epidemiological studies find that the risk remains high even after adjusting for other factors like smoking intensity and duration. Often these patients present with advanced disease at a younger age but may have

comparable survival compared with similarly matched lung cancer patients without HIV infection. Smoking cessation is the most effective preventive strategy.

Introduction

The advent of highly active antiretroviral therapy (HAART) (► [Antiretroviral Medications, Care and Treatment](#)), consisting of multiclass antiretroviral drug regimens, has dramatically decreased HIV-associated morbidity and mortality in the economically developed world. As HIV infection has transformed from a fatal disease to a chronic condition, there has been renewed public and clinical interest in long-term morbidities, including malignancies (► [Cancers Related to HIV](#)) that occur disproportionately within this population. HIV-infected individuals have an increased propensity to develop both AIDS-defining malignancies (ADM) (► [Overview: AIDS-Related and Non-AIDS Related Cancers](#)), such as Kaposi's sarcoma (KS) (► [Presentation and Pathogenesis of Kaposi Sarcoma](#)), non-Hodgkin lymphoma (NHL), and ► [cervical cancer](#), and a number of non-AIDS-defining malignancies (NADM) (► [Overview: AIDS-Related and Non-AIDS Related Cancers](#)), including lung cancer, ► [anal cancer](#), liver cancer, and ► [Hodgkin's lymphoma](#). Lung cancer is the most common NADM and the leading source of NADM mortality among HIV-infected individuals. Lung cancer risk appears to be two to five times greater in the HIV-infected persons compared to the general population, and most studies show that the risk remains high even after adjusting for other factors, such as smoking intensity and duration (Kirk et al. 2011). Often these patients present with advanced disease at a younger age and yet may have a comparable survival compared with stage- and histology-matched lung cancer patients without HIV infection. This entry briefly describes the epidemiology, risk factors, clinical characteristics, treatment, and outcome of HIV-associated lung cancer, as well as potential approaches to decrease the burden of lung cancer through smoking cessation initiatives.

Epidemiology

Epidemiologic studies of lung cancer among the HIV population commonly involve linkage of HIV/AIDS registries, observational databases, or clinical HIV cohorts to cancer registries. The risk of lung cancer is estimated by calculating the standardized incidence ratio (SIR), resulting in comparisons of observed cases occurring among an HIV-infected study population to the expected number of estimated cases from general population rates. These analyses have primarily been conducted in the United States and Europe. In the majority of the registry studies, SIRs are in the range of a two- to fivefold increase in lung cancer associated with HIV infection (Kirk et al. 2011). Two meta-analytic studies estimated the increased risk for lung cancer with HIV infection to be 2.6-fold (Grulich et al. 2007; Shiels et al. 2009). The risk of lung cancer in the setting of HIV infection appears to have remained relatively stable in the pre-HAART and HAART era.

Risk Factors

Smoking

Although there is a clear and consistent demonstration of elevated lung cancer risk among HIV-infected individuals compared with the general population, it is difficult to determine whether this elevated risk is due solely to the increased incidence of cigarette smoking in the HIV-infected population. Among HIV-infected individuals living in the United States, prevalence estimates of smoking range from 35% to 70% compared to approximately 20% in the general population (Kirk et al. 2011; Rahmanian et al. 2011). Tobacco use may be even more common among HIV-seropositive intravenous drug users. Five studies directly accounted for individual smoking exposure among HIV-infected persons, and the risk for lung cancer associated with HIV infection was 1.2–3.6-fold higher compared to epidemiologically valid comparison populations who were not infected with HIV (Kirk et al. 2011). In four of the studies, the smoking-adjusted increase attributable to HIV infection was

statistically significant, although in one it was not. In the largest cohort study in the HAART era, with individual level smoking data involving greater than 110,000 US military veterans, the incident rate of lung cancer was 1.7 times higher in the HIV-infected veterans compared to uninfected veterans and remained significant after multivariable adjustment (Sigel et al. 2012). The true test of an independent effect of HIV infection on lung cancer risk would be to observe the effects of HIV among nonsmokers. Such an analysis may not be feasible due to the very low incidence of lung cancer among nonsmokers and the high prevalence of smoking among HIV-infected persons (Kirk et al. 2011).

HIV-Related Factors

Unlike the prototypical ADMs, KS, and NHL, where the risk increases as immunosuppression becomes more pronounced, lung cancer may occur at any point in the course of HIV infection. The risk of HIV-associated lung cancer generally is not closely linked to a low CD4+ cell count or to an elevated HIV viral load, although there is evidence that prolonged moderate immunosuppression as seen in the HAART era may contribute to the risk. Organ transplant recipients on immunosuppressive agents have a lung cancer incidence rate comparable to HIV-infected patients (Grulich et al. 2007).

HIV itself might have a direct oncogenic role. Though limited experimental data suggest that HIV *tat* (trans-activator of transcription) (► [Tat Expression and Function](#)) gene product may modulate the expression of proto-oncogenes and tumor suppressor genes, amplification of HIV sequences in lung carcinoma has not been demonstrated. Other potential mechanisms for the role of HIV in the pathogenesis of lung cancer includes accelerated lung damage through recurrent pulmonary infections and increased susceptibility to tobacco carcinogens through genomic instability (Kirk et al. 2011; Mani et al. 2012).

Clinical Characteristics

Lung cancer typically is diagnosed a decade or more earlier among HIV-infected persons

compared to those without HIV infection (Pakkala and Ramalingam 2010; Mani et al. 2012). However, after adjustment for difference in the age compositions of populations at risk, the difference in the age at diagnosis of lung cancer (50 vs. 54 years) was relatively modest between persons with AIDS and the general population (Shiels et al. 2010). The majority of patients with HIV-associated lung cancer are symptomatic at diagnosis (Mani et al. 2012). This is often due to the advanced stage of disease by the time lung cancer is diagnosed. Respiratory complaints are particularly frequent and most notably include cough (40–86%), chest pain (25–75%), and dyspnea (10–57%). Fatigue is ubiquitous, and as many as 10–30% of patients also will have hemoptysis. The signs and symptoms of lung cancer among HIV-infected persons mirror those of stage-matched lung cancer controls.

Non-small-cell lung cancer (NSCLC) represents 67–86% of primary lung cancers in patients with HIV; small-cell lung cancer comprises another 6–14% with unidentified subtypes accounting for the remaining percentage (Pakkala and Ramalingam 2010). Of the NSCLC, the most common histologic diagnosis is adenocarcinoma (30–67%) followed by squamous cell (17–39%), large cell (3–16%), and bronchoalveolar (2–3%) cancer in HIV-positive patients. The majority of patients present with either advanced stage III or IV disease. Recent HAART-era case series and cohort studies with morphology and stage data have described similar distributions of morphologic type and stage at presentation in HIV-infected lung cancer patients compared with HIV-indeterminate or uninfected patients (D'jaen et al. 2010; Sigel et al. 2012).

Radiological features of lung cancer in HIV-infected patients appear to be similar to those in HIV-negative patients and include a parenchymal mass, more often peripheral rather than central, mediastinal lymphadenopathy, and pleural effusions. A low clinical suspicion for malignancy, particularly in younger patients, and overreliance on nondiagnostic chest radiographs may result in delayed diagnosis of lung cancer in HIV-positive individuals (Brock et al. 2006).

Treatment and Outcome

Recommendations for the management of HIV-associated lung cancer are evolving. Historically, individuals with HIV-associated lung cancer have been excluded from participating in ► [clinical trials](#) (Persad et al. 2008). Surgery with curative intent remains the treatment of choice for localized disease, and there is increasing experience in using of radiation therapy and systemic chemotherapy for patients who do not have surgical options.

Data on the management of HIV-associated lung cancer are principally derived from uncontrolled retrospective analyses rather than prospective clinical trials. In a recent study, HIV-infected NSCLC patients who underwent surgery with curative intent were more likely to have pulmonary and extrapulmonary postoperative complications, more rapid progression to disease recurrence, and poorer postoperative survival when compared to HIV-indeterminate patients (Hooker et al. 2012). HIV-infected lung cancer patients with CD4 + counts less than 200 cells/mm³ had shortened median survival compared with patients with higher counts. This finding runs contrary to prior studies that advocated surgery for HIV-infected patients regardless of immune status (Cadranet et al. 2006). However, the lack of precision inherent in a small sample size precludes drawing any strong recommendations from these studies.

In the general population, patients with advanced NSCLC usually are treated with a combination of a platinum drug (cisplatin or carboplatin) and a third-generation, non-platinum drug (docetaxel, gemcitabine, irinotecan, paclitaxel, pemetrexed, or vinorelbine), resulting in a slight increase in survival and relief of cancer-related symptoms. Strategies to further improve survival of patients include the addition of targeted drugs to cytotoxic chemotherapy, such as epidermal growth factor receptor or anti-vascular endothelial growth factor monoclonal antibodies and receptor protein kinase inhibitors, and pursuing maintenance therapy with pemetrexed after first-line chemotherapy (Makinson et al. 2011). There are potential drug-drug interactions

and cumulative toxicity when HAART is combined with systemic chemotherapy (Rudek et al. 2011). Etoposide, taxanes, vinca alkaloids, and anilinoquinazolines erlotinib and gefitinib are metabolized by cytochrome P450. All protease inhibitors inhibit CYP4503A4, but ritonavir, even in low doses, is the most potent inhibitor in the class. Also, ritonavir inhibits the P-glycoprotein efflux pump protein, driving chemotherapeutic agents like vinca alkaloids and taxanes outside the tumor cells. Protease inhibitors may be associated with a greater incidence of grade 4 hematological toxicity when used in conjunction with the aforementioned chemotherapy regimens and, in the case of nelfinavir and lopinavir, a heightened risk of diarrhea (Makinson et al. 2011). The nucleoside analog zidovudine can exacerbate myelosuppression, while other drugs in this class (stavudine, zalcitabine, and didanosine) may aggravate peripheral neuropathy associated with cisplatin and taxane derivatives (Rudek et al. 2011).

In the HIV-infected population with NSCLC, the factors associated with increased survival include Eastern Cooperative Oncology Group Performance status less than 2, HAART during chemotherapy, and CD4+ count >200 cells/μl at NSCLC diagnosis (Lavole et al. 2009; Makinson et al. 2011). Initial studies of patients with HIV and lung cancer implied that they may have a shortened survival compared to HIV-negative lung cancer controls. During 1996 to 2000, 24-month survival with lung cancer was only 10% among people with AIDS, compared to 31% in the general population in New York (Biggar et al. 2005). In Italy during 1999–2006, the risk of death among patients with lung cancer and AIDS was sixfold higher when compared to the general population (Zucchetto et al. 2010). Recent studies in the HAART era have yielded variable results. In a retrospective study of HIV-infected lung cancer patients collected from several clinics with experience in taking care of this population, the median survival for those with advanced cancer was 9 months and was comparable to the median survival of Surveillance, Epidemiology, and End Results (SEER) lung cancer participants (D'jaen et al. 2010). A recent analysis of HIV-infected and HIV-negative lung cancer

patients collected from a SEER database between the years 2000 and 2005 showed no significant difference in clinical outcomes (Rengan et al. 2012). In addition, survival after curative resection in early stage patients was similar in HIV-infected individuals and uninfected controls. These data suggest that HIV status should not be the most important determinant in therapeutic decision-making in NSCLC.

Prevention

Smoking Cessation

As there are no unique clinical practice guidelines to implement smoking cessation efforts among HIV-positive persons, evidence-based treatments with demonstrated efficacy in the general population must be incorporated into the care of HIV-positive smokers.

There are a number of barriers and complicating factors that compromise the success of smoking cessation in the HIV-infected individuals. Smokers are more likely to be abusers of alcohol and illicit drugs, and tobacco use may increase when persons are under the influence of these substances; substance abuse (Substance use) also can be a risk factor for smoking cessation failure. HIV-infected individuals may have a number of psychological stresses and mental health challenges that contribute to smoking and make cessation more difficult. Some HIV-infected individuals may use tobacco to manage HIV-related symptoms and pain and perceive smoking as one way to cope with the stress of living with a difficult illness (Rahmanian et al. 2011; Lifson and Lando 2012). In addition, HIV-infected individuals may feel that they will ultimately die from AIDS, making smoking cessation less of a priority.

US Public Health Service guidelines recommend brief individual smoking cessation counseling with five components (known as the “5 As”) at each clinical encounter. Providers are advised to systematically *ask* about tobacco use, *advise* smokers to quit, *assess* willingness to quit, *assist* with quitting, and *arrange* follow-up. Smokers’ telephone quit lines are cost-effective

interventions with broad reach and demonstrated efficacy for long-term smoking cessation. Motivational interviewing is effective in increasing quit attempts. Cognitive behavioral interventions are additional strategies to help smokers quit or reduce cigarette smoking. These interventions are designed to modify critical cognitions and actions that maintain behaviors such as smoking by promoting the thoughts and skills necessary to create behavioral change. Given the widespread availability of cell phones throughout the world, delivery of interventions through cell phones may be feasible even in many resource-limited settings. When cell phone counseling is used in conjunction with nicotine replacement therapy, HIV-infected smokers were 3.6 times more likely to quit than those who received usual care with physician advice, written materials, and nicotine replacement therapy (Lifson and Lando 2012).

Medications approved by the US Food and Drug Administration for smoking cessation include nicotine replacement therapy (patch, lozenges, inhalers, gum, and nasal spray), bupropion, and varenicline. There are no known interactions between nicotine replacement therapy and HAART. Bupropion is metabolized by the hepatic cytochrome P450 CYP2B6 system, and its metabolism has been shown to be inhibited by protease inhibitors (nelfinavir, ritonavir) and a non-nucleoside reverse transcriptase inhibitor (efavirenz) in vitro. Short-term ritonavir administration does not significantly alter bupropion pharmacokinetics in healthy volunteers, and no medication-associated adverse events were observed in a case series of 10 HIV-positive persons using either ritonavir, nelfinavir, or efavirenz with bupropion (Nahvi and Cooperman 2009). The results and tolerance recorded for varenicline in HIV-positive individuals are similar to those published in relation to seronegative patients, and no drug interactions have been described to date with HAART and varenicline. Both bupropion and varenicline are associated with significant neuropsychiatric symptoms, including behavioral changes, hostility, agitation, depressed mood, suicidal ideation, and attempted suicide. These symptoms have occurred in patients without preexisting psychiatric illness.

Despite the high prevalence of smoking and significant barriers to quitting among those with HIV, 30–60% of HIV-positive smokers are contemplating quitting or preparing to quit smoking, and so the importance of stopping smoking needs to be an ever present message during our clinical encounters (Rahmanian et al. 2011; Lifson and Lando 2012).

Screening

Lung cancer screening is a rapidly evolving field that has the potential to significantly reduce the burden of lung cancer. Early randomized control trials in lung cancer screening evaluated chest radiography with or without sputum cytology and showed no reduction in lung cancer mortality.

The lack of a clear result from chest X-ray screening and the refinement of computerized tomographic (CT) scanning techniques led to the evaluation of CT scanning for lung cancer screening. The National Lung Screening Trial (NLST) compared the effects of low-dose helical CT and standard chest X-ray on lung cancer mortality. The randomized national trial involved more than 53,000 current and former US smokers between the ages of 55 and 74 years old who had at least a 30-pack per year smoking history. Among trial participants screened with low-dose helical CT, there were 20% fewer lung cancer deaths (National Lung Screening Trial Research Team et al. 2011). The possible disadvantages of helical CT include the cumulative effects of radiation from multiple CT scans, surgical and medical complications in patients who prove not to have lung cancer but who need additional testing to make that determination, and overdiagnosis from the detection of cancers that never would have become symptomatic.

At the time of this writing (August 2013), the American College of Chest Physicians, the American Society of Clinical Oncology, and the American Thoracic Society recommend screening for lung cancer using LDCT based primarily on results from NLST, with eligibility criteria that model closely on NLST (current or former smokers ages 55–74 years with a ≥ 30 pack-year

history of cigarette smoking and ≤ 15 years since quitting). The recommendations also stipulate that screening should be offered only in clinical settings similar to those in the trial. The US Preventive Services Task Force's final recommendations are pending, and its draft recommendation supports annual screening for lung cancer with LDCT in persons at high risk based on age and cumulative tobacco smoke exposure.

The high prevalence of infectious and inflammatory conditions in HIV-infected patients can generate suspicious findings and increase the false-positive rate, resulting in significant anxiety and expense. The NLST study population, while ethnically representative of the high-risk US population of smokers, was a highly motivated and primarily urban group that was screened at major medical centers. In part for these reasons, the results may not accurately predict the effects of recommending low-dose helical CT scanning for other populations, including HIV-infected individuals. Also, other lung pathologies are more common in HIV-infected individuals, and these may yield more false-positive scans and make it particularly hard to apply the results of the NLST population to HIV-infected patients. Additional studies are needed to address this issue, perhaps by modeling the findings using studies of helical CT scans in an HIV-infected population.

Conclusion

Lung cancer risk is about two to five times greater in HIV-infected persons than in the general population, even after adjusting for smoking intensity and duration. HIV-associated lung cancer typically is diagnosed a decade or more earlier among HIV-infected persons. Adenocarcinoma is the most common histological type, and the majority of patients are diagnosed with locally advanced or metastatic disease (stage IIIB or IV). As pulmonary infections are common among HIV-infected individuals, clinicians may not suspect lung cancer in this patient population, and this may lead to a delay in the cancer diagnosis. Surgery with curative intent remains the treatment of choice for localized disease, and there is

increasing experience in using radiation therapy and systemic chemotherapy for patients who do not have surgical options. Though retrospective studies conducted during the pre- and early HAART era reported shorter survival times among HIV-lung cancer patients than HIV-indeterminate or negative lung cancer patients, in the most recent of studies performed in the HAART era, these two groups appear to have comparable survival times. Screening with low-dose CT scans is not yet routine in this high-risk population. As smoking plays a significant role in the development of HIV-associated lung cancer, people with HIV should be reminded of the hazards of smoking and encouraged to stop. Smoking cessation strategies with demonstrated efficacy in the general population should when possible be routinely incorporated into the care of HIV-positive smokers.

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Lymphocyte Apoptosis

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Definition

Decades ago, when cell death was observed in cells or tissues, it was assumed that the cause was either toxic or traumatic and that there was little cellular control over whether a given cell or tissue would undergo death in response to a given stimulus. Consequently naïve terms such as cytotoxicity or necrosis were used. In the intervening years much has been learned, and it is now recognized that cell death is a tightly coordinated event that can proceed through distinct processes of apoptosis, autophagy, and necrosis and on occasion through mechanisms which share features of more than one process such as necroptosis which has characteristics of both apoptosis and necrosis.

In parallel, much has been learned about how cell death occurs during HIV infection, which, at its most fundamental of levels, causes a disease of dysregulated cell death. For example, it is now understood that HIV infection of CD4 T cells causes the death of most of the cells which are infected, but those cells which resist death become a long lived reservoir for HIV. In addition HIV infection causes polyclonal activation of both immune and nonimmune cells, and this activation in turn favors death of those uninfected cells – resulting in bystander death of CD4 and CD8 T cells, B cells, neurons, hepatocytes, and other cell types. Moreover every HIV-encoded protein impacts the cell death/survival balance, often in a situational-dependent manner; for example, Tat can favor or induce resistance to apoptosis depending upon concentration and cell type.

Understanding how cell death pathways are altered in HIV infection is of direct clinical relevance, as numerous correlations exist between rates of cell death and rates of either CD4 decline or outcome from HIV infection. Indeed, study of patients with slower rates of HIV disease progression (LTNP and/or elite controllers) has illuminated novel insights into control of the immune system and suggests novel therapeutic strategies aimed towards curing HIV infection.

Cell Death and Apoptosis Regulation

Cell death plays a critical role in human development and tissue homeostasis, but when dysregulated, both excessive and insufficient cell death favor the development of disease. Cell death in humans occurs by one of three main pathways: autophagy, necrosis, or apoptosis. Accelerated CD4 T-cell death by apoptosis is an example of excessive cell death which contributes to the immunodeficiency seen during human immunodeficiency virus (HIV) infection.

Autophagy plays a role in both the innate and adaptive immune responses. Autophagy results when a double-membrane vesicle engulfs part of the cytoplasm becoming an autophagosome, which then fuses with a lysosome. The components of the autophagosome are broken down in the acidic environment of the lysosome. In HIV infection, single-stranded viral RNA triggers toll-like receptors (TLR), which recruit adaptor proteins such as myeloid differentiation factor 88 and toll-interleukin-1 receptor domain-containing adaptor-inducing interferon- β (TRIF) resulting in Beclin being sequestered away from Bcl-2 and induction of autophagy. Key morphologic characteristics which distinguish an autophagic cell are an accumulation of double-membrane-lined vacuoles and an absence of chromatin condensation.

Necrosis involves cell swelling and rupturing of the plasma membrane, resulting in an inflammatory response. Classically necrosis is a traumatic or complement mediated death; however, some forms of necrosis are tightly regulated and termed necroptosis. Death receptors and toll-like

receptors can induce necroptosis, and such death is dependent on receptor-interacting protein 1 (RIP1). For example, tumor necrosis factor (TNF) ligation of the TNF receptor 1 recruits TNF receptor-associated death domain (TRADD) and Fas-associated death domain (FADD), which in the presence of a caspase 8 inhibitor (i.e., cFLIP), RIP1 and RIP3 are phosphorylated, resulting in necrosis. When RIP1 activity is blocked by necrostatin 1, necroptosis is blocked. RIP3 knockout mice have less tissue necrosis and inflammation following vaccinia virus infection than WT mice. In the context of HIV, Vpr interacts with RIP1, and both HIV-infected and bystander CD4 T cells undergo necroptosis in certain contexts as discussed in greater detail below.

Both HIV-infected and uninfected T cells also die by apoptosis in an HIV-infected individual. Apoptosis is a mechanism of programmed cell death wherein the cell is broken down in a tightly regulated and stepwise manner. The term was first coined in 1972 phenotypically as involving nuclear condensation and membrane blebbing. Excessive apoptosis can lead to tissue atrophy, while insufficient apoptosis favors malignancy formation and/or progression. Apoptosis is most often induced via one of two major pathways, (1) the intrinsic or mitochondrial pathway or (2) the extrinsic or death receptor pathway. Neither pathway is exclusive, with cross talk existing between both.

Caspases are cysteine proteases that cleave proteins at aspartic acid residues. The first caspase was described in the 1990s as cleaving interleukin-1- β . Since then, ten human caspases have been described and have been found to contribute to cell proliferation, inflammation, and apoptosis. Caspases are synthesized as inactive zymogens with an N-terminal prodomain, a large subunit which contains the active cysteine, and a smaller subunit. The N-terminal prodomain is responsible for protein-protein interactions with adaptor proteins (discussed more in depth later) and functions to connect the death stimulus to the caspase. Once a stimulus is detected, two sequential cleavage events occur, first cleaving and freeing the smaller subunit, followed by cleavage and release of the larger subunit. The

larger and smaller subunits heterodimerize and form an active tetramer. For a protein to be cleaved, it must contain an aspartic acid with three specific preceding amino acids; not all proteins containing this recognition site will be cleaved, indicating that the tertiary or quaternary structure of the target protein might also play a role. Caspases can be classified as either an initiator caspase, which most often cleaves other apoptosis regulatory proteins, or as an effector caspase which typically cleaves structural proteins.

Initiator caspases (such as caspase 8) cleave and activate effector caspases such as caspase 3 and/or activate Bcl-2 family members such as BID. They are activated either by receptor clustering or by specific cofactors. For example, cytochrome c is required for activating caspase 9, a mechanism that is discussed further in depth later. In a healthy cell, caspase 9 is found in the cytosol, and cytochrome c is localized to the mitochondria; however, cytochrome c is released into the cytosol after a death stimulus and its release activates caspase 9. Active caspase 9 then cleaves and activates the effector caspases. The effector caspases are responsible for the phenotypic changes of apoptosis by cleaving a wide range of proteins including structural proteins actin and laminin and tumor suppressor proteins like PTEN and amplifying the apoptotic signaling cascade with cleavage/activation of additional caspases or Bcl-2 family members. Regulation of effector caspases involves specific inhibitors such as IAPs (inhibitors of apoptosis). One IAP, x-linked IAP (XIAP), specifically inhibits caspase 3 and 7 through direct interaction.

The Bcl-2 family is a group of proteins involved in apoptosis regulation involving the mitochondria. The first Bcl-2 family member, B-cell lymphoma 2 (Bcl-2), was identified in the middle of the 1980s and was originally being investigated for its role in oncogenesis. Ultimately Bcl-2 does not promote cell growth but protects the cell from death. To date, over 15 Bcl2 family members have been identified on the basis of containing one or more alpha helical Bcl-2 homology (BH) domains. Bcl-2 family members can be subdivided based on their functional activities

into three groups: antiapoptotic, proapoptotic, or BH3 only.

Antiapoptotic Bcl-2 family members are Bcl-2, Bcl-xL, Bcl-w, A1, Mcl-1, Bcl-RAMBO, Diva, and Bcl-G and inhibit cell death, by either (1) binding BH3-only proteins, sequestering them away from the proapoptotic family members, or (2) directly binding proapoptotic family members, inhibiting BH3 proteins from docking and activating them. Antiapoptotic family members contain a hydrophobic groove that allows proteins containing BH3 domains to bind.

The proapoptotic Bcl-2 family member subgroup consists of Bax, Bak, and Bok. There are two theories on how Bak and Bax are activated: (1) direct activation by a BH3-only protein or (2) indirect activation through inhibition of antagonists. Once activated, Bax and Bak can hetero- or homo-oligomerize to form pores in the outer mitochondrial membrane. Through these pores, inter-mitochondrial proteins are released into the cytosol and the cell is destined to die. While no crystal structure of this pore has been made, crystal structures of individual Bak and Bax show that each has a BH1, BH2, and BH3 domain that forms a hydrophobic groove which allows BH3-only proteins to bind and activate Bak and Bax.

The BH3-only subgroup of apoptosis-inducing proteins includes: Bid, Bim, Bad, BMF, Noxa, HRK, Puma, Bik, and MULE, which share a common BH3 domain, which in the presence of Bcl-2 paralogs forms an alpha helix that binds pro- or antiapoptotic family members via their BH3 grooves. BH3-only proteins induce apoptosis either by binding antiapoptotic Bcl-2 family members, resulting in the release of activator proteins like Bid or Bim that activate Bak or Bax. Alternatively, BH3-only proteins may sequester antiapoptotic Bcl-2 family proteins away from Bak and Bax, allowing Bax/Bak then to become active.

The intrinsic apoptosis pathway involves mitochondrial outer membrane permeabilization (MOMP). There are two channels proposed to open to result in MOMP, the first being mitochondrial permeability transition pore (PTP) composed of voltage-dependent anion channel (VDAC) and adenosine nucleotide transporter (ANT) and hexokinase which opens in response to apoptotic

stimuli. Another proposed pore involves oligomerization of Bak and/or Bax. Bak is expressed on the mitochondria, while Bax is cytosolic and only translocates to the mitochondria once a death stimuli is present. When Bax/Bak are oligomerized and inserted into the mitochondrial outer membrane, MOMP occurs, and the inner mitochondrial proteins, cytochrome c and Smac/DIABLO, are released into the cytosol, where in the presence of ATP and cytochrome c apoptotic protease activating factor 1 (APAF-1) oligomerizes and activates caspase 9 to form the "apoptosome." In the apoptosome, caspase 9 becomes active and cleaves downstream caspase 3 and 7, while Smac/DIABLO bind IAPs, derepressing the inhibition exerted by IAPs.

Death receptor ligation also initiates apoptosis as well as proliferation and differentiation. Death receptors are type II transmembrane proteins with a conserved 80 amino acid cytosolic region deemed the death domain. There are three classical death receptor classes: tumor necrosis factor receptors (TNFR), Fas/CD95 receptor, or TNF-related apoptosis-inducing ligand (TRAIL) receptors. Following receptor binding to its cognate ligand, TNF for TNFR receptors, Fas for CD95 receptors, or TRAIL for TRAIL receptors, the receptor trimerizes and recruits proteins which constitute the death-inducing signaling complex (DISC). For Fas and TRAIL receptors, the DISC is composed of Fas-associated death domain (FADD) which in turn recruits and activates procaspase 8 binds via its death-effector domain (DED). TNFR differs slightly from this because first TNFR-associated death domain (TRADD), then FADD, and procaspase 8 bind. In type I cells, active caspase 8 directly cleaves caspase 3, initiating the caspase cascade and the cell dies. In type II cells, active caspase 8 cleaves BID to its active form, tBID, and induces death via the mitochondrial pathway.

Associations of Apoptosis with HIV Disease Progression

One hallmark of HIV infection is a depletion of CD4 T cells over the course of disease. However,

observations over the past three decades have shown that not all HIV-infected individuals decrease the CD4 T-cell counts and progress to the acquired immune deficiency syndrome (AIDS) at the same rate. A subset of untreated HIV-infected individuals will maintain normal CD4 T-cell counts over periods longer than 7 years and are referred to as long-term non-progressor (LTNP). These patients also have a relatively low but detectable HIV viral burden in the lymphoid tissue and in the peripheral blood. It is estimated that 2–5% of people living with HIV are LTNPs. Even smaller subsets of individuals (<1%) infected with HIV are able to maintain undetectable levels of HIV replication without antiretroviral therapy, are categorized as elite controllers (EC), and maintain normal CD4 T-cell counts as a result of their excellent HIV control. There are mechanisms by which some human hosts rapidly decrease CD4 T-cell counts, while others have a very long, slow decrease in CD4 T-cell counts, and still others maintain normal CD4 T-cell counts which is unclear and an area of active investigation.

Although the mechanisms by which CD4 T cells die during HIV infection are likely multifactorial, it is clear that the CD4 T cells are dying by accelerated apoptosis. Furthermore, there are lower levels of spontaneous CD4 T-cell apoptosis in LTNPs compared to normal progressors (NP) (Gougeon et al. 1996). More specifically, caspase 9 and caspase 3 activity in CD4 T cells is higher and caspase 9/bcl-2 ratios are higher in progressors compared to LTNPs and noninfected individuals (Gougeon et al. 1996). When CD4 T cells from LTNPs, with a normal CD4 T-cell counts for over 7 years, were compared to AIDS patients, the LTNPs had a lower frequency of apoptotic CD4 and CD8 T cells. The T cells of LTNPs also had lower expression of Fas and Fas L and, therefore, were less susceptible to Fas-mediated apoptosis. The patients with AIDS in this cohort also had greater spontaneous mitochondrial depolarization and generated greater reactive oxygen species compared to LTNPs. Interestingly, CD4 T cells from LTNPs have similar levels of apoptosis despite higher levels of markers of immune activation than HIV-negative

individuals, suggesting that LTNPs may have a factor protecting their CD4 T cells from activation-induced cell death (AICD), in which activation of T-cell receptors results in cell death.

PD-1 is a cell-surface marker which belongs to the CD28 family and is expressed on activated CD4 and CD8 T cells. When PD-1 is bound by its ligands PD-L1 or PD-L2 during chronic viral infection, an immunosuppressive pathway is activated that suppresses T-cell function, including effector T-cell function, and leads to exhaustion of virus-specific CD8 T cells. LTNPs exhibit lower levels of PD-1 on HIV-specific CD8 T cells than progressors, suggesting that progressors have greater levels of HIV-specific T-cell exhaustion. Since blocking the PD-1/PD-L1 binding can reverse cognate T-cell exhaustion, there is great interest in therapeutically inhibiting PD-1/PD-L1 signaling. This work hints that LTNPs may have more functional anti-HIV defenses and are able to maintain preserved immune numbers as well as function.

Although most work has looked to the host for an explanation of slowed disease progression, some evidence suggests that the virus may also be responsible. The (H[F/S]RIG)₂ domain of the viral protein R (Vpr) of HIV induces apoptosis by binding to the adenine nucleotide translocator (ANT) in CD4 T cells. Viruses isolated from LTNPs have a higher frequency of the *vpr* R77Q mutation than viruses isolated from progressors (Lum et al. 2003). Pseudotyped HIV containing *vpr* R77Q infection of CD4 T cells in vitro resulted in less T-cell apoptosis than wild-type virus. In a cohort of 19 LTNPs followed over 6 years, seven of the patients were infected with viruses with the *vpr* R77Q mutation, whereas none of the progressors were infected with viruses carrying this mutation. Therefore, naturally occurring viral mutations may create viruses that are less able to induce T-cell apoptosis, resulting in a slowed disease progression.

Decreased T-cell apoptosis in LTNPs compared to progressors may also be due to alterations in T-cell regulation. CD4⁺ CD25⁺ regulatory T cells (Treg) suppress T-cell responses and maintain immune tolerance in a healthy state. Early investigations into the role of Tregs in the

pathogenesis of HIV disease suggests that numbers and function of Tregs may influence the state of immune activation during HIV infection, dysfunction of HIV-specific T cells, and other host-specific immune responses (Eggena et al. 2005). Treg numbers decrease significantly in progressors during the course of their HIV infection but are maintained at near-normal levels for LTNPs. The progressive decline in Treg cells correlated with elevated markers of immune activation and increased levels of spontaneous CD8 T-cell apoptosis *in vitro*. Antiretroviral therapy reversed this, increased Treg cell numbers and decreased immune activation and anti-CD3-induced CD8 T-cell apoptosis.

With mounting evidence suggesting that host factors dictate the progression of HIV disease, genetic differences between LTNPs and progressors have been sought. Genetic variants of cytokines that modulate HIV disease progression and HIV replication have been identified to differ between LTNPs and progressors. The CCR5 $\Delta 32$ allele, TNF- α -238A, and noncoding proteins associated with HLA-B*-5701 were positively associated with LTNPs in an Italian cohort of HIV-infected individuals. Array analysis revealed overexpression of genes in LTNPs involved in cytokine interactions and negative control of apoptosis, whereas progressors had overexpression of CD38 which is associated with immune activation and cell death. Furthermore, genome-wide transcriptome analysis of primary CD4 and CD8 T cells isolated from progressors and LTNPs with HIV infection was analyzed by Illumina microarray. A gene set enrichment analysis (GSEA) demonstrated several signal transduction pathways enriched in progressors, including tricarboxylic acid cycle and oxidative phosphorylation, which represent mitochondrial energy and metabolism pathways as well mitochondrial-mediated apoptosis. In contrast, LTNPs gene sets were enriched with cell survival pathways such as mitogen-activated protein kinase (MAPK), WNT, and protein kinase B (AKT) pathways. Collectively, these results give insight into differences in cell types, cytokine milieu, and intracellular signal transduction pathways which result in variances in levels of apoptosis between people who

have a normal progression of HIV disease and that of LTNPs.

The majority of ECs maintain a normal CD4 T-cell counts as a result of complete viral suppression, despite similar reductions in naïve T-cell counts as seen in progressors. The ECs, however, are distinguished from progressors by preserved thymic function and extra-thymic production of recent thymic emigrants. Furthermore, HIV-specific CD8 T cells from ECs are more resistant to apoptosis than similar cells from progressors, as measured by cleaved caspase 3 after the CD 8 T cells underwent HIV cognate peptide stimulation. This was associated with significant upregulation of Bcl-2 in HIV-specific CD8 T cells in EC compared to progressors.

Ten to 15% of HIV-infected individuals progress rapidly to AIDS within 1 year. These rapid progressors (RP) have an earlier emergence of CXCR4-utilizing strains of HIV, higher concentrations of IP-10, and single nucleotide polymorphisms in toll-like receptors and IL-7R α . However, the mechanistic basis by which these individuals have a more rapid progression of disease has remained unknown. In PBMCs from a large prospective cohort in China, there was a distinct cluster of genes expressed in RPs, unique from progressors that were associated with cell survival and apoptosis pathways; for example, 5 miRNAs were upregulated in RPs which impacted mitochondrial transport or the proapoptotic proteins Bid, Bmf, and Bim. This data suggests that the RP phenotype is impacted by proapoptotic factors.

The treatment for HIV replication and disease progression is antiretroviral therapy (ART). After HIV-infected patients are treated with effective ART, there is a dramatic decrease in plasma viremia, an increase in CD4 T-cell counts, and a decrease in spontaneous CD4 and CD8 T-cell apoptosis. However, about 1 in 10 patients on ART will maintain a paradoxically high CD4 T-cell counts despite ongoing viral replication. The percent of CD4 T-cell apoptosis in this unique population is significantly less than patients with virologic and immunologic ART failure (Meroni et al. 2002). The decreased CD4 T-cell apoptosis despite HIV replication may be due to intrinsic

antiapoptotic features of the ART. HIV protease inhibitors, in particular, can bind to the adenine nucleotide translocator protein in the mitochondrial transmembrane and prevent cytochrome c release, thereby preserving the mitochondrial transmembrane potential and blocking apoptosis. It remains unclear whether using ART with intrinsic antiapoptotic properties affects long-term clinical outcomes.

Taken together, this data demonstrates that a greater apoptotic response to HIV results in a rapid decline in CD4 T cells and rapid progression of disease; a host protective, antiapoptotic response to HIV results in normal CD4 T-cell counts and a slower disease course. Although a small-number people possess genetic protective factors that guard them from progressive HIV disease, alterations in apoptotic signaling may be a therapeutic approach to HIV infection in the majority of individuals with a normal disease progression.

HIV Infection of Lymphocytes

With the advent of highly active antiretroviral therapy (HAART) in the mid-1990s, the morbidity and mortality of HIV infection dramatically decreased, and now HIV-infected individuals have lived for almost two decades with normal CD4 T-cell counts and minimal spontaneous CD4 T-cell apoptosis as a consequence of suppressed viral replication. Nonetheless, stopping antiretroviral therapy (ART) still results in a viral rebound within 3–4 weeks (Finzi et al. 1997), due to persistence of HIV within a viral reservoir where HIV remains latent. There are several immunologic reservoirs for persistent virus, including resting CD4 T cells, macrophages, follicular dendritic cells, and hematopoietic stem cells. There are also anatomic sites for HIV to evade ART such as the central nervous system and the male urogenital tract. It is unclear whether HIV persists in these hidden sanctuaries despite effective ART because of episodic reactivation of the reservoir or because of ongoing low-level replication within the reservoir. Some evidence and mathematical modeling suggests that current ART is

capable of completely blocking even low-level replication within the reservoir, and therefore intensification of ART using newer agents might be capable of eventually eliminating HIV. However, if the reservoir is stably integrated latent virus, novel approaches will be needed to target the residual virus in order to eliminate HIV.

HIV latency occurs when HIV DNA is inserted into the host genome and remains transcriptionally silent, with up to 1 per 10^6 cells isolated from the peripheral blood containing an integrated and transcriptionally silent HIV provirus (Finzi et al. 1997; Chun et al. 1997). These integrated proviruses do not show viral evolution, suggesting that much of the reservoir is seeded early in infection and also suggesting that proviral integration serves as a survival feature for the retrovirus to maintain replication-competent virus over time.

It remains unclear how latency is established. The two predominant hypotheses are that HIV infects either (1) resting CD4 T cells or (2) activated CD4 T cells which escape death or revert to a resting state. De novo infection of resting CD4 T cells is difficult and inefficient compared to infection of actively replicating cells. There is in vitro evidence that HIV-infected actively proliferating cells are able to revert to a latent state with integrated proviral DNA after treatment with selective cytokines. Once in a resting state, these HIV-infected cells can reactivate to produce replication-competent virus. Since proliferating CD4 T cells contain latent HIV provirus, the two hypotheses remain: (1) HIV infects and integrates into actively proliferating cells and then cells create transition into a resting state or (2) HIV directly infects resting cells.

In order to fully understand proviral latency, it must be determined whether the mechanism of establishing latency is due to cellular factors (*trans* effects), viral genetic defects (*cis* effects), or viral and host factors (*cis/trans* effects). A number of proposed mechanisms involve *trans*-acting factors. Since HIV transcription depends on several steps beginning with the HIV transcription factors SP-1, NFAT, and NF-KB, it is possible that there may not be sufficient nuclear concentration of these factors to support active replication of the HIV. In addition, there is

evidence that negative regulatory transcription factors such as YY1 can bind the HIV LTR and increase the number of cells that become inactive, resulting in latently infected cells. Transcript elongation is also required for HIV replication; therefore, insufficient amounts of elongation factors, including positive elongation factor b, may limit HIV replication and promote latency. Studies of CD4 T-cell clones that have a single integration demonstrate that integration site location may influence whether the virus becomes latent or transcriptionally active. The clones with proviruses in the same chromosomal region had similar silent or inducible gene status. Lastly, micro-RNAs, which target certain gene sequences and degrade the viral RNA by the RNA interference pathway, may be upregulated and act to prevent HIV replication in certain cells, thereby promoting a latent state (Huang et al. 2007).

Both cis- and trans-acting factors affect chromatin at the HIV promoter. There are significant differences in the chromatin organization in transcriptionally active versus inactive HIV promoters notably by epigenetic silencing. In vitro work in transformed T-cell lines has demonstrated that HIV provirus can integrate into the heterochromatin of actively transcribing genes as well as quiescent genes. Nucleosome modifications and site of binding can limit the access of transcription factors to HIV. Furthermore, DNA methylation is found in a large number of latently HIV-infected cells and can also alter viral promoter sequences. These mechanisms suggest that treating latently infected cells with small-molecule inhibitors of the family of histone-modifying enzymes (HDACs) would alter the chromatin formation at the HIV promoter, allow the provirus better access to transcription factors, and possibly reactivate HIV out of latency. When resting CD4 T cells from virologically suppressed HIV-infected patients were treated with the HDAC inhibitor, vorinostat, there was an increase in biomarkers of acetylation and a 4.8-fold increase in HIV RNA expression (Archin et al. 2012), providing a proof of concept that HDAC inhibitors can reactivate latent HIV infection.

Another possible mechanism of latency occurs by cis-acting mechanisms or mutations

of the virus. Viral proteins are required for HIV replication, such as the HIV protein Tat which acts by binding to an RNA stem-loop structure, the trans-activating response element (TAR) of the 5' end of the transcript before initiating transcription elongation. By Tat binding to HIV, it activates transcription by creating a transcriptionally active elongation complex. A mutation in the TAR motif inhibits HIV transcription and results in HIV proviruses carrying this mutation remaining transcriptionally inactive in resting CD4 T cells. In order for this mechanism to be responsible for establishing the viral reservoir, the viral mutations would need to be minimal in order to allow for replication-competent virus upon reactivation.

Viral and host cellular (*cis* and *trans*) factors in combination may also be responsible for establishing HIV latency. As an example, since HIV prefers to integrate into transcriptionally active genes, when an upstream host gene is being transcribed, its transcription may obstruct the HIV 5'LTR transcription if both transcription factors are initiating in the same direction. Furthermore, the antisense strand of siRNA may affect histone methylation and result in gene silencing by chromatin remodeling of the HIV 5'LTR promoter. Whatever the mechanism of establishing the reservoir, silencing transcription and maintaining viral latency, it is clear that latently HIV-infected cells are protected from HIV-induced apoptosis and remain stable for months to years.

Although latently HIV-infected cells are resistant to apoptosis, most transcriptionally active cells produce virions and die as a result of the replicating virus. There are three predominant theories as to how infected cells die during HIV infection. Firstly, a body of work in CD4 T cells shows that during productive and acute infection, the HIV protein protease cleaves the host protein caspase 8, creating a unique protein fragment called Cas8p41. Once produced, Cas8p41 translocates to the mitochondria where it activates NF- κ B and induces mitochondrial permeability which leads to apoptosis of the infected cell (Nie et al. 2007). Since transcriptionally inactive HIV-infected cells would not be producing the

HIV protease protein, the latently infected cells would not die. Furthermore, short-term antigen stimulation of a CD4 T cell results in host procaspase 8 upregulation, whereas chronically stimulated cells downregulate procaspase 8, acquire a memory phenotype, and become resistant to apoptosis, and this may explain how an activated infected cell is transformed into a resting latently infected cell. Secondly, HIV proviral integration can cause death of cells prior to replicating virus. Viral integration into the host genome involves activating the DNA damage response, including DNA-dependent protein kinase (DNA-PK), which phosphorylates p53 and histone H2AX. Blocking HIV integration with the integrase inhibitor raltegravir or infecting cells with an integrase-deficient mutant virus abrogated cell death, whereas replenishing HIV Integrase restored cell death. Furthermore, inhibiting DNA-PK resulted in HIV-infected cells that did not die, although it is unclear whether these cells exhibited a transcriptionally inactive resting state. Thirdly, accumulated unintegrated HIV provirus transcripts can induce HIV-infected cell death. Resting HIV-infected CD4 T cells have full-length unintegrated viral DNA that is distinct from the genome-integrated proviral DNA. Replication of limited numbers of virus from a resting CD4 T cell would result in only a few viral DNA molecules and would not be sufficient to cause apoptosis, whereas prolific viral replication (as seen in an activated cell) would be adequate to cause infected cell apoptosis.

Once HIV infects a cell, it can integrate into the host genome, kill the cell, and/or shift into a resting latent state. What determines how, whether, or when any of these steps occurs remains unknown, although convincing arguments suggest that actively replicating infected cells can be transformed into a resting latent state. Moreover, the presence of viral proteins, induced during viral replication, may result in HIV-infected CD4 T-cell death. Understanding these processes will direct approaches to reactivating HIV out of a latent state or prevent it from ever entering a latent state and allow for HIV-infected cell apoptosis as a means of eradicating HIV infection.

HIV Infection of Myeloid Cells

Myeloid cells, including monocytes, dendritic cells, and macrophages, play important roles in the adaptive and innate immune responses. They are some of the first cells to be exposed to HIV in the mucus membranes, and since they express CD4 receptors and the necessary chemokine coreceptors, they are vulnerable to infection. However, these cells are resistant to HIV-induced apoptosis but can induce death of bystander, infected, and uninfected cells.

When a monocyte differentiates into a macrophage, it becomes apoptosis resistant. This phenotype can be attributed to altered regulation of death receptors and the mitochondrial apoptotic regulators. At the cell membrane in macrophages, cFLIP, the inhibitor of caspase 8, is upregulated while caspase 8 expression is downregulated. This allows molecules of cFLIP to bind and sequester caspase 8 away from FADD, resulting in less DISC formation and downstream activation of caspase 3, whose levels are also decreased in macrophages. In order to inhibit mitochondrial depolarization and apoptosis, macrophages upregulate Bcl-xL, thereby inhibiting oligomerization and activation of Bak. Downstream of the mitochondria in macrophages, XIAP, a protein that binds and inhibits caspases 9, 3, and 7 activation, is upregulated while caspase 9 expression is decreased.

HIV infection of myeloid cells also induces an apoptotic resistant phenotype. Monocytes from HIV-1 infected patients express a differential gene signature than T cells from these same individuals resulting in death ligand resistance (Giri et al. 2009). This may be due to HIV infection of monocytes and macrophages causing NF- κ B activation. NF- κ B affects multiple pathways but in macrophages allows the cells to become resistant to TNF-induced apoptosis. In addition, HIV-infected macrophages are resistant to TRAIL; the HIV glycoprotein gp120 stimulates the production of macrophage colony-stimulating factor (M-CSF) which downregulates TRAIL receptor 1 and upregulates Mcl-1. Similarly, the HIV protein Tat upregulates Bcl-2 miRNA and protein

levels in a dose-dependent manner (Zheng et al. 2007). Furthermore, Nef causes Bad phosphorylation, thereby inhibiting the proapoptotic BH3 protein from binding Bcl-2 or Bcl-xL and sequestering them away from Bak and Bax (Wolf et al. 2001). These events, paired with those that already make myeloid cells resistant to cell death, help to establish an HIV reservoir which is difficult to eliminate.

As HIV-infected myeloid cells become resistant to apoptotic stimuli, they develop the ability to induce death of bystander cells by upregulating death receptor ligands, possibly explaining why noninfected T cells undergo apoptosis in an HIV-infected patient. Nef and Tat recruit T cells to antigen-presenting macrophages, whereupon the death ligands expressed by the infected macrophages interact with death receptors on activated T cells, thereby causing T-cell death. Nef expression in macrophages increases the production of macrophage inflammatory protein-1 (MIP-1), which is a T-cell chemotactic. Tat expression in dendritic cells drives production of other chemoattractants, interferon gamma-induced protein 10, and monocyte chemoattractant protein 2, luring T cells and macrophages closer to the infected myeloid cell. Additionally, Tat expression in macrophages increases expression of TRAIL. TNF- α production on macrophages is increased by gp120 or HIV infection, and following interaction with TNFR2 has been proposed to cause death of CD8 T cells in HIV-infected individuals. Furthermore, Monocyte-derived macrophages from healthy donors express more FasL upon infection with HIV. Inhibiting the interaction of FasL with its receptor decreases death of bystander T cells killed by those infected macrophages. This increase of FasL, and subsequent induction of apoptosis in bystander T cells, is specific to monocyte-derived macrophages as opposed to monocyte-derived dendritic cells. While infected macrophages and dendritic cells may recruit T cells, it is due to the increase in death receptor ligand expression on macrophages that attributes to their death. Therefore, as cells are lured to the infected macrophage, they are exposed to the death-stimulating ligands, resulting in their death.

HIV Proteins and Apoptosis

A number of HIV proteins have been directly or indirectly associated with regulation of apoptosis, particularly Gp120, Vpr, Tat, Nef, integrase, and protease. The purpose of this section is to review the reported mechanisms associated with HIV protein regulation of apoptosis, focusing mainly on lymphocyte death. However, several limitations in the relevant literature should be noted first. The use of immortalized cell lines is common but difficult to interpret, since they often have abnormal apoptosis regulatory mechanisms a priori. Single protein overexpression systems may not accurately reflect true in vivo intracellular protein concentrations, which are by and large unknown. Single- or multiple-gene deficient viral constructs may not replicate normally, which is important in accurately modeling in vivo infection. Furthermore, few observations have been rigorously replicated, and even fewer have been conclusively demonstrated in in vivo infection, so the reported effects may be model and context dependent. What remains, though, is a likely picture of pleiotropic and overlapping effects of HIV proteins on apoptosis, suggesting a complex regulatory system with inherent redundancies highlighting the importance of apoptosis in the survival and propagation of the HIV virus.

Gp120

The HIV-1 envelope protein Gp120 has been shown in numerous models and cell types to induce apoptosis. Gp120 exists in both a membrane-bound form, expressed on virions and the cell surface of infected cells, and insoluble form, which is detectable in plasma and tissues in vivo. As Gp120 engagement with the CD4 receptor and associated coreceptors is necessary for viral entry, Gp120-induced apoptosis has been mostly described as contributing to uninfected, so-called bystander, cell death, through direct cell-to-cell contact, syncytium formation, or trans-signaling events. For instance, exposure to exogenous Gp120 can increase lymphocyte susceptibility to apoptosis through upregulation of death receptor expression, such as Fas and

TRAIL receptors 1 and 2, or downregulation of Bcl-2 expression. Gp120 also induces cells to express death ligands, including Fas and TNF- α (154). Gp120-induced proapoptotic signaling through cellular protein kinases results in activation of MTOR and (Castedo et al. 2001; Perfettini et al. 2004) as well as. Other reported proapoptotic effects of Gp120 include induction of cell cycle arrest and generation of reactive oxygen species. In vitro and in some instances mouse models confirm Gp120-induced cytotoxicity in many cell types, including neurons, cardiomyocytes, vascular endothelial cells, hepatocytes, renal tubular epithelial cells, and osteoblasts, implicating this protein in the pathogenesis in many non-AIDS-associated morbidities.

Vpr

The accessory viral protein Vpr, contained within mature virions, infected cells and secreted into the extracellular space, has been reported to have both pro- and antiapoptotic effects. The main function of Vpr is to induce G(2)/M cell cycle arrest in infected cells, and the off-target effects likely differ before and after that important event in the viral life cycle. Early, low-level expression of Vpr results in suppression of NF- κ B-dependent cytokine induction, decreased Bax, and increased Bcl-2 expression, rendering cells resistant to death receptor-mediated apoptosis. Coincident with the G(2)/M arrest, VPR induces expression of other antiapoptotic molecules, including surviving. On the other hand, later in the viral life cycle, Vpr can directly induce apoptosis through mitochondrial depolarization by binding either Bax or ANT and VDAC. Others have shown that intracellular expression of Vpr in renal tubular epithelial cells results in sustained ERK activation and activation of procaspase 8, although this has not been demonstrated in lymphocytes.

Vpr is one of the few HIV proteins where the reported apoptotic regulatory effects have a direct correlation in human infection. As discussed above, a naturally occurring polymorphism in Vpr (R77Q) is overrepresented in long-term non-progressing patients compared to normal progressors; furthermore, this mutation is associated

with decreased apoptosis induction by Vpr in vitro (Lum et al. 2003).

Tat

The HIV-1 transactivator protein, Tat, has been described to have a number of pro- and antiapoptotic effects. Produced in infected cells, Tat is secreted into the extracellular environment and enters uninfected bystander cells through clathrin-mediated endocytosis. Low concentrations of exogenous Tat are generally antiapoptotic and result in resistance to death receptor-induced apoptosis, associated with increased expression of Bcl-2 and cFLIP. In fact, the C terminus of Tat has been shown to directly interact with the Bcl-2 promoter and induce transcription. However, higher concentrations of Tat can induce apoptotic sensitivity through increasing expression of FasL (Westendorp et al. 1995), caspase 8, and Bax. Some of these effects may be dependent on Tat-mediated activation of p53 through inhibition of SIRT1. Endogenous Tat expression generally results in an antiapoptotic phenotype. Therefore, Tat may have a role in bystander killing. In fact, exogenous treatment of monocytes and macrophages with Tat results in increased surface expression of TRAIL, which can then participate in killing of neighboring uninfected cells.

Nef

The net effect of Nef expression in HIV-infected cells on apoptosis regulation is unclear, as a number of quite varied functions have been attributed to this accessory protein. Intracellular expression of Nef has been associated with increased Fas and FasL expression, increased PD-1 expression, and decreased expression of antiapoptotic Bcl-2 proteins. Nef has also been shown to cause lysosomal permeabilization, releasing cathepsin D into the cytosol and thereby initiating mitochondrial-dependent cell death (70). However, a number of antiapoptotic functions have been described as well. Through direct interactions, Nef can inhibit the activity of both ASK-1 and, indirectly inhibit the function of Bad (Wolf et al. 2001). More recently, it has been shown that intracellular expression of Nef results in secretion of the protein in exosomes, which can then contribute

to activation-induced cell death in uninfected bystander cells.

Protease

Although the primary substrate for HIV-1 protease is the Gag/Pol polyprotein, the acute and significant cellular toxicity associated with endogenous overexpression of HIV-1 protease suggests significant degenerate substrate specificity. In fact, HIV-1 protease has been shown to specifically cleave Bcl-2 between phenylalanine 112 and alanine 113, inactivating Bcl-2 and resulting in apoptosis of protease-expressing cells. We have shown that HIV-1 protease also specifically cleaves procaspase 8 between phenylalanines at positions 355 and 356 resulting in a pro-apoptotic fragment called Casp8p41 (Nie et al. 2007). Casp8p41 interacts with the mitochondria resulting in depolarization and apoptosis that is dependent on Bax/Bak (Sainski et al. 2011).

HIV-1 protease is the other HIV protein where there is specific evidence of importance in apoptosis regulation in human infection. Two mutations in HIV-1 protease, I54V and V82A, are overrepresented in infected patients who are failing antiretroviral therapy but have sustained elevations in CD4 T-cell counts, suggesting impairment in HIV-induced cell death. We have shown that these mutations also impair the ability of HIV-1 protease to cleave procaspase 8 to generate Casp8p41, whereas they do not impair the ability of protease to cleave the Gag/Pol polyprotein (Natesampillai et al. 2010). Furthermore, higher levels of Casp8p41 expression in memory CD4 T cells in HIV-infected patients are associated with lower CD4 T-cell counts.

Integrase

A recent study implicates the activities of HIV integrase in HIV-induced lymphocyte apoptosis prior to progeny virus replication, as apoptosis failed to occur in cells infected with integrase-deficient virus or treated with the integrase inhibitor raltegravir. DNA breakage, as a necessary event for HIV proviral integration into the host genome, initiates a cellular DNA damage response, characterized by activation of DNA-dependent protein kinase (DNA-PK). DNA-PK-

dependent phosphorylation and activation of p53 was necessary for HIV-induced apoptosis in that particular *in vitro* model. However, it is clear that a substantial portion of infected cells must survive this toxic DNA damage response in order to establish both latent and productive infection. This suggests that additional events in the post-integration phase of the viral life cycle, such as those described above, also contribute to the lymphocyte apoptosis seen during productive HIV infection.

HIV-Mediated Apoptosis of Nonimmune Cells

With the advent of HAART, people infected with HIV are living longer, have healthier lives, and are no longer suffering from opportunistic infections and cancers. For reasons that are not entirely clear, effectively treated HIV-infected individuals are now experiencing non-AIDS-related causes of morbidity and mortality at an accelerated rate (Deeks et al. 2013). Most of these are a result of end-organ diseases such as liver disease, kidney disease, and neurocognitive disease. Accumulating evidence suggests that HIV may directly or indirectly cause apoptosis of end-organ, non-immune cells, contributing to the disease of the organ.

Liver Disease

End-stage liver disease is one of the most common causes of hospitalized death of HIV-infected individuals in the United States. Because hepatocyte apoptosis will eventually lead to cell repair, inflammation, and ultimately fibrosis of the liver, understanding hepatocyte cell death during HIV infection is critical. Although liver disease is more common in HIV-infected individuals with viral coinfections such as hepatitis B and hepatitis C, there is evidence that HIV alone can cause liver damage. Early work has shown histologic damage to the liver during HIV infection and the presence of HIV p24 and HIV cDNA in liver tissue. Mechanistically, the X4 HIV gp120 can bind to CXCR4 receptors on human hepatocytes and induce a low level of transformed or primary hepatocyte

apoptosis, without infecting the cell, since the CD4 T-cell receptors are not expressed on non-immune cells. When the chemokine receptor-linked G_i protein was inhibited, HIV/CXCR4-mediated hepatocyte apoptosis was abrogated; however, caspase inhibition did not affect this low-level direct HIV-hepatocyte death. Thus, HIV proteins may be directly cytopathic to hepatocytes.

HIV causes chronic activation of the immune system, which contributes to the increased T-cell turnover and the pathogenesis of HIV. Therefore, it is possible that increased activation of intrahepatic circulating immune cells may contribute to liver damage and hepatocyte apoptosis. Liver biopsies from HIV/HBV-coinfected patients were compared to liver biopsies from HBV mono-infected patients and analyzed for markers of T cell, monocyte, natural killer cells, and hepatic stellate cell (HSC) activation before and after ART. Compared to HBV mono-infected samples, the HIV/HBV-coinfected samples had significantly increased hepatocyte apoptosis but fewer intrahepatic T cells, Kupffer cells, and HSC as well as lower levels of immune cell activation. These results were not altered by ART. Therefore, HIV-mediated liver disease appears to occur independent of the presence of immune cells or their activation state.

Tumor necrosis factor apoptosis-inducing ligand (TRAIL) and its receptors TRAIL-R1 and TRAIL-R2 are members of the tumor necrosis receptor (TNF) death receptor family. When TRAIL binds TRAIL-R1 or TRAIL-R2, a death-inducing signaling complex (DISC) forms which contains the death receptor and adaptor proteins which in turn signal a caspase-dependent cell death. HIV gp120 signals through CXCR4 on hepatocytes which upregulates TRAIL-R2 expression, making the previously TRAIL-resistant hepatocytes susceptible to TRAIL-mediated killing. Moreover, HCV upregulates TRAIL expression. Therefore, the accelerated hepatocyte apoptosis found during HIV/HCV coinfection might be due to a TRAIL-dependent death process. When HIV gp120 primed hepatocytes were co-incubated with hepatocytes expressing HCV core, there was selective

apoptosis in the HIV-primed hepatocytes which was inhibited by blocking TRAIL-R2. This was correlated with liver biopsy samples from HIV and HCV mono- or coinfecting patients which showed higher TRAIL expression in HCV-infected livers, greater TRAIL-R2 expression in HIV-infected livers, and higher levels of apoptosis in HIV/HCV coinfecting livers than either mono-infected liver. Additional work showed increased caspase 3/7 activity in HCV-infected hepatocyte cell lines after treatment with heat-inactivated HIV that was abrogated by pharmacologic caspase inhibition. These findings suggest that HIV-mediated hepatocyte apoptosis during states of increased TRAIL production, such as HCV coinfection, is due to a TRAIL-mediated apoptosis.

Kidney Disease

End-stage renal disease (ESRD) occurs in approximately 1% of HIV-infected individuals in the United States with 10–15% of those infected experiencing some form of renal disease. In fact, HIV-associated kidney disease is now the third leading cause of ESRD in African Americans in the United States. The majority of this kidney disease is due to HIV-associated nephropathy (HIVAN) which is characterized by focal glomerulosclerosis and tubulointerstitial lesions that occur with tubular epithelial cell injury as a result of apoptosis. HIV RNA has been isolated from human kidney samples; however, the mechanism of by which HIV causes apoptosis in the renal cells remains under investigation.

In one model of HIV-associated renal cell apoptosis, lentiviral expression of X4 HIV gp120 caused apoptosis of transformed human proximal tubular cell lines that was associated with an increase in Fas and FasL expression on the tubular cells, after HIV gp120 treatment. The HIV gp120/renal cell death was inhibited by pretreating the cells with antagonistic-FasL antibody or caspase 8 inhibitor. Additionally, soluble HIV gp120 causes renal tubular cell apoptosis that was inhibited by blocking gp120 binding to the cell and by pharmacologically inhibiting p38 kinase phosphorylation. Together, this work alludes to possible signaling mechanisms for the HIV

gp120-dependent renal tubular apoptosis, including signaling which occurs through the Fas/FasL and/or p38 signaling. Transcription of Fas and FasL is regulated by NF- κ B, yet NF- κ B also transcribes a number of genes that mediate immune and inflammatory processes. HIV transgenic mice with HIVAN had higher expression of the FasL promoter than transgenic mice with normal renal architecture. In addition, p65-containing (RelA) complexes were bound to the FasL promoter in HIVAN cells and NF- κ B decreased FasL RNA levels in the same cells. This data indicates that the HIV-mediated apoptosis of renal tubular cells can result from signaling through Fas/FasL binding and is controlled by NF- κ B transcription regulation.

In addition to HIV gp120, other HIV proteins have also been reported to signal renal cell apoptosis. HIV Vpr inhibits cell division of proximal tubule cells and induces renal tubular apoptosis. Human renal tubular epithelial cells (RTEC) undergo apoptosis after treatment with lentiviral vectors expressing HIV Vpr but not control lentiviruses, manifested by caspase 3/7 activation, PARP-1 cleavage, and mitochondrial injury. Vpr also induced ERK phosphorylation and activation, which can be associated with cell cycle arrest in certain cell types, as well as cleavage of BID to tBID and caspase 8 activation; all indicators of an extrinsic pathway apoptosis (Snyder et al. 2010). These results have been confirmed by evidence of tubular apoptosis, increased Fas levels, and ERK phosphorylation in human biopsy tissue from patients with HIVAN compared to HIV-infected individuals without kidney disease. With a better understanding of HIV-mediated apoptosis of renal tubular cells, interventions can be designed to prevent HIVAN and ESRD in HIV-infected individuals.

Neurologic Disease

HIV-infected individuals often experience neurocognitive decline which is referred to as HIV-associated neurocognitive disorders (HAND). Clinically, these neurocognitive findings can range from subclinical changes to HIV-associated dementia (HAD). Before effective ART, the prevalence of HAD was 20–30% in

some populations, but with HAART, the incidence is closer to 1%. However, less severe forms of HAND such as minor cognitive-motor disorder (MCMD) have been reported in as high as 37% of infected individuals even after ART. Autopsy specimens from HIV patients with neurologic changes have shown apoptosis of central nervous system neuronal and nonneuronal cells in excess of control specimens. This was associated with damage to synapses and death of hippocampal and basal ganglion neurons, suggesting that neuronal apoptosis may be contributing to HAND. The mechanism of HIV-mediated neuronal apoptosis is likely multifactorial with HIV directly and indirectly triggering neuronal damage.

Similar to HIV-induced liver and kidney disease, HIV-caused apoptosis in neurons is due in part to direct effects of HIV proteins (Singh 2004, p. 141). HIV gp120, in particular, induces neuronal apoptosis in cell culture, ex vivo in hippocampal slice preparations and in mouse models including direct stereotactic intracranial injection of HIV gp120 (Toggas et al. 1994). The apoptosis was dependent on surface expression of CXCR4 and protein kinase C activation. Because K⁺ channel blockade has prevented neuronal death in other models of apoptosis, and since HAND may be partially reversible with ART, the role of K⁺ channels in HIV-mediated neuronal death has been under investigation. Work thus far has demonstrated that treating cortical neurons with HIV gp120 resulted in enhancement of A-type transient outward K⁺ currents and eventually in neuronal apoptosis. It is unclear whether this occurred as a result of indirect activation of bystander glial cells which produced a soluble substance that signaled cell death or via direct mechanisms. Furthermore, prolonged HIV gp120 or SDF-1 α /CXCR4 binding also altered neuronal K⁺ channel efflux and induced apoptosis; however, in this rat model of neuronal damage, the channel effects were also blocked by p38 kinase inhibition.

In addition to HIV gp120, the HIV Tat protein also causes alterations in K⁺ channel efflux and neuronal apoptosis. Rat microglial cells treated with HIV Tat resulted in the release of TNF- α ,

IL-1 β , and ROS, suggesting a state of microglial activation, which was coincident with an enhanced outward K⁺ channel current. Altogether, this work suggests that controlling K⁺ channel activity may alter HIV-associated neuronal apoptosis.

Other indirect mechanisms of HIV-associated neuronal damage have been proposed that involved astrocytes which can maintain low levels of infection. Astrocyte infection with X4 HIV results in inositol triphosphate-regulated calcium release which signals apoptosis in neighboring astrocytes, but the HIV-infected astrocytes themselves did not die. In this system, HIV infection conferred a protective antiapoptotic state to the actively infected cells, caused surrounding bystander cell death, and enabled the astrocytes to serve as sanctuary for the HIV reservoir. The same may be true in other myeloid cells.

Altering Apoptosis Pathways and the HIV Cure Initiative

Over recent years, spurred by the report of one and possibly other HIV “cures,” much attention has been devoted to understanding ways in which HIV might be eradicated. Careful examination of how cure was affected in those cases may provide guidance as to how this might be recapitulated on a broader scale.

In the initial report, an HIV-infected patient with long-term antiretroviral-mediated HIV suppression was treated for AML with chemotherapy, followed by auto- then allogeneic bone marrow transplantation using a donor with CCR5delta 32 mutation. Several years following treatment there is no detectable replication-competent HIV despite cessation of antiretroviral therapy (Allers et al. 2011). There has been much speculation as to why HIV was cleared in this case, with the predominant theories being that either (i) the cytotoxic chemotherapy eradicated cells harboring HIV, and ARV prevented repopulation of the HIV reservoir, (ii) the transplanted allogeneic bone marrow mediated a graft versus HIV, (iii) the transplanted CCR5delta 32 cells precluded productive infection posttransplant, thereby

preventing reestablishment of the HIV reservoir, or (iv) a combination of these effects.

Considering these possibilities on a mechanistic basis, cytotoxic chemotherapy (in particular using the agents used in the Berlin patient – i.e., fludarabine, amsacrine, and ara-C with anti-Thymoglobulin (ATG) GVHD prophylaxis) causes the death of both cancerous and non-cancerous cells including lymphoid and myeloid subsets by induction of mitochondrial-dependent apoptosis in the case of the chemotherapeutics and by combination of complement-dependent lysis and caspase-mediated apoptosis in the case of ATG. Moreover recent evidence implicates TRAIL as a critical effector mechanism involved in graft versus tumor effects, raising the possibility that any graft versus HIV effect might be mediated by TRAIL as well. Furthermore TRAIL-mediated immune control by CD8 T cells has been described in the case of influenza infection (Brincks et al. 2008), suggesting that it may be important as well during HIV infection. As outlined above, TRAIL has also been implicated in the immunopathogenesis of HIV.

The concept that inducing apoptosis in cells from HIV-infected patients can cause eradication or at least reduction in viral burden is not new. Spurred by initial observations that inhibiting apoptosis in HIV-infected cells increase HIV replication (Chinnaiyan et al. 1997) and the corollary that inducing apoptosis by cytotoxic chemotherapy can reduce HIV replication, we tested the hypothesis that apoptosis induction in HIV-infected cells might reduce viral burden by killing those cells which produce the virus. Indeed, more than a decade ago we reported that exogenous treatment of cells from HIV-infected patients with recombinant TRAIL caused a reduction in viral burden, often to undetectable levels (Lum et al. 2001), without negative quantitative or qualitative effects on bystander cells. At the time this was an attractive and promising preclinical strategy as a variety of clinical grade compounds were being developed as therapy for a variety of cancers; however, in the interim the development of these compounds have suffered from weak delivery, the recognition of TRAIL-resistant cancers, and disappointing early phase clinical trial results.

Conclusion

Looking to the future, how can the premise that inducing apoptosis in HIV-infected cells reduce HIV burden be exploited towards the goal of achieving a cure for HIV?

- (i) **Apoptosis-inducing agents to reduce the burden of HIV-infected cells.** In the early 1990s there was interest in evaluating OKT3 (an apoptosis-inducing anti-CD3 antibody) in the setting of IL-2 to reactivate HIV expression along with antiretroviral therapy. This treatment resulted in decreased HIV RNA levels and undetectable HIV on viral outgrowth assays from CD4 T cells in a proportion of patients. This proof of concept study demonstrates the ability of anti-cellular therapies to impact HIV burden. It would be of interest to study the anti-HIV effect of other anti-cellular therapies – such as traditional chemotherapeutic agents. Alternately ATG which was used in the Berlin patient and which targets numerous epitopes including CD2, CD3, CD4, CD8, CD11a, CD28, and, CD45 might reduce cellular reservoirs of HIV occurring in both CD3 T cells and CD4⁺ monocytes/macrophages and DC. This is particularly appealing today given the superiority of current antiretroviral agents over those used in the 1990s, which may prevent repopulation of the viral reservoir during cytotoxic treatment.
- (ii) **Apoptosis sensitizers to enhance the likelihood of HIV dying in response to expressing HIV proteins.** Latently infected resting memory CD4 T cells are proving to be difficult to eliminate because they are resistant to the cytotoxic and proapoptotic effects of viral reactivation (Shan et al. 2012). In latently infected CD4 T cells which have been induced to reactivate virus, there are a wide variety of proapoptotic stimuli expressed intracellularly including Tat, Nef, Env, Vpr, and HIV protease. In the different settings of acute infection of an activated CD4 T cell, these stimuli are sufficient

to contribute to the death of infected cells. However, in the setting of HIV reactivation, these stimuli are insufficient to cause the death of cells which express these proteins. Therefore, we have proposed the model of using chemosensitizing drugs that have been tested in oncology to alter the susceptibility of a latently HIV-infected CD4 T cell that has become resistant to the proapoptotic effects of intracellular HIV replication, so that the latently infected resting memory CD4 T cell becomes susceptible again to the proapoptotic effects of productive HIV replication. This novel paradigm of “**prime, shock, and kill**” involves chemosensitization to render latently infected cells susceptible to the cytotoxic effects of proapoptotic HIV proteins and viral reactivation with agents that do not cause immune activation (e.g., vorinostat), in order to cause intracellular expression of the proapoptotic HIV proteins Tat, Nef, Env, Vpr, and protease. While this model has yet to be fully tested, it may explain observations that agents such as auranofin which is a cancer-chemosensitizing agent may have anti-SIV effect in chronically SIVmac251-infected macaques under highly intensified ART

At the most fundamental of levels, HIV infection is a disease of altered cell death, with too many acutely infected and bystander CD4 T cells dying leading to immunodeficiency and too few latently infected cells dying allowing persistence of the infection. Therefore, enhanced understanding of the death pathways that occur in HIV, as well as the counter regulatory mechanisms which are induced to prevent death of cells destined to become the reservoir of HIV, will offer insights towards death-modifying treatment approaches to eradicate HIV.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [CXCR4, Coreceptors](#)
- ▶ [Global NeuroAIDS](#)

- ▶ [HAND Adjunctive Therapies: Reversing Neuronal Injury](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [HIV-1 Virion Structure](#)
- ▶ [Host Genetics and Genomics](#)
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***M. avium* Complex and Other Nontuberculous Mycobacteria and HIV**

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***Mycobacterium avium* Complex**

Definition

Mycobacterium avium complex (MAC) comprises two closely related acid-fast bacteria, *M. avium* and *M. intracellulare*. The most common clinical presentation of disease due to these organisms in adults is infection of the respiratory tract, primarily seen in immunocompetent individuals with underlying chronic pulmonary disease, while disseminated infection is primarily a complication of advanced human immunodeficiency virus (HIV) infection. Although the incidence of disseminated MAC infection (dMAC) has declined significantly with the widespread use of potent combination antiretroviral therapy (ART), it still remains an important complication of advanced HIV infection.

Epidemiology

Mycobacterium avium complex organisms are thought to have little virulence in the normal host, and prior to the onset of the HIV epidemic

had mostly been recognized as a cause of localized pneumonia in persons with chronic pulmonary disease. Reports of dMAC infection were extremely rare prior to 1980, when there were only 24 cases reported in the literature (Horsburgh 1991). After the onset of the HIV epidemic, MAC infection became increasingly recognized as a cause of illness in individuals with advanced HIV, as defined by a CD4 T cell count of less than 100 cells/mm³, and the incidence of dMAC infection increased as the HIV epidemic worsened. MAC typically presents as disseminated disease in HIV-infected individuals not on ART, although localized MAC disease can occur in HIV-infected individuals on ART, presenting as localized lymphadenitis, pericarditis, osteomyelitis, skin and soft tissue infection, or central nervous system infection.

MAC has been isolated from water, soil, foods, and a variety of animal species, and is thought to be ubiquitous in the environment (Gordin and Horsburgh 2005). Water is the main source for infection and MAC has been isolated from tap drinking water, ice machines, swimming pools, hot tubs, and spas (Corti and Palmero 2008). The ubiquitous nature of MAC in the environment has made infection difficult to prevent. It is estimated that 7–12% of adults have been previously infected with MAC (Benson 1994b), and before the widespread availability of ART up to 40% of patients with acquired immune deficiency syndrome (AIDS) developed dMAC. With the widespread availability of ART and MAC

prophylaxis, the incidence of dMAC has significantly decreased and occurs rarely in those on ART even with low CD4 T cell counts.

The most significant risk factor for dMAC is a CD4 cell count less than 50 cells/mm³. No environmental exposure or specific behavior has consistently been associated as a risk factor (Karakousis et al. 2004). Prior opportunistic infections including *Pneumocystis jirovecii* pneumonia (PJP), severe anemia, and interruptions in antiretroviral therapy may increase the risk for dMAC (Benson 1994b), as do high plasma HIV RNA levels (>100,000 copies/mL) and previous colonization of the respiratory or gastrointestinal (GI) tract with MAC (Benson 1994b). Neither gender nor racial/ethnic group appears to be associated with dMAC (Benson 1994b). It should be noted that assessment of risk has largely been done prior to the availability of effective ART, and most risk factors were evaluated in individuals not receiving effective ART.

Pathogenesis

MAC is made up of two closely related organisms, *M. avium* and *M. intracellulare*, which can be distinguished by polymerase chain reaction (PCR) and other molecular testing. *M. avium* complex bacilli are acid-fast, slowly growing bacteria that are comprised of 28 serogroups, with serogroups 4 and 8 most frequently isolated from HIV-infected persons. *M. avium* appears to be a more important pathogen in disseminated disease, while *M. intracellulare* is a more important pathogen in causing respiratory disease (Guthertz et al. 1989; Han et al. 2005). There is no difference in treatment of these two organisms, so they are not routinely differentiated at diagnosis. MAC isolates from individuals with advanced HIV differ from both isolates in individuals without HIV and isolates from environmental sources, and have been shown to be more virulent in vitro and in animal models (Horsburgh 1991).

Person-to-person transmission of MAC does not appear likely, and those living in the same household or close contacts of those with MAC disease do not appear to be at increased risk for infection (Horsburgh 1999). The primary mode of transmission is via inhalation,

ingestion, or inoculation with bacilli via the respiratory or GI tract, with the GI tract being the most common portal of entry (Corti and Palmero 2008). In individuals with intact immune systems, asymptomatic colonization of the respiratory or GI tract can occur without further dissemination.

In individuals with advanced HIV, after MAC exposure and mucosal entry, transient MAC bacteremia occurs, followed by tissue invasion and colonization. Bacteria are taken up by polymorphonuclear leukocytes (PMNs), mononuclear phagocytes, fibroblasts, and endothelial cells and disseminated primarily to reticuloendothelial organs such as the liver, spleen, and bone marrow (Karakousis et al. 2004). In the absence of an effective immune response, persistent bacteremia occurs with ongoing mycobacterial replication and increasing tissue infiltration (Corti and Palmero 2008).

Disseminated MAC may be secondary to primary infection and subsequent dissemination in an immunocompromised host, or secondary to reactivation of MAC acquired previously, followed by dissemination (Gordin and Masur 1994). Cell-mediated immunity is probably the most important factor in immunological control of MAC and protection from infection. In a prospective study of individuals with HIV and CD4 cell counts less than 50 cells/mm³, the risk of developing MAC bacteremia within 1 year was 60% in those colonized with MAC in the respiratory or GI tract, with a greater risk in those with colonization of the GI tract (Corti and Palmero 2008).

Clinical Manifestations

In individuals not infected with HIV, MAC is most commonly a localized infection of the respiratory tract and causes pneumonia in individuals with chronic lung disease from etiologies such as chronic obstructive pulmonary disorders (COPD) and silicosis. In individuals infected with HIV who are not on effective ART, disseminated disease is most common, although localized disease of the pulmonary system can occur, presenting as pulmonary nodules, infiltrates, or cavities. Localized MAC can also present as isolated pericarditis,

intra-abdominal or soft tissue abscesses, skin lesions, osteomyelitis, or central nervous system (CNS) lesions (Benson 1994b). MAC can also present as a syndrome resembling Whipple's disease with localized involvement of the small intestine, although this is likely a localized manifestation of disseminated disease (Corti and Palmero 2008). Thus, in HIV-infected individuals not receiving effective ART, MAC is classically a disseminated disease that involves multiple organ systems.

Clinical symptoms generally precede bacteremia by a number of weeks and are nonspecific but include fever, night sweats, weight loss, fatigue, abdominal pain, and diarrhea (Horsburgh 1991; Corti and Palmero 2008; Benson 1994b). All of these symptoms are nonspecific and can occur with other opportunistic infections or advanced HIV disease. Approximately 40% of individuals with HIV and MAC have diarrhea and 20% intractable abdominal pain, which although nonspecific may point toward a diagnosis of dMAC (Corti and Palmero 2008). Other signs of dMAC on physical exam include lymphadenopathy and hepatosplenomegaly. Significant anemia, neutropenia from bone marrow infiltration, elevated alkaline phosphatase levels, and increased lactate dehydrogenase (LDH) levels offer evidence of dMAC infection on laboratory analysis (Corti and Palmero 2008; Benson 1994b).

Distinguishing dMAC infection from other opportunistic infections, particularly *Mycobacterium tuberculosis* (MTB) infection, can be challenging. Although MAC is ubiquitous in the environment, there is an inverse geographical relationship between MTB prevalence and MAC prevalence (Corti and Palmero 2008), which may aid in differentiating these two pathogens. In addition, night sweats, significant peripheral lymphadenopathy, and lung involvement are more common signs and symptoms of disseminated MTB infection, while leukopenia, elevated alkaline phosphatase levels, and hepatosplenomegaly are more common in dMAC (Corti and Palmero 2008).

In individuals with HIV on ART with good immunological and virological response to therapy, the most common presentation of dMAC

infection is “unmasking” immune reconstitution inflammatory syndrome (IRIS) from a previously undiagnosed infection. Previously diagnosed or treated dMAC can also worsen after ART initiation, termed “paradoxical” IRIS. IRIS secondary to underlying MAC disease is clinically indistinguishable from active MAC disease, although the temporal association of signs and symptoms compatible with dMAC after the introduction of ART points toward a diagnosis of MAC IRIS (Corti and Palmero 2008). IRIS from underlying MAC disease may be relatively benign and self-limiting, or may result in severe symptoms necessitating treatment with anti-inflammatory therapy or corticosteroids at doses similar to the treatment of IRIS secondary to MTB (Phillips et al. 2005). IRIS associated with MAC typically presents with localized lymphadenitis or soft tissue abscess, so any abscess associated with IRIS should prompt consideration for underlying MAC infection. Treatment of both “unmasking” and “paradoxical” IRIS should include continuation of ART, treatment of underlying MAC infection, and if needed symptomatic treatment with anti-inflammatory therapy or corticosteroids (Corti and Palmero 2008).

Diagnosis

The clinical presentation of dMAC infection can closely resemble disseminated MTB infection, so careful consideration and workup for disseminated MTB is critical, as the treatment of MAC and MTB differ considerably.

Diagnosis of dMAC infection is based on consistent clinical signs and symptoms, along with isolation of MAC from blood, lymph node aspirate, bone marrow aspirate, or cultures from other normally sterile sites (Benson 1994a). Within 14 days of the onset of symptoms, MAC bacteremia occurs in 86–98% of individuals with dMAC (Corti and Palmero 2008), so isolation of MAC from blood cultures is typically the easiest and least invasive way to diagnosis dMAC. Patients with dMAC often have continuous, high-grade bacteremia with high colony counts, making one or two blood cultures usually adequate for diagnosis (Woods 1994).

Hepatomegaly, splenomegaly, paratracheal, retroperitoneal, para-aortic, or peripheral lymphadenopathy may be found with dMAC disease and is often easily visualized by computed tomography (CT) scan. In cases of negative blood cultures or when further workup of lymphadenopathy is warranted, enlarged lymph nodes are potential sites for biopsy. Except for peripheral blood culture, all specimens obtained should be prepared for acid-fast bacilli (AFB) smear prior to submission for mycobacterial culture (Woods 1994). Since the GI tract is the most frequent anatomical site involved, duodenal or mucosal biopsies can be diagnostic as well, with white mucosal lesions visualized in the duodenum in about 80% of individuals with GI involvement on endoscopy (Corti and Palmero 2008). MAC can also be found on liver biopsy with disseminated disease. In one retrospective study, 501 HIV-infected individuals with either abnormal liver function tests, fever greater than 2 weeks, or hepatomegaly underwent percutaneous liver biopsy. The most common diagnosis on biopsy was MAC, seen in over 17% of biopsies (Poles et al. 1996).

Isolation of MAC by conventional mycobacterial culture on solid medium generally takes 3–4 weeks, but can be decreased to 5–12 days with culture on liquid media, such as with the radiometric BACTEC TB system. It is generally recommended that specimens be sent for culture on both liquid and solid media. Solid media allow for visualization of colony morphology, growth rates, and quantification, while liquid media allow for a higher mycobacterial yield and quicker diagnosis. In addition, commercial DNA probes are now available for confirmation of MAC within hours.

Since MAC often colonizes the respiratory and GI tract, sputum and stool cultures can represent colonization rather than true infection; positive cultures may be difficult to interpret. While colonization with MAC can be predictive of future dMAC infection, routine screening with GI or respiratory tract cultures is not recommended as there is a lack of data supporting prophylaxis for asymptomatic patients harboring MAC at these sites.

Prevention of MAC Infection

Because individuals who are profoundly immunosuppressed are at significantly increased risk for developing dMAC infection, primary prophylaxis is indicated in adults and adolescents infected with HIV who have a CD4 cell count less than 50 cells/mm³. Before initiating primary prophylaxis for MAC disease, both dMAC and MTB infection should be ruled out. In some settings routine primary prophylaxis is not initiated if ART is started immediately, even in cases where the CD4 cell count is less than 50 cells/mm³, as the risk of dMAC is thought to be minimal in patients with rapid immune reconstitution to ART (Lange et al. 2004). There is currently no evidence to support this strategy and the recommendation is still to initiate primary prophylaxis if indicated based on CD4 cell count.

Rifabutin, a rifamycin that has activity both in vitro and in animal models against MAC and MTB, was among the first antimycobacterial agents to be evaluated in individuals with HIV/AIDS. Two randomized, double-blind, placebo-controlled trials of rifabutin 300 mg daily versus placebo for prevention of dMAC disease in persons with HIV demonstrated decreased incidence of MAC in the rifabutin arms, although there was no significant difference in survival compared to placebo (Nightingale et al. 1993). However, potential drug interactions, high cost, limited availability, potential for development of cross-resistance in individuals coinfecting with MTB, and requirement for daily dosing prompted the investigation of other alternatives to rifabutin, including macrolide and azalide antibiotics also shown to have in vitro and in vivo activity against MAC.

A randomized, double-blind trial compared azithromycin 1200 mg weekly, rifabutin 300 mg daily, and azithromycin 1200 mg weekly plus rifabutin 300 mg daily. After 1 year of follow-up the risk of MAC was reduced in the azithromycin arm by almost 50% (MAC incidence rate of 15.3% in the rifabutin arm compared to 7.6% in the azithromycin arm). The azithromycin plus rifabutin combination arm had the greatest efficacy in preventing MAC with a MAC rate of 2.8% at 1 year of follow-up, but dose-limiting toxicity

was significantly more common in the combination arm (Havlir et al. 1996).

Another randomized, double-blind, placebo-controlled trial compared clarithromycin 500 mg twice daily, rifabutin 450 mg daily, and clarithromycin 500 mg twice daily plus rifabutin 450 mg daily. After a median follow-up of almost 2 years, 9% of individuals developed MAC in the clarithromycin arm versus 15% in the rifabutin arm and 7% in the combination therapy arm. While there was an increased risk of developing MAC in the rifabutin arm, there was no significant difference in MAC incidence between the clarithromycin and combination clarithromycin and rifabutin arms (Benson et al. 2000).

Thus, the preferred prophylactic regimen includes either azithromycin 1200 mg weekly or clarithromycin 500 mg twice daily (or 1000 mg extended-release tablets) in patients with a CD4 cell count less than 50 cells/mm³ (Table 1). Azithromycin has the advantage of weekly dosing rather than daily dosing and fewer drug-drug interactions than clarithromycin and rifabutin. In patients who cannot tolerate either azithromycin or clarithromycin, rifabutin 300 mg daily is the recommended alternative. Clarithromycin is associated with an increased risk of birth defects in animal studies and miscarriage in humans (Anderson et al. 2013), so azithromycin

is recommended for primary prophylaxis in pregnant women.

MAC prophylaxis can be safely discontinued for patients on effective ART once they have achieved viral load suppression and immune reconstitution. A randomized, double-blind, placebo-controlled trial of 520 individuals found no cases of MAC in either the placebo arm or the azithromycin 1200 mg weekly arm after 12 months in individuals who had an increase in CD4 cell count to above 100 cells/mm³ for greater than 3 months on ART (El-Sadr et al. 2000). A similar randomized, placebo-controlled trial of azithromycin 1200 mg weekly versus placebo found that after 16 months of follow-up, there were only 2 cases of dMAC out of 321 in the placebo group versus 0 cases out of 322 in the continued azithromycin group, with no statistically significant difference between the two groups (Currier et al. 2000). Thus, it appears safe to discontinue MAC prophylaxis once the CD4 cell count has increased above 100 cells/mm³ for greater than 3 months. Primary prophylaxis should be reintroduced if the CD4 cell count decreases to less than 50 cells/mm³.

M. avium Complex and Other Nontuberculous Mycobacteria and HIV, Table 1

Recommendations for primary and secondary prophylaxis of disseminated MAC disease in HIV infection

<p><i>Indications for initiating primary prophylaxis:</i> If the CD4 cell count is <50 cells/mm³, after ruling out disseminated MAC and <i>Mycobacterium tuberculosis</i> infection</p>	<p><i>Preferred therapy:</i> Azithromycin 1200 mg PO once weekly, <i>or</i> Clarithromycin 500 mg PO BID</p> <p><i>Alternative therapy:</i> Rifabutin 300 mg PO daily</p> <p><i>In pregnancy:</i> Azithromycin 1200 mg PO once weekly</p>
<p><i>Indications for discontinuing primary prophylaxis:</i> If on effective ART, once the CD4 cell count is >100 cells/mm³ for greater than 3 months</p>	
<p><i>Indications for restarting primary prophylaxis:</i> If the CD4 cell count decreases to <50 cells/mm³</p>	

Treatment of MAC Infection

Given the risk for the development of resistance and subsequent relapse with monotherapy, initial treatment of MAC disease should consist of two or more antimicrobial drugs. It is recommended that MAC isolates be tested for clarithromycin or azithromycin susceptibility in all patients (Griffith et al. 2007).

In a randomized, double-blind study investigating clarithromycin for treatment of MAC bacteremia in HIV-infected individuals, clarithromycin 500 mg twice daily had equivalent efficacy toward clearance of bacteremia and decreased mortality rates at 6 weeks compared to higher clarithromycin doses (1000 mg and 2000 mg twice daily). The lower-dose clarithromycin arm was also better tolerated; there was a statistically significant increase in adverse effects with the 2000 mg twice daily dose. Emergence of clarithromycin-resistant strains was a significant problem with all doses and occurred in 46% of subjects at 16 weeks (Chaisson et al. 1994).



In another randomized, open-labeled trial, individuals with dMAC were randomized to receive either clarithromycin 500 mg twice daily or 1000 mg twice daily plus weight-based ethambutol, plus either rifabutin 300 mg daily or clofazimine 100 mg daily. After a mean follow-up of 4.5 months, 22% of patients receiving low-dose clarithromycin died compared to 43% of those receiving high-dose clarithromycin, leading to the discontinuation of the high-dose clarithromycin arms. There were no differences in clinical or bacterial outcomes between the remaining arms. Thus, clarithromycin doses greater than 1000 mg/day should be avoided due to increased mortality risk (Cohn et al. 1999).

In a small randomized, open-labeled trial comparing azithromycin 600 mg daily plus weight-based ethambutol and clarithromycin 500 mg twice daily plus weight-based ethambutol for the treatment of MAC bacteremia, at 16 weeks the clarithromycin-ethambutol regimen cleared bacteremia in 86% of patients versus 38% of patients in the azithromycin-ethambutol arm. There was no statistically significant difference in resolution of symptoms, laboratory abnormalities, or adverse events between the two regimens (Ward et al. 1998).

Another randomized, double-blind study investigated the treatment of MAC bacteremia in HIV-infected individuals with weight-based ethambutol plus either azithromycin 250 mg daily, azithromycin 600 mg daily plus, or clarithromycin 500 mg twice daily. After 24 weeks of therapy there was no significant difference in clearance of bacteremia, relapse, or mortality rates between the high-dose azithromycin arm and the clarithromycin arm, although the trend favored clarithromycin. The low-dose azithromycin arm had poor clearance of bacteremia and was dropped midway through the study (Dunne et al. 2000).

Lastly, in a multicenter, randomized, open-label trial of patients with AIDS and MAC bacteremia, individuals were randomized to either clarithromycin 500 mg twice daily plus ethambutol 15 mg/kg/day, clarithromycin 500 mg twice daily plus rifabutin 450 mg daily, or clarithromycin 500 mg twice daily plus

ethambutol 15 mg/kg/day plus rifabutin 450 mg daily. After 12 weeks of follow-up there was no significant difference in microbiological response between the three arms, although there was a higher relapse rate in the clarithromycin plus rifabutin arm compared to the other two arms. There was improved survival and a lower relapse rate in the three-drug arm, and no significant difference in adverse events among the three arms (Benson et al. 2003).

Based on the aggregate of these data, the treatment recommendation for dMAC is either clarithromycin 500 mg twice daily plus ethambutol 15 mg/kg day, or azithromycin 500 mg daily plus ethambutol 15 mg/kg daily (Table 2). Rifabutin 300–450 mg daily is the recommended agent if a third drug is to be added. The addition of a third or fourth agent should be considered in patients with advanced immunosuppression (CD4 cell count less than 50 cells/mm³), high mycobacterial loads (>2 log₁₀ colony-forming units/mL of blood), absence of effective ART, or where emergence of drug resistance is likely. Other alternative agents including amikacin, streptomycin, or a

***M. avium* Complex and Other Nontuberculous Mycobacteria and HIV, Table 2** Recommendations for the treatment of disseminated MAC disease in HIV infection

Testing for drug susceptibility to clarithromycin or azithromycin is recommended Standard therapy should include at least two drugs, with consideration for a third or fourth agent with: Advanced immunosuppression (CD4 count <50 cells/mm ³) High mycobacterial loads (>2 log ₁₀ colony-forming units/mL of blood) Absence of effective ART If the emergence of drug resistance is likely	<i>Preferred therapy:</i> Clarithromycin 500 mg PO BID <i>plus</i> ethambutol 15 mg/kg PO daily, <i>or</i> Azithromycin 500 mg PO daily <i>plus</i> ethambutol 15 mg/kg PO daily <i>Recommended third agent:</i> Rifabutin 300–450 mg PO daily
<i>Other agents:</i> Use should be based on drug susceptibility testing	Amikacin, streptomycin, levofloxacin, or moxifloxacin

fluoroquinolone such as levofloxacin or moxifloxacin may be considered, although randomized clinical trials evaluating these agents are lacking. To minimize the occurrence of drug reactions and treatment-associated IRIS, in most cases ART and antimycobacterial therapy should not be initiated concurrently. ART should be initiated as soon as possible following the first 2 weeks of dMAC treatment to reduce the occurrence of other opportunistic infections.

For clarithromycin-resistant MAC, the recommendation is to treat with a fluoroquinolone such as moxifloxacin or levofloxacin plus ethambutol plus rifabutin. Multiple studies looking at clofazimine have not demonstrated improved microbiological response and its use has been associated with increased mortality, so it is not recommended in the treatment of MAC infection (Karakousis et al. 2004). Treatment with azithromycin and ethambutol is the preferred regimen for pregnant women, given the association of birth defects in mice and rats with clarithromycin (Corti and Palmero 2008).

An improvement in fever curve should be expected within 2 to 4 weeks after starting treatment, although in very extensive disease or advanced immunosuppression clinical response may be delayed. Repeat blood cultures for MAC should be obtained 4–8 weeks after initiating therapy in patients who fail to have an adequate clinical response to treatment. It is reasonable to discontinue therapy after individuals have completed at least 12 months of therapy, have no signs or symptoms of MAC, and have sustained a CD4 cell count greater than 100 cells/mm³ for 6 months after ART initiation (Aberg et al. 2003).

If individuals have a poor clinical response and persistent mycobacteremia after 4–8 weeks of treatment, repeating susceptibility testing for azithromycin and clarithromycin is recommended. Treatment regimens should be based on repeat susceptibility testing, and should consist of antimicrobials from at least two new drug categories not previously used. Whether or not continuing clarithromycin or azithromycin despite proven resistance is of additional benefit is unknown.

Other Nontuberculous Mycobacteria (NTM) and HIV

Mycobacterium kansasii

M. kansasii is the second most common cause of nontuberculous mycobacterial disease in the United States, and after MAC, the second most common cause in individuals with advanced HIV. Like MAC, it is primarily an environmental pathogen and tap water is likely its primary reservoir (Griffith et al. 2007).

M. kansasii is a slow-growing NTM that causes pulmonary disease in geographic clusters around the world, including the south and central United States. Risk factors for pulmonary disease include underlying chronic lung disease, malignancy, and alcoholism. In individuals with advanced HIV, *M. kansasii* can present as disseminated disease, typically in individuals with a CD4 cell count less than 50 cells/mm³. Unlike with dMAC infection, pulmonary disease is also seen in over 50% of individuals with disseminated disease, and bacteremia is uncommon, found in only 25% of individuals (Griffith et al. 2007). *M. kansasii* is typically considered one of the most pathogenic NTM, and represents true infection in 50% of patients with positive respiratory cultures (Marras and Daley 2004).

Treatment for pulmonary and disseminated disease should involve three agents, including rifampin 10 mg/kg/day, ethambutol 15 mg/kg/day, and isoniazid 5 mg/kg/day (maximum 300 mg daily), along with pyridoxine 50 mg daily. In patients with rifampin-resistant disease, a three-drug regimen is recommended based on drug susceptibility testing, but should include a macrolide (clarithromycin or azithromycin), moxifloxacin, ethambutol, sulfamethoxazole, or streptomycin. In pulmonary disease, treatment duration should entail 12 months of therapy after conversion of sputum cultures to negative. The treatment duration for disseminated *M. kansasii* disease is similar to that of disseminated MAC and includes at least 12 months of therapy if signs or symptoms have resolved and sustained immune reconstitution has occurred on ART (Griffith et al. 2007).

Mycobacterium chelonae

M. chelonae is a rapidly growing environmental mycobacterium that can cause isolated skin, soft tissue, and bone infections, and has been implicated as a cause of keratitis in contact lens wearers and those that have undergone ocular surgery. It can also present as disseminated disease in individuals with advanced HIV (Kunin et al. 2014).

An open-labeled, single-arm study of 14 patients with *M. chelonae* infection investigated monotherapy with clarithromycin 500 mg twice daily. Ten patients had disseminated disease, and eleven patients completed at least 6 months of therapy. Clarithromycin was subsequently discontinued in 9 of the 14, with no evidence of relapse. Two other patients died of causes unrelated to infection and one self-discontinued therapy after 3.5 months with subsequent relapse (Wallace et al. 1993).

For significant skin, soft tissue, bone, or pulmonary disease, combination therapy with clarithromycin plus another agent is recommended to minimize selection for clarithromycin-resistant organisms. Antimicrobial choice should be guided by drug susceptibility testing. At least 4 months of treatment is recommended for skin and soft tissue infections, 6 months for bone infection, and 12 months from sputum culture conversion for pulmonary infection. Surgery may be indicated as an adjunct for skin, soft tissue, or bone infection if disease is extensive or when antimicrobials alone fail; removal of involved foreign bodies or prosthetic devices is crucial for cure (Griffith et al. 2007).

Mycobacterium genavense

M. genavense is a slow-growing NTM that causes both localized and disseminated disease in HIV-infected individuals (Bottger et al. 1992). It has been isolated from the cervical lymph node of a dog as well as a number of pet birds, including psittacine birds (Kiehn et al. 1996). Disseminated disease has been noted in individuals with advanced HIV, and *M. genavense* has been recovered from multiple reticuloendothelial sites including blood, bone marrow, liver, and spleen. *M. genavense* is likely acquired through ingestion

and appears to have a predilection for colonizing the GI tract (Doggett and Strasfield 2011).

Optimal treatment of *M. genavense* infections is unknown, but multidrug regimens that include clarithromycin are recommended. Most isolates are susceptible to amikacin, rifamycins, fluoroquinolones, macrolides, and streptomycin. Ethambutol has poor activity against *M. genavense* (Griffith et al. 2007). Studies evaluating outcomes of therapy in HIV-infected individuals are limited.

Mycobacterium gordonae

M. gordonae is a slow-growing, environmental NTM that is ubiquitous in the environment and frequently recovered from freshwater sources, soil, and tap water, including water from hospitals. When found in cultures it is typically considered a nonpathogenic contaminant, as it can easily be introduced into clinical specimens during collection and processing (Weinberger et al. 1992) and can colonize the GI and respiratory tract (Mizoshita et al. 2011). All of these factors may make it difficult to differentiate as a contaminant or true pathogen.

Occasionally *M. gordonae* has been associated with clinically significant infections and has been associated with disseminated disease, pulmonary infection, keratitis, peritonitis, and skin and soft tissue infections, as well as in individuals with underlying immunosuppression such as advanced HIV. Infections have also been associated with foreign bodies, which may even have been the portal for infection (Weinberger et al. 1992).

There is a paucity of data available on optimal treatment regimens. Ethambutol, rifabutin, clarithromycin, linezolid, and the fluoroquinolones most consistently have in vitro activity against *M. gordonae* (Griffith et al. 2007).

Conclusion

Disseminated *Mycobacterium avium* complex (dMAC) infection is primarily a complication of advanced HIV infection in the absence of effective ART. More common prior to the advent and widespread use of potent combination ART, the

incidence of dMAC has declined considerably over the past two decades, although individuals with very low CD4 cell counts still remain at risk.

MAC is ubiquitous in the environment, and a CD4 cell count less than 50 cells/mm³ is the most significant risk factor for disseminated infection. Prophylaxis with azithromycin or clarithromycin is recommended in persons with a CD4 cell count less than 50 cells/mm³ and should be continued until the CD4 cell count is maintained above 100 cells/mm³ for greater than 3 months. Optimum treatment for dMAC includes treatment with at least two agents including clarithromycin or azithromycin plus ethambutol, with the addition of rifabutin as a third agent for those with more extensive disease. Although the incidence of dMAC has declined significantly with the widespread use of effective ART, it still remains an important complication of advanced HIV infection.

Environmental mycobacteria other than *M. tuberculosis* and *M. avium* complex can cause infection in humans. For many of these NTM, chronic pulmonary disease is a risk factor for infection of the respiratory system, although immunodeficiency from advanced HIV infection is a risk factor for a number of NTM as well. Treatment differs based on the pathogen and in some cases has not been well elucidated or studied, making accurate diagnosis crucial and treatment challenging.

Cross-References

► Tuberculosis and HIV

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Macrophages in HIV Immunopathogenesis

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Definition

Blood monocytes and tissue macrophages are professional phagocytic cells that play important roles in tissue homeostasis and the innate defense against microbial infections. Along with CD4⁺ T cells, monocytes and macrophages are a major target for HIV-1 infection. These cells play crucial roles in viral transmission and spread to body tissues, in the persistence of HIV-1 infection, and in HIV-1 immunopathogenesis and neuropathogenesis.

Monocyte and Macrophages: Origin, Heterogeneity, and Functions

Monocytes (Mos) and macrophages (Mφs) are myeloid cells that belong to the mononuclear phagocytic system (Murray and Wynn (2011) and references herein). Monocytes are generated in the bone marrow from hematopoietic stem cell precursors. It is generally believed that tissue Mφs originate from circulating monocytes that mature and differentiate after being recruited into tissues. However, recent studies in mice, supported by studies of patients affected by congenital monocytopenia, have challenged this paradigm. Indeed, tissue-resident Mφs have been shown to renew locally during adult life, thus suggesting that resident Mφs do not derive from blood monocytes, at least in some tissues, but can self-maintain and repopulate locally. Mos/Mφs are professional phagocytic cells that play crucial roles both in tissue homeostasis and in innate defenses against bacterial, viral, and parasitic infections. Mos/Mφs are extremely sensitive to any stress, malfunction, or danger signals due to the expression of numerous receptors on their surfaces, including toll-like receptors (TLR), scavenger receptors, and receptors for the Fc segment of immunoglobulins (FcR).

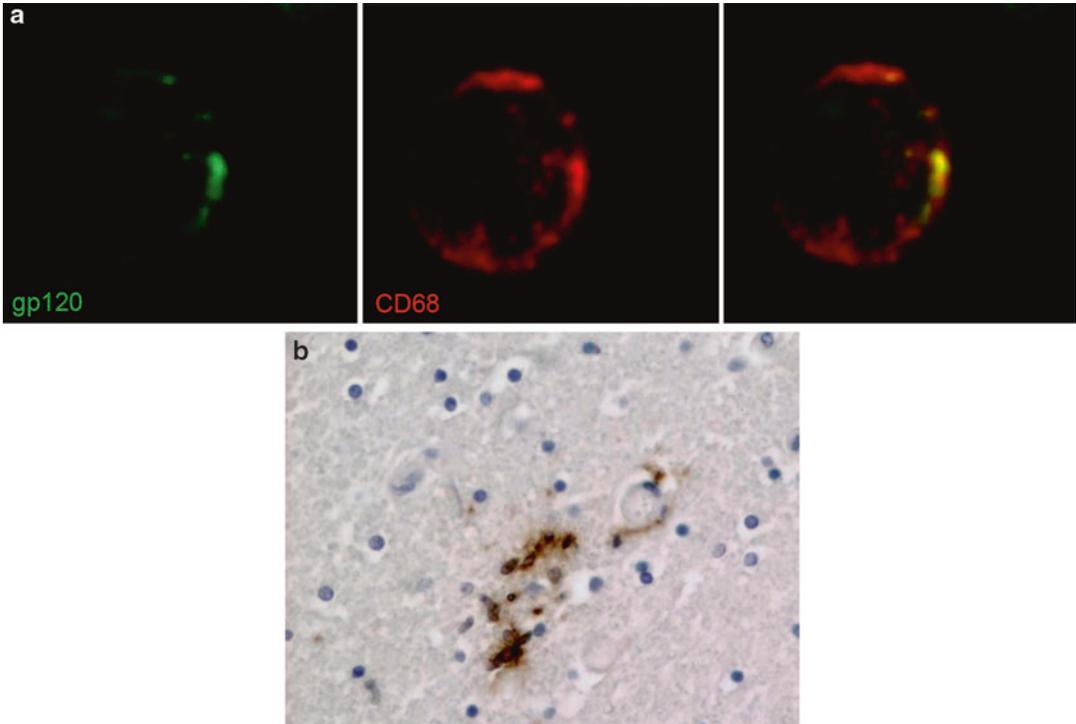
Mos circulate in the blood for several days and can extravasate into tissues, where they differentiate into Mφs in situations of inflammation and tissue injury. Three subsets of blood Mos can be distinguished phenotypically based on the differential expression of two surface molecules: the coreceptor to bacterial lipopolysaccharides (LPS) CD14 and the FcR for IgG₃ (FcγIII_R) CD16. Classical monocytes are CD14⁺⁺CD16⁻, intermediate monocytes are CD14⁺⁺CD16⁺, and nonclassical monocytes are CD14⁺CD16⁺⁺ (Ziegler-Heitbrock et al. 2010). In particular, nonclassical CD14⁺CD16⁺⁺ monocytes patrol blood vessels and respond to viral infections by producing proinflammatory cytokines such as TNFα and IL-1 via the activation of TLR7 and TLR8 (Cros et al. 2010).

Mφs are highly plastic cells that can acquire distinct phenotypes and functions based on the neighboring microenvironment. Tissue-resident Mφs, such as Kupffer cells in the liver, microglia in the brain, and alveolar Mφs in the lung,

differentiate and exert specialized functions according to their tissue localization. Infiltrating Mφs are derived from inflammatory Mos that extravasate to sites of inflammation or infection, and their accumulation in tissues is associated with the increased turnover of circulating monocytes. Mφs contribute to the first line of defense against pathogens either by phagocytizing and degrading them or by secreting chemokines and cytokines. These soluble molecules either exert direct antimicrobial effects or serve to recruit and activate other innate and adaptive immune cells. Based on their phenotype and effector functions, by analogy with the T helper type 1 and T helper type 2 (T_H1-T_H2) polarization, Mφs have been further classified as classically activated Mφs (M1 Mφs), which exhibit microbicidal properties and secrete proinflammatory cytokines and alternatively activated Mφs (M2 Mφs), which play a crucial role in resolving inflammation.

General Considerations for Monocytes and Macrophages in HIV Infection

Mos and Mφs are, along with CD4⁺ T cells, major cell targets for HIV-1 infection. Unlike T cells, Mos/Mφs are relatively resistant to the cytopathic effects of HIV-1 infection and can survive for long periods of time following infection. Although Mos isolated from the blood do not support productive HIV infection *in vitro*, due to the blocking of early steps in the viral replication cycle before the integration of proviral DNA, Mos-bearing HIV-1 DNA and RNA have been detected in the blood of HIV-1-infected patients, and HIV-1 variants distinct from those present in CD4⁺ T lymphocytes have been found in circulating Mos (Bergamaschi and Pancino (2010) and references herein). In addition, HIV-1 transcripts and signatures of viral evolution in viral genome sequences have been described in monocytes of patients undergoing effective combined antiretroviral therapy (cART). Monocytes harboring HIV-1 proteins can be detected in peripheral blood mononuclear cells (PBMC) (Fig. 1a). Altogether, these data indicate that infected Mos in HIV-1-infected patients can participate in viral dissemination into tissues.



Macrophages in HIV Immunopathogenesis, Fig. 1 HIV-1 infected monocytes and macrophages from HIV-1 infected patients. (a) Confocal microscopy immunolocalization of the HIV-1 protein gp120 (green) and the macrophages marker CD68 (red) in PBMC from HIV-1-infected patient. Original magnification: 63 \times .

(b) Immunohistochemical analysis of HIV-1 p24 protein expression (brown precipitate) on frontal cortex sections from patient with HIV-1-related encephalitis. Original magnification: 20 \times (Images kindly provided by Dr. Roberta Nardacci, National Institute for Infectious Diseases, IRCCS “L. Spallanzani.” Rome, Italy)

In particular, the magnitude of the nonclassical CD14⁺CD16⁺⁺ Mo subset, which comprises less than 10% of blood Mos in healthy individuals, is increased with HIV infection and can expand to 40% of Mos in AIDS patients (Thieblemont et al. 1995). CD14⁺CD16⁺⁺ Mos express higher levels of the HIV-1 coreceptor C-C chemokine receptor type 5 (CCR5) than other Mo subsets and have been reported to be more prone to HIV infection.

In contrast to Mos, M ϕ s are permissive to HIV-1 infection *in vitro*. For *in vitro* studies, M ϕ s are differentiated from blood monocytes by culturing for 6–7 days in the presence of macrophage colony-stimulating factor (M-CSF) or human serum. Monocyte-derived macrophages (MDMs) have been a valuable model for studies of the HIV-1 replication cycle and interactions between viral determinants and cellular factors. However, MDMs are not representative of the

heterogeneity of M ϕ s in body compartments. Unfortunately, *in vivo* studies in humans have been dampened by the fact that tissue M ϕ s are difficult to isolate from solid organs. However, immunohistochemistry and *in situ* hybridization studies have provided evidence for the infection of resident M ϕ s in different organs, including the brain, lymph nodes, liver, and lungs of HIV-1 patients (Fig. 1b) (Koenig et al. 1986). Valuable information regarding Mo and M ϕ infection *in vivo* and their role in pathogenesis has been obtained from nonhuman primate (NHP) models. Infection of Asian macaques with pathogenic SIVmac results in a disease similar to HIV-1 infection in humans. Together with macaque infections by SHIVs, which are chimeric SIV viruses bearing HIV envelope (Env) glycoproteins, this model recapitulates key features of human HIV infection. In particular, SIV-NHP

infection has been the best model for AIDS and neuroAIDS.

The following sections will address specific aspects of the HIV-1 replication cycle in Mφs and the role of Mφs in virus transmission and spread and in the immunopathology of the disease.

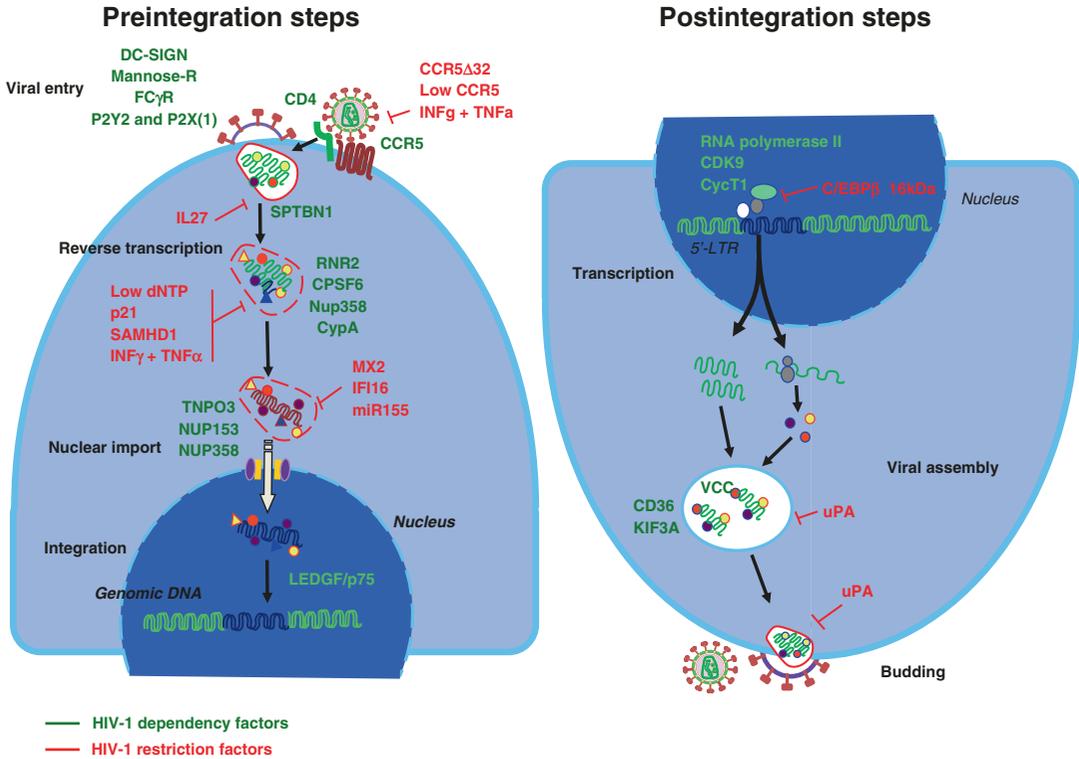
HIV Replication Cycle in Macrophages

HIV-1 tropism is determined at the level of viral entry via interactions between the gp120 envelope glycoprotein (Env) and receptors expressed on the cell surface, including the primary receptor CD4 and coreceptors that are strain and target specific (► [Tropism and Properties of the HIV-1 Envelope Glycoprotein in Transmission; CXCR4, Co-receptors](#)). R5 strains of HIV-1 use C-C chemokine receptor 5 (CCR5) as their coreceptor, whereas X4 strains of HIV-1 use CXCR4 coreceptors. R5/X4 HIV-1 isolates have dual tropisms and use both CCR5 and CXCR4 coreceptors. Macrophage-tropic (M-tropic) viruses can infect Mφs and CD4⁺ T lymphocytes and primarily use CCR5, T-cell-line-tropic (T-tropic) viruses can infect CD4⁺ T lymphocytes and T-cell lines using CXCR4, and dual-tropic viruses can infect both CD4⁺ T cells and Mφs. However, not all R5 HIV-1 strains are M-tropic, and some X4 viruses can efficiently infect Mφs, indicating that cellular tropism involves Env-coreceptor interactions that are not completely determined by coreceptor specificity. In particular, Mφs express lower CD4 levels than T lymphocytes, and it has been reported that M-tropic viruses isolated from the brain demonstrate lower CD4 dependence for entry, possibly due to higher Env affinity for CD4. In addition, gp120 Envs from some M-tropic viruses also appear to have higher affinities for the CCR5 coreceptor. Other molecules expressed on the Mo/Mφ surface, including heparan sulfate proteoglycans, mannose receptors, and FcRs, can serve as attachment receptors for HIV-1, therefore increasing virus binding to the cells and favoring interactions between viral Env, CD4, and coreceptors and consequently viral entry (Fig. 2 for a schematic

representation of HIV-1 replication cycle in Mφ and (Bergamaschi and Pancino 2010) for review).

The HIV-1 replication cycle begins with the binding of the viral envelope glycoprotein (Env) gp120 to the primary receptor CD4 and to the coreceptor CCR5 (► [HIV Life Cycle: Overview](#)). These interactions trigger conformational changes that lead to the fusion of the cellular and viral membranes and the delivery of the viral core into the cytoplasm. Recent reports have shed the light on the role of purinergic receptors in HIV-1 entry into CD4 T cells and Mφs (Paoletti et al. 2012). It has been shown that the binding of the gp120 viral envelope to the CD4 receptor leads to rapid cellular ATP release. ATP then acts on purinergic receptors, including P2Y2 and P2X (1), to induce transient plasma membrane depolarization through proline-rich kinase 2 (pyk2) activation and stimulate fusion between viral and cellular membranes. Immunohistochemical analyses of frontal brain sections containing microglial cells from HIV-infected, untreated patients and from patients with HIV-associated encephalitis showed that P2Y2 and phosphorylated pyk2 were expressed at higher levels compared to uninfected donors, supporting a role for this signaling pathway in HIV pathogenesis.

Following entry, the viral core is partially disassembled, and viral RNA is reverse-transcribed into DNA inside nucleoprotein complexes formed by viral and host cell proteins called the reverse transcription complex (RTC) and pre-integration complex (PIC). Reverse transcription, a hallmark step of retroviruses, converts the HIV-1 RNA genome into double-stranded DNA and is catalyzed by the reverse transcriptase enzyme; reverse transcription requires the availability of an intracellular pool of deoxynucleoside triphosphates (dNTP) (► [Uncoating and Nuclear Entry](#)). The reverse transcription process appears to be a limiting step in HIV-1 replication in Mφs (► [Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis](#)). Although the reverse transcriptase of lentiviruses is able to catalyze DNA synthesis, even at low dNTP concentrations, HIV-1 reverse transcription in Mφs exhibits slow kinetics compared to activated CD4⁺ T cells. This is attributed to the limited availability of dNTP in Mφs.



Macrophages in HIV Immunopathogenesis, Fig. 2 HIV-1 replication in macrophages. The virus can be captured on macrophage surface by different attachment receptors, including FcγR, mannose receptor, or DC-SIGN. The viral envelope protein (Env) of M-tropic HIV-1 binds the primary receptor CD4, undergoes a conformational change, then binds the chemokine receptors CCR5, and enters cells by fusion of the viral and cellular membranes. The viral entry is promoted by purinergic receptors (P2Y2 and P2X (1)) activation. Viral reverse transcription, which has slow kinetics due to the low dNTP intracellular levels, is affected by p21 and SAMHD1 restriction factors. Viral pre-integrative steps are also counteracted by IL27, INFγ + TNFα, MX2, IFI16, and miR155. HIV-1 capsid recruits cellular factors (CPSF6, Nup358, and CypA) to avoid activation of

immune sensors. The HIV-1 PIC then enters the nucleus of nondividing cells through the help of cellular proteins (TNPO3, Nup153, Nup358). Integrase catalyzes the insertion of the viral cDNA into the host genome. Provirus transcription to viral mRNAs, which is stimulated by the viral protein Tat and cellular factors (CycT1, CDK9, RNA polymerase II) is inhibited by C/EBPβ small isoform. The viral protein Rev transports partially spliced and unspliced genomic transcripts from the nucleus to the cytoplasm. Viral structural and enzymatic proteins are synthesized and assembled at the level of macrophage-specific compartments (VCCs) with the help of CD36 and KIF3A. Viral particles bud into VCCs where they are stored and released in the extracellular macrophage milieu through a yet unclear mechanism. Viral assembly and release can be counteracted by uPA

Indeed, the average dNTP concentration (26 nM) in MDMs is approximately 200 times lower than in PHA-activated T cells. Recent reports indicated that the anabolic and catabolic dNTP cellular pathways in MDMs are regulated by two cellular proteins, p21 and the SAM domain and HD domain, 1 (SAMHD1), respectively, leading to the inhibition of HIV-1 reverse transcription in these cells (see below). Importantly, HIV-1 is

able to replicate in primary human Mφs without stimulating the innate immune response despite reverse transcription of genomic RNA into double-stranded DNA, an activity that might be expected to trigger innate pattern recognition receptors. In an attempt to understand the mechanisms involved in this process, a recent study demonstrated that HIV-1 recruits the cellular factors CPSF6 and cyclophilins Nup358 and CypA

to cloak its replication via its capsid, permitting evasion of innate immune sensors in macrophages (Rasaiyaah et al. 2013). The authors found that capsid mutant viruses defective for the binding of CPSF6 and cyclophilins cannot replicate in MDMs because their reverse transcripts trigger innate sensors, leading to the production of type I interferons (IFN) and the induction of an antiviral state.

Lentiviruses, including HIV-1, are the only retroviruses able to infect nondividing cells such as Mφs, dendritic cells, and resting CD4⁺ T cells; this has been attributed to the ability of their cDNA to enter nuclei through an intact nuclear membrane. HIV-1 capsid is thought to be the major determinant of HIV-1 nuclear import in nonproliferating cells, interacting with several cellular proteins, including transportin 3 (TNPO3) and the nucleoporins NUP153 and NUP358 (Matreyek and Engelman 2013) (► [Role of Transportin-SR2 \(Transportin-3, TRN-SR2, TNPO3\) in HIV Replication](#)).

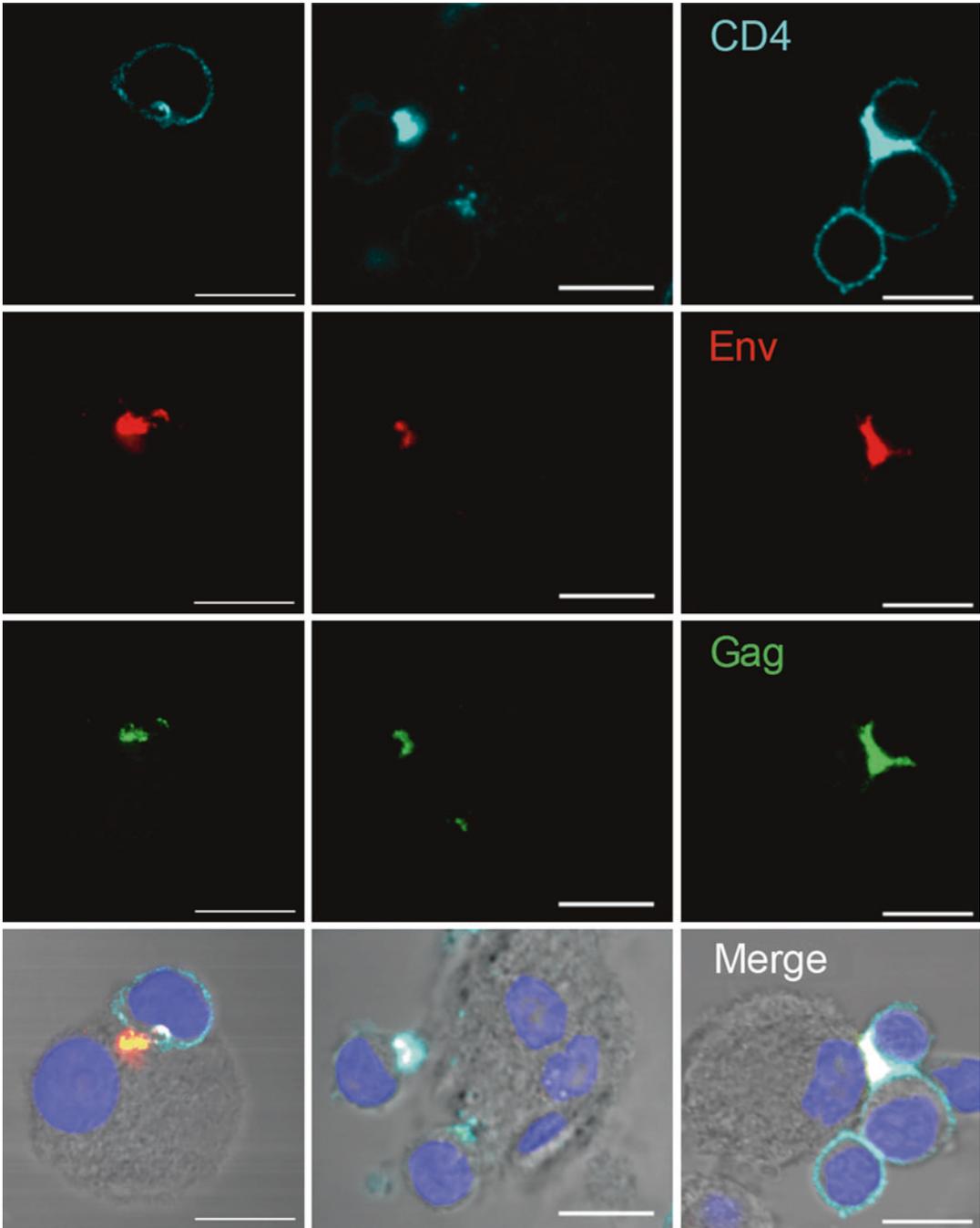
Following nuclear import of the viral PIC, HIV-1 integrase preferentially catalyzes the insertion of the linear double-stranded viral DNA into the host cell chromosome in transcribed genes, although not in close proximity to the start of transcriptional units, which is different than oncogenic retroviruses (► [Nuclear Import: HIV-1 Goes NUPs](#)).

Once integrated into the host genome, the provirus behaves like any human gene, with transcription being initiated at the 5' end and terminating at the 3' end. The 5' LTR contains enhancer and promoter sequences, with binding sites for several transcription factors (NFκB sites, C/EBP sites, SP1 sites, and a TATA cassette) (► [Integration; Transcription \(Initiation, Regulation, Elongation\)](#)). These transcription factors position RNA polymerase II at the 5' LTR to initiate transcription. The viral Tat is then indispensable for transcription elongation, recruiting the positive elongation transcription factor b (P-TEFb) complex, which contains cyclin T1 (CycT1) and a cyclin-dependent kinase 9 (CDK9) heterodimer, resulting in the phosphorylation of the carboxy-terminal domain of RNA polymerase II by CDK9 and a dramatic

stimulation of transcription elongation. In Mo, CycT1 is not expressed, and enhanced permissivity to HIV-1 infection was found to be associated with the increased expression of CycT1 during Mo differentiation into Mφs. Although CycT1 expression is later down-regulated in differentiated Mφs, its expression is enhanced by HIV-1 infection.

Synthesized viral RNAs are then exported from the nucleus to the cytoplasm for translation and packaging. The Env glycoprotein precursor, gp160, is synthesized in the endoplasmic reticulum and transported to the plasma membrane via the secretory pathway. In contrast, Gag and Gag-Pol polyprotein precursors are synthesized on free ribosomes in the cytosol and are targeted to the plasma membrane. HIV-1 assembly in CD4⁺ T cells takes place at the plasma membrane, and nascent viral particles bud off from the cell surface, facilitated by cellular endosomal sorting machinery complexes (ESCRTs), which normally function to promote the budding of vesicles into late endosomes to form multivesicular bodies (► [HIV-1 Assembly Cofactors](#)). Concomitant with virus release, the viral protease cleaves the Gag and Gag-Pol precursors into their respective protein domains, leading to virion maturation.

In contrast to T lymphocytes, where HIV-1 assembly occurs exclusively at the plasma membrane, in macrophages, newly formed virions are also assembled at the membrane of preexisting cytoplasmic compartments called virus-containing compartments (VCCs) (Tan and Sattentau 2013) and references herein) (Fig. 3) (► [Virus Assembly](#)). HIV-1 assembled virions then bud into VCCs, which may facilitate their protection from the immune response and antiviral drug treatments and contribute to the establishment of a viral reservoir in macrophages (discussed below). The VCCs appear to be macrophage-specific compartments and are clearly distinct from the endocytic pathway, as they have a neutral pH in contrast to endosomes, which are strongly acidic. VCCs share similar expression levels of the membrane marker CD44 with the plasma membrane, suggesting their cell surface origin, while endosomes have virtually no CD44. Further supporting their plasma membrane



Macrophages in HIV Immunopathogenesis, Fig. 3 HIV-1 transmission from infected MDMs to CD4⁺ T cells. Laser scanning confocal microscopy of HIV-1_{BaL}-infected MDM co-cultured for 3 hours with autologous CD4⁺ T cells preincubated for 30 min with non-blocking anti-CD4 (L120, mouse IgG₁), permeabilized, and stained for HIV-1 Gag (KC57-FITC) and Env (2G12-biotin) +/- appropriate secondary

antibodies. Nuclei were stained with Hoechst and images acquired using an Olympus FV1000 microscope with 60× oil-immersion objective lens. Scale bars represent 10 μm (Images kindly provided by Dr. Christopher Duncan at the Sir William Dunn School of Pathology, The University of Oxford, UK. Dr. Duncan was supported by the Wellcome Trust (094449/Z/10/Z)

origin, VCCs can remain accessible to the external medium via conduits or narrow microchannels; however, such connections are transient, and the dynamics of formation generate enclosed VCCs. A recent study showed that CD36, a scavenger receptor that is not expressed in lymphocytes, is localized to the VCC membrane and is essential for the recruitment of newly synthesized Gag to VCCs for viral assembly (Berre et al. 2013). Interestingly, anti-CD36 antibodies inhibited the extracellular release of HIV-1 virions from infected MDMs and the transmission of the virus to CD4⁺ T cells via the retention of HIV-1 virions within VCCs. A role for the kinesin KIF3A, a molecular motor that propels cargo along microtubules, in the translocation of VCCs at the macrophage surface and virus release was described (Tan and Sattentau 2013). The migration of VCCs to the virological synapses of infected macrophages with T cells has been reported, suggesting a role for VCCs in cell-to-cell transmission of HIV-1. However, other studies have suggested that the assembly and release of HIV-1 into macrophages may occur primarily at the plasma membrane. In particular, the analysis of macrophages in various organs from HIV-1-infected patients by electron microscopy suggested that a majority of virions use the plasma membrane rather than internal VCCs for budding (Tan and Sattentau 2013).

Restriction Mechanisms of HIV-1 Replication in M ϕ s

As described in previous sections, M ϕ s are permissive to HIV-1 infection both in vitro and in vivo. However, several factors, including the cytokine environment and exogenous stimuli, modulate their capacity to support HIV-1 replication. Furthermore, several host factors constituting an innate intracellular defense against the virus, called intrinsic immunity (► [Cell Intrinsic Immunity; Cellular Restriction Factors](#)), contribute to limit HIV-1 replication, affecting different steps of the viral life cycle (Fig. 2 and (Bergamaschi and Pancino 2010) references herein).

Differential Restriction of HIV-1 in M1- and M2a-Polarized M ϕ s

The polarization of MDMs into M1 cells, which are involved in the Th1 immune response, by interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) stimulation leads to the inhibition of HIV-1 entry, impaired viral cDNA synthesis, delayed viral cDNA integration, and reduced proviral transcription. M1 polarization was found to be associated with the downregulation of CD4 receptors and increased secretion of CCR5-binding chemokines (CCL3, CCL4, and CCL5). In contrast, the polarization of MDMs into anti-inflammatory M2a, which is involved in the Th2 response, via exposure to IL-4 inhibits HIV-1 infection at a late post-integration step. The removal of the polarization stimulus was associated with a renewed capacity to support HIV-1 infection, suggesting that M1/M2a polarization may represent a mechanism that permits macrophages to cycle between latent and productive HIV-1 infection (Cassol et al. 2010).

Restriction of HIV-1 Viral Entry

The absence of the expression of the CCR5 molecule on the cell surface, linked to the homozygous CCR5 Δ 32 mutation, blocks the entry of R5 HIV-1 into both M ϕ s and CD4⁺ T cells. Stimulation with lipopolysaccharide (LPS), a major constituent of gram-negative bacteria, activates MDMs and strongly inhibits HIV-1 infection. Viral entry restriction by LPS was correlated with the downregulation of CCR5 expression due to a defect in its recycling. In vivo exposure of macrophages to LPS might limit HIV-1 infection, as suggested by the observation that M ϕ s from the mucosa of the gastrointestinal tract, which is exposed to gram-negative bacteria and LPS, do not express CCR5 on their surface and are resistant to HIV-1 infection.

Restriction of HIV-1 Pre-integration Steps

IL-27, a cytokine that belongs to the IL-12 family, is mainly produced by activated antigen-presenting cells, including dendritic cells (DCs), Mos, and M ϕ s. IL-27 was recently shown to induce Mo differentiation into MDMs that are resistant to HIV-1 replication, due to a post-entry

block (Dai et al. 2013). IL-27 specifically confers HIV resistance to M ϕ s by downregulating the expression of the protein spectrin β non-erythrocyte 1 (SPTBN1), an HIV-dependent host factor that binds viral Gag and is required for an early step in HIV-1 replication.

SAMHD1 is a restriction factor that inhibits HIV-1 reverse transcription in DCs, M ϕ s, and resting CD4⁺ T cells by degrading dNTPs (Laguette et al. 2011) (► [SAMHD1](#)). Indeed, SAMHD1 is a dGTP-stimulated triphosphohydrolase that converts dNTPs into their constituent deoxynucleosides and inorganic triphosphates. SAMHD1 exerts its antiviral activity only in non-dividing cells. The most recent studies have shown that the restriction activity of SAMHD1 is lost upon its phosphorylation at residue T592 by specific cyclin-dependent kinases in activated and proliferating cells. However, SAMHD1's dNTPase activity is not affected by its phosphorylation. These data challenge the role of the dNTPase activity of SAMHD1 in the restriction of HIV-1 in M ϕ s. Recently, SAMHD1 has been found to have RNA exonuclease activity and it has been proposed that it restricts HIV-1 by degrading the viral genomic RNA as it is reverse transcribed (Ryoo et al. 2014). The relative contribution of the two mechanisms is still debated. Primate lentiviruses that express the accessory protein Vpx, such as HIV-2 and SIV, are able to escape this restriction, as Vpx targets SAMHD1 to the degradation machinery.

The M ϕ intracellular dNTP pool is also the target of viral restriction mediated by p21, an inhibitor of cyclin-dependent kinases, which inhibits HIV-1 reverse transcription in MDMs by suppressing the expression of ribonucleotide reductase subunit 2, RNR2, an enzyme indispensable for dNTP de novo biosynthesis in MDMs (Allouch et al. 2013). p21 also restricts lentiviruses expressing Vpx (HIV-2 and SIV), which is able to degrade SAMHD1. Interestingly, Fc γ R engagement on the MDM surface strongly inhibits HIV-1 reverse transcription by upregulating p21 expression.

Type I interferons can inhibit HIV-1 replication at different steps. MX2 is a recently described restriction factor of HIV-1 and related primate

lentiviruses (Goujon et al. 2013) (IFN Signalling and Induction of Restriction Factors). MX2 is inducible in MDMs and other cell types (CD4⁺ T cells, THP1 monocytic cell lines, and the glioblastoma line U87-MG) by IFN- α stimulation. MX2 did not affect HIV-1 reverse transcription but strongly reduced levels of 2-long terminal repeat circular DNA (2-LTR circles), a marker for HIV-1 cDNA nuclear localization, and of integrated proviruses, indicating a blockade of HIV-1 nuclear import or the degradation of viral cDNA. Susceptibility to MX2 restriction appears to be determined by the HIV-1 capsid. IFI16 (IFN-inducible protein 16) was recently described as an innate immune response inhibitor of HIV-1 replication in MDMs (Jakobsen et al. 2013). IFI16 senses HIV-1 reverse-transcribed cDNA and stimulates the innate immune response via a pathway that is dependent on stimulator of IFN genes (STING). The activated antiviral state stimulated by IFI16 targets a step after HIV-1 entry but prior to HIV-1 integration.

miR-155 is a microRNA induced in MDMs following stimulation of TLR3 and TLR4 by poly I:C (a synthetic analog of dsRNA) or LPS, respectively, and inhibits HIV-1 replication at post-entry, pre-integration events (Swaminathan et al. 2012). Overexpression of miR-155 in MDMs induces downregulation of the expression of mRNA and proteins involved in trafficking and/or nuclear import of PIC (ADAM10, TNPO3, Nup153, LEDGF/p75), suggesting that miR-155 antiviral activity targets HIV-1 cofactor mRNA for degradation.

Restriction of HIV-1 Post-integration Steps

The C/EBP binding sites in the HIV-1 LTR promoter recruit the C/EBP β transcription factor and are required for HIV-1 replication in macrophages but not in T cells. However, C/EBP β has two isoforms: a large isoform of 30–37 KDa that stimulates gene transcription and a small isoform of 16–21 KDa that exerts repressive activity. In HIV-1-infected patients, lung alveolar macrophages do not exhibit active viral replication, whereas they represent a major source of virus in pulmonary tuberculosis coinfection. The viral latency of HIV-1 in alveolar macrophages was

correlated with upregulated expression of the small inhibitory isoform of C/EBP β , which is suppressed after *Mycobacterium tuberculosis* infection. Furthermore, IFN- β , a cytokine that is produced in the brain and lung tissues during acute HIV/SIV infection, was shown to enhance the expression of the inhibitory isoform of C/EBP β and suppress SIV replication in macrophages of rhesus macaques.

Urokinase-type plasminogen activator (uPA) signaling has been shown to inhibit a late step in HIV-1 assembly, affecting the maturation and release of HIV-1 from infected MDMs and monocyte cell lines. uPA is a serine protease that interacts with a specific GPI-anchored receptor, uPAR (CD87), at the cell surface. uPAR is expressed by inflammatory cells, including macrophages. The uPA-uPAR interaction, involving the engagement of β 1, β 2, and Mac-1 integrins, was found to mediate uPA antiviral effects on HIV-1 release in macrophages, activated PBMCs, and ex vivo cultures of lymphoid tissues that were infected in vitro. Importantly, the loss of control of HIV-1 in the central nervous system (CNS) was correlated with deregulation of uPA and uPAR expression. These studies suggested that HIV-1 infection induces the overexpression of uPAR and consequently the overproduction of soluble uPAR (suPAR) in cerebrospinal fluid (CSF). The excess suPAR in the CSF binds most of the extracellular uPA, preventing it from binding to cell surface-associated uPAR and consequently inhibiting HIV-1.

Free-Virus and Cell-to-Cell HIV Transmission

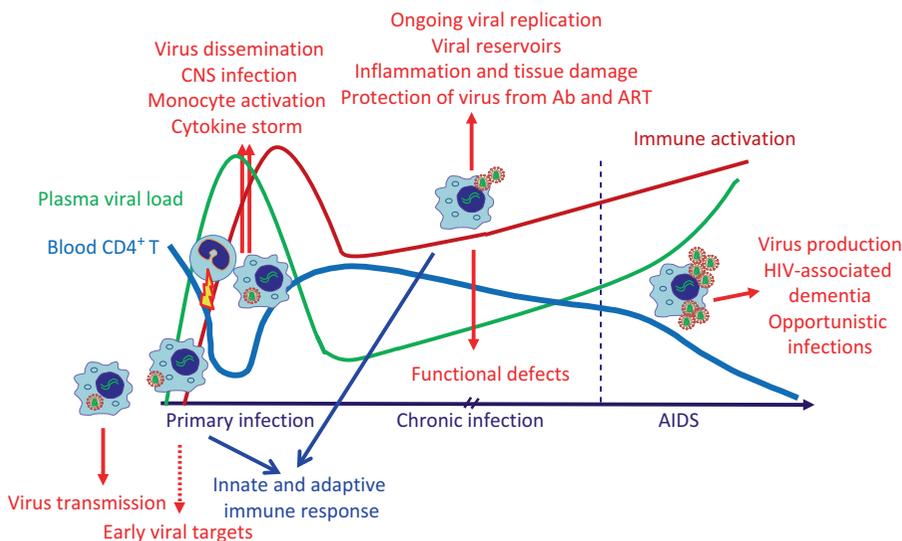
The free-virus transmission mode of HIV-1 was the first to be studied; however, it does not appear to be the most efficient mode of virus transmission. Indeed, following the fusion of HIV-1 Env from an infected cell with the receptors of a target cell, the formation of a cellular junction triggers the formation of membrane nanotubes, and the juxtaposition of the same HIV-1 Env molecular complex with the engaged receptors (CD4, CXCR4, CCR5), integrins (such as integrin

α 4 β 7), tetraspanins (such as CD81), adhesion proteins such as LFA1, ICAM-1, ICAM-3 and tubulin, and the actin cytoskeleton results in the formation of the virological synapse. This membrane structure mediates the cell-to-cell transmission of the virus to target cells (► [HIV-1 Transmission; Cell-Types Associated with](#)). HIV-1 transmission from infected MDMs to uninfected activated CD4 T cells through virological synapses (Fig. 3) (Groot et al. 2008) has been shown to be 20–250 times more efficient than cell-free infection. The formation of the virological synapse between infected MDMs and CD4 T cells occurs via the binding of viral gp120 Env with CD4 on target cells, a process that is dependent on actin and is facilitated by interactions between ICAM-1 and LFA-1. Whereas in cell-to-cell transmission between CD4 T cells, the microtubule-organizing center (MTOC) plays an important role in the formation of the virological synapse, in MDMs the actin cytoskeleton appears to have a more important role in the formation of these cellular junctions. Live fluorescence imaging has revealed that filopodia, which are long membrane projections produced by Mos/M ϕ s and other myeloid cells to probe their environment, protruding from infected MDMs are rich in HIV-1 Gag proteins and likely permit the virus to reach and be disseminated into neighboring cells. Cell-to-cell transmission of HIV-1 from HIV-1-infected MDMs to CD4 T cells is susceptible to epitope-specific broadly neutralizing monoclonal antibodies (bNmAbs) targeting the CD4 binding site (CD4bs) and glycans or glycopeptides but demonstrates resistance to bNmAbs targeting the Env gp41 subunit. In addition, whereas cell-free infection from HIV-1-infected MDMs to autologous CD4 T cells is efficiently inhibited by antiretroviral drugs, corresponding cell-to-cell transmission was reported to be resistant to the action of these drugs. This resistance has been attributed to the high multiplicity of infection of the target CD4 T cells via the cell-to-cell transmission mode. These findings suggest that in tissues where M ϕ s and CD4⁺ T cells are densely packed, antiretroviral penetrance might be reduced due to the increased probability of HIV cell-to-cell transmission.

Monocytes and Macrophages in HIV-1 Pathogenesis

Mos and Mφs are involved in various stages of HIV-1 infection (Fig. 4). They likely contribute to virus transmission via the mucosal route. Indeed, although less frequently infected than CD4⁺ T cells, Mφs account for 20–30% of leukocytes in semen, whereas T lymphocytes comprise only 5%. Semen Mφs may transmit infection at the mucosal surface by different mechanisms. Infected Mφs produce free viruses that may be transmitted to target cells in the lamina propria by crossing the epithelial barrier through breaks in the epithelium. Infected Mφs may also transmit virus to epithelial cells that permit the virus to cross the epithelial barrier via a mechanism called transcytosis, without being directly infected

themselves. Infected Mφs can also migrate through intact mucosa to the lamina propria, where they encounter CD4⁺ T cells and dendritic cells. Mφs are localized to mucosal sites of exposure to HIV-1 during sexual intercourse and may be early targets for the virus. However, the question of whether Mφs are among the first cells infected by the incoming virus is controversial. While some studies suggest that resident macrophages in the female genital mucosa or in the male urethra may serve as early targets for HIV-1, studies in macaque models suggest that the first targets during SIV exposure are primarily CD4⁺ T cells and DCs. Nevertheless, Mφs throughout the body are exposed to HIV-1 very early during infection. In infected macaques, macrophages accounted for 10–12% of SIV-infected lymph node cells 7–12 days after infection (Zhang et al. 1999).



Macrophages in HIV Immunopathogenesis, Fig. 4 Monocytes/macrophages in HIV-1 pathogenesis. Mos and Mφs are involved in all the stages of HIV-1 infection. They likely contribute to virus transmission via the mucosal route. Mφs are localized to mucosal sites of exposure to HIV-1 during sexual intercourse and may be early targets for the virus. Mos/Mφs play a crucial role in viral dissemination to body tissues and transmission to adjacent CD4⁺ T cells. Activated Mos crossing the blood-brain barrier are probably the main vehicles for HIV infection of the central nervous system. Perivascular Mφs and resident microglia are likely responsible for the persistence of low levels of viral replication and abnormal levels of

immune activation in the brain favoring HAND in HIV-infected patients and HAD in the late stages of the disease. Infected Mφs could serve as reservoirs of infectious virus. In addition, Mφs can survive for long periods of time and may be a source of viral rebound after therapy interruption. Mos/Mφs also play an important role in HIV/SIV immunopathogenesis, strongly contributing to immune activation and tissue damage. The defective phagocytic capacity of Mφs in HIV-1-infected patients favors opportunistic infections that are common at the AIDS stage of HIV infection. HIV-1 infection also affects the capacity of Mos/Mφs to induce adaptive responses and their adjuvant functions for adaptive immune cells

Mos/M ϕ s play a crucial role in viral dissemination to body tissues and transmission to adjacent CD4⁺ T cells. Indeed, infected M ϕ s store replication-competent HIV-1 in VCCs for the long term and can efficiently transmit virus to adjacent CD4⁺ T cells and other susceptible target cells (Sharova et al. 2005). Activated Mos crossing the blood-brain barrier have been suggested to be the main vehicles for HIV infection of the central nervous system. Mos in blood and M ϕ s in lymph nodes rapidly increase after SIV infection. Increased trafficking of Mos to the gut occurs in HIV infection and is associated with enhanced expression of homing molecules (Integrin β 7) and chemokine receptors (CCR2/CCL2 axis) (Acute HIV Infection and the CNS). Accordingly, M ϕ number is increased in the goat mucosa of untreated HIV-1-infected patients.

Mos/M ϕ s also play an important role in HIV/SIV immunopathogenesis, strongly contributing to immune activation and tissue damage. During SIV infection of macaques (► [Overview: Immunopathogenesis](#)), the rate of monocyte turnover increases, which suggests tissue infiltration and replacement of damaged M ϕ s. The levels of monocyte turnover in the acute phase of SIV infection predict disease progression (Hasegawa et al. 2009). In the late stages of disease, when CD4⁺ T cells are deeply depleted, HIV-1 can be detected in association with M ϕ s in nonlymphoid organs such as lung, colon, brain, liver, and kidney (Donaldson et al. 1994). When opportunistic coinfections occur, M ϕ s can produce huge amounts of virus and sustain high levels of viremia (Orenstein et al. 1997) (Opportunistic Infections of Global Significance). In macaques infected with a highly pathogenic SHIV that causes a rapid loss of CD4⁺ T cells, long-lived infected resident M ϕ s in tissues sustained high levels of virus production for months. In addition, chronically infected tissue M ϕ s were refractory to treatment with RT inhibitors (Igarashi et al. 2001). Depletion of CD4⁺ T cells before infection of rhesus macaques with SIV increased viral replication in M ϕ s and favored the emergence of SIV viruses harboring envelopes that were able to infect CCR5⁺ cells independently of the CD4 receptor (Ortiz et al. 2011).

M ϕ Dysfunction in HIV Infection

HIV-1 infection provokes functional defects and the destruction of M ϕ s, with consequent impact on innate defenses against microbes, including phagocytosis and the intracellular lysis of pathogens. In vitro HIV-1 infection of MDMs affects FcR-mediated phagocytosis by interfering with FcR signaling. In addition, impaired phagocytosis by Mos and mucosal M ϕ s was described in untreated HIV-1 patients. Similarly, SIV-infected monkeys exhibit gut mucosal M ϕ s with deficient bacterial clearance functions. The defective phagocytic capacity of M ϕ s likely favors opportunistic infections that are common at the AIDS stage of HIV infection. HIV-1 infection also affects the capacity of Mos/M ϕ s to induce adaptive responses and their adjuvant functions for adaptive immune cells. Indeed, defects in antigen uptake and presentation as well as the altered production of cytokines by Mos/M ϕ s have been reported in HIV-1-infected patients.

Relatively few studies have addressed the effects of HIV infection on Mo/M ϕ metabolic activity. It has been shown that HIV-1 affects cholesterol metabolism by Mos/M ϕ s, resulting in the presence of fatty M ϕ s in vessels and likely contributing to cardiovascular disease in HIV-infected patients. Interestingly, accumulation of CD68⁺CD163⁺-activated M ϕ s and high levels of sCD163 (see below) has been described in cardiac tissues of HIV-infected humans with coronary atherosclerosis.

Role of Mos/M ϕ s in Inflammation and Immune Activation in HIV Infection

Primary HIV-1 acute infection causes a strong cytokine storm and high levels of generalized immune activation. Levels of T-cell immune activation following primary infection are stronger predictors of disease progression than plasma viral load (Acute HIV Infection and the CNS). Mos/M ϕ s exert effector functions on the inflammatory response to viruses and nucleic acids and play a key role in driving immune activation in HIV-1-infected patients and SIV-infected macaques. Viral Env binding to the CD4 receptor and viral RNA sensing by TLRs 7 and 8 after viral entry resulted in the secretion of inflammatory

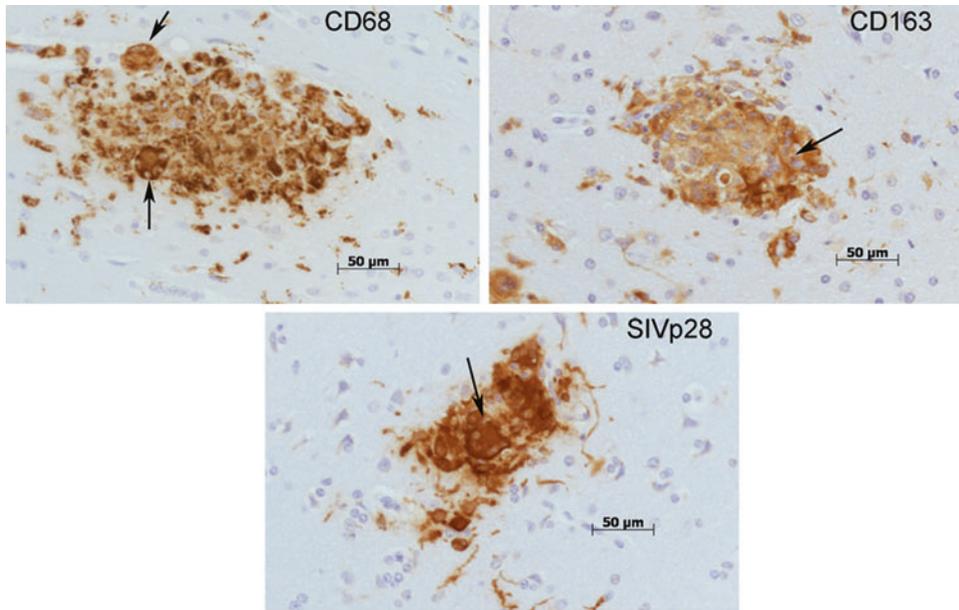
cytokines such as IL-1 and TNF- α . The expansion of monocytes exhibiting a skewed profile toward activated subsets (CD14⁺CD16⁺/CD14⁺CD16⁺⁺) in acute HIV-1 infection was documented long ago (Thieblemont et al. 1995) and has been confirmed by numerous studies. It has recently been shown that levels of the monocyte activation markers IL1RA and sCD14 in plasma are increased during primary infection and that early levels of IL1RA are strong predictors of a T-cell activation set point (Chevalier et al. 2013). Early immune activation in HIV patients and SIV-infected macaques has been proposed to be driven by an early disruption in intestinal epithelial barrier function associated with the massive depletion of intestinal CD4⁺ T cells and microbial translocation. However, using a mutated SIV virus that caused neither CD4⁺ depletion in the gut lamina propria nor microbial translocation (► [Microbial Translocation](#)), levels of immune activation equivalent to those induced by infection with the wild-type virus were detected. Viral replication itself is likely driving Mo/M ϕ activation in primary HIV-1 infection, and Mo/M ϕ activation may contribute to generalized T-cell activation at this early stage. Microbial translocation, favored by intestinal M ϕ destruction and dysfunction, later fuels further activation of the immune system.

A subset of nonclassical CD14⁺CD16⁺⁺ Mos expressing the M-DC8⁺ marker was found to be significantly expanded during chronic HIV infection with active viral replication (Dutertre et al. 2012). M-DC8⁺CD14⁺CD16⁺⁺ Mos express CX3CR1 and CCR2 chemokine receptors and are able to migrate and traffic between body compartments in response to chemokines. These cells are able to secrete TNF α in response to bacterial LPS and may play a major role in the maintenance of chronic immune activation in the gut mucosa.

Role of Mos/M ϕ s in Neuropathogenesis

HIV-1 infection of the central nervous system (CNS) can lead to neurological disease, causing cognitive impairments and eventually leading to HIV-1-associated dementia (HAD) in untreated patients (for review see Burdo et al. (2013) and references herein) (► [HIV-2 Transmission](#)).

Despite the fact that neurons are not directly infected, neuronal damage occurs early in the infected CNS, as indicated by the decreased levels of the CNS metabolite N-acetylaspartate, which is indicative of neuronal loss or dysfunction within weeks of HIV and SIV infection. Diverse factors have been implicated as causes of neuronal damage, including viral proteins such as the gp120 envelope glycoprotein or Tat, proinflammatory cytokines such as TNF- α , which is released by activated Mos/M ϕ s, and microglial activation and the subsequent release of neurotoxins. Although HAD has almost disappeared since the introduction of cART, a substantial proportion of treated patients still exhibit HIV-associated neurocognitive disorders (HAND) despite effective viral suppression. Among the potential causes of HAND, early CNS damage prior to therapy (► [HAND in the Pre-ART and Post-ART Eras](#)), the persistence of low levels of viral replication in the brain, and abnormal levels of immune activation have been suggested. HIV-1 replication in the CNS occurs in perivascular M ϕ s and resident microglia (Fig. 5). Microglia constitutes the stable resident population, and perivascular M ϕ s are repopulated from the bone marrow. Perivascular macrophages expressing the scavenger receptor CD163 are the primary cell type that is productively infected by SIV in the brain. Administering the thymidine analog bromodeoxyuridine (BrdU) to monkeys permits the monitoring of lymphocyte and Mo/M ϕ proliferation and traffic in vivo. It has been shown that BrdU is taken up by monocyte precursors in the bone marrow and is then found in blood monocytes. In monkeys that demonstrated a rapid progression toward AIDS, BrdU⁺ monocyte numbers increased by 8 days post-infection. A second wave of activated monocytes in the blood was detected preceding immunodeficiency and AIDS. Earlier work had shown a correlation between the magnitude of circulating CD14⁺CD16⁺ monocyte subsets and HAD. In the macaque model, soluble CD163 (sCD163) released from activated Mos/M ϕ s and particularly CD14⁺CD16^{+/+++} Mos was highly associated with an increased number of BrdU⁺ monocytes. In addition, levels of sCD163 are elevated in the plasma of HIV-infected patients in the early stages



Macrophages in HIV Immunopathogenesis, Fig. 5 SIV encephalitic lesions in the frontal cortex of an SIV-infected CD8-depleted rhesus macaque: SIVE lesions contain infected macrophages and multinucleated giant cells (*arrows*). Resident (CD68⁺) and perivascular

(CD163⁺) macrophages are present in SIVE lesions and are active sites of productive viral replication (SIVp28⁺) (These images were kindly provided by Drs. Tricia Burdo and Kenneth Williams. TB and KW are supported by NIH grants R01NS082116 (TB) and R01NS040237 (KW))

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of infection and remain high in chronically infected patients, while they decrease in cART-treated patients. However, plasma sCD163 levels were found to be associated with neuropsychological impairment in HIV-1-infected patients under cART. Plasma sCD163 has been proposed to be an early marker of Mo/M ϕ activation that correlates with HIV replication and pathogenesis.

Macrophages as Viral Reservoirs

Despite the paramount success of cART in containing HIV-1 infection and disease, HIV-1 cannot be cured. Abnormal levels of immune activation and HAND persist under cART. The principal obstacle to HIV-1 eradication by cART is the establishment of viral reservoirs, where the virus is not reachable by antiretroviral drugs. Quiescently infected CD4⁺ T memory cells, in which the integrated provirus remains latent and does not express viral proteins, are considered the major HIV-1 reservoir. However, infected M ϕ s could serve as reservoirs of infectious virus. M ϕ s can survive for long periods of time and may be a

source of viral rebound after therapy interruption. Accordingly, macrophage-tropic HIV-1 populations were found to be compartmentalized in cerebrospinal fluid (CSF) and have been associated with the slow decay of virus in the CSF after the initiation of cART, suggesting residual viral replication in long-lived cells, such as M ϕ s. The persistence of infected M ϕ s harboring infectious virus under cART may be attributable to different mechanisms. At the cellular level, as described above, HIV-1 accumulates in VCCs, where it may be protected not only from Abs but also from antiretroviral molecules. Macrophages can transmit the virus directly from VCCs to adjacent cells, and it has been reported that antiretrovirals exhibit reduced inhibitory effects on cell-to-cell transmission (Sigal et al. 2011). It is noteworthy that the activity of antiretroviral drugs, such as nucleoside RT inhibitors or HIV-1 protease inhibitors, was strongly reduced in persistently infected MDMs compared with their activity in acutely infected MDMs (Perno et al. 1988). At the tissue level, viral persistence may rely on ongoing viral

replication in M ϕ s at anatomical sites where the penetrance of antiretroviral molecules may be suboptimal (sanctuaries), as has been reported for the brain, semen, and the gut.

Conclusions

HIV-1 infection subverts the natural roles of Mos/M ϕ s in innate defenses and tissue homeostasis, transforming them into vehicles for dissemination and shelters for persistence. Infection of Mos/M ϕ s affects their functions and thus contributes to HIV-1-associated morbidity, including cardiac and CNS diseases. Overall, together with CD4⁺ T-cell depletion, Mo/M ϕ infection drives the progression of HIV-1 infection to AIDS. Therapeutic strategies for the treatment of HIV-1 infection should improve the focus on Mos/M ϕ s as fundamental targets.

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Macrophage-Specific Aspects of HIV-1 Infection

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Definition

Macrophages are a heterogeneous population of myeloid leukocytes that perform diverse innate and adaptive functions of the vertebrate immune system. They are principally defined as being differentiated, tissue-resident forms of monocytes, with a phagocytic capacity, relatively large size, and expression of various functional markers on their cell surface. Notably, macrophages express the HIV/SIV entry receptors CD4 and CCR5/CXCR4 and have long been known as *in vivo* and *in vitro* targets of infection. However, HIV and SIV that infect CD4⁺ T cells vary in their capacity to infect macrophages (macrophage

tropism). This variability can be attributed to macrophage-specific characteristics and differences among virus variant, principally in the ability to enter macrophages, as well various levels of restriction outlined in this entry and adaptations that the virus uses to overcome these restrictions.

Introduction

While CD4⁺ T cells constitute the majority of infected cells during *in vivo* HIV infection, other important targets of infection are myeloid cells, predominantly macrophages. T cells and macrophages are vastly different in morphology, proliferative capacity, gene expression profile, and interaction with HIV, yet both can become infected and support viral replication.

In the central nervous system (CNS), the majority of infected cells are myeloid cells (macrophages and microglia), and these are responsible for neurological aspects of disease. Outside the CNS, macrophages are involved in events at mucosal surfaces during transmission, can serve as long-lived reservoirs, and may contribute substantially to virus production especially in late-stage disease when CD4⁺ T cells are largely depleted (Blankson et al. 2002). Thus, comparative studies of HIV infection of macrophages versus T cells continue to be of great interest. Much of the current understanding of HIV molecular pathogenesis comes from studies using tissue culture-adapted viruses and transformed cell lines that resemble neither T cells nor macrophages. It is therefore essential to consider the HIV life cycle in the context of the very different primary cell types these viruses can infect. While the primary focus of this review is HIV-1, critical insights have been gained through studies of SIV, and so we will indicate where data is extrapolated from SIV to the context of HIV-1.

Macrophage Models Used to Study HIV/SIV

Much of the understanding of HIV biology has been generated through studies in transformed

cell lines, but while useful they do not always reflect unique features of HIV/primary cell interactions. Monocyte-/macrophage-like cell lines exist that are sometimes used for HIV infection studies (U937, THP-1, MonoMac, HL60), but since they may not faithfully recapitulate primary cell interactions with the virus, primary cells are often used. As cell-specific experimental targets for HIV, monocyte-derived macrophages (MDMs) are most often generated by *ex vivo* culture and differentiation of blood-derived monocytes. In addition, more physiologic and phenotypically diverse tissue-associated macrophages can be isolated directly from human and nonhuman primate mucosal regions such as the gut, lung, and vaginal tract, as well as from the secondary lymphoid organs, liver, kidneys and central nervous system (Cassol et al. 2006).

Phenotypically, macrophages are distinguished from other leukocytes by their propensity to adhere to culture surfaces, their large irregular morphology, and surface expression of MHC (major histocompatibility complex) class II as well as myeloid markers such as CD14, CD16, CD68, and CD156. Functionally, macrophages are highly diverse, with important roles ranging from innate immune control, inflammation, phagocytosis, and antigen presentation to anti-parasitic and wound healing.

Traditionally, HIV-1 isolates have been phenotypically classified as macrophage tropic (capable of infecting primary macrophages and primary CD4⁺ T cells but not transformed T-cell lines), T-cell-line tropic (capable of infecting primary CD4⁺ T cells and transformed T-cell lines but not primary macrophages), or dual tropic (capable of infecting all three target cell types *in vitro*). The term “macrophage tropic” was formerly used interchangeably with “CCR5 tropic” in reference to HIV entry receptors, a correlation that is now recognized to be highly imperfect, as will be discussed in detail below (Goodenow and Collman 2006). Today, “macrophage tropism” in the context of lentiviral infection generally refers to the ability of a virus to infect and replicate in *ex vivo* cultured macrophages and/or the ability of viral components (such as the envelope glycoprotein) to mediate

infection of macrophages in a single-round assay (Duncan and Sattentau 2011).

Interestingly, monocytes are much more resistant to HIV-1 infection *in vitro* than differentiated macrophages. *In vivo*, HIV DNA can be found in circulating CD14⁺ monocytes, although typically at levels considerably lower than CD4⁺ T cells. Monocytes impact HIV pathogenesis partially because they are directly infected by the virus and may be involved in trafficking virus into tissues such as the brain, and partially through bystander and indirect effects associated with immune activation (Kuroda 2010).

Macrophage-Specific HIV Entry

The viral envelope (Env) glycoprotein of HIV-1 (as well as HIV-2 and SIV) is comprised of the surface subunit gp120 and transmembrane subunit gp41, which are organized as trimeric units (trimer of heterodimers) on the virus surface. Entry is mediated by gp120 engagement of cellular CD4, which results in structural changes to gp120 that enable interactions with one of two principal cellular coreceptors, CCR5 or CXCR4. Further structural changes in gp41 then lead to ► [fusion](#) of the viral and cellular membranes and viral entry (Wilens et al. 2012).

Entry Receptor Use as Determinant of Macrophage Tropism

Early studies on HIV-1 suggested that macrophage-tropic viral isolates used CCR5 as a coreceptor, whereas viruses that could not infect macrophages used CXCR4. However, it is now recognized that CCR5 is the principal coreceptor for most HIV-1 strains, and a gradation of macrophage tropism exists among them that is determined by the ability to utilize CCR5/CD4 in the context of their expression levels on the surface of primary cells. Conversely, macrophages also express low levels of CXCR4, and while T-cell-line-tropic HIV-1 strains that use CXCR4 for entry are usually not able to enter macrophages efficiently, some primary isolates that use CXCR4 for entry can use macrophage CXCR4. Dual-tropic HIV-1 variants, which use both CCR5 and

CXCR4, may enter macrophages through either or both pathways. Despite this plasticity in macrophage coreceptor use, however, it is true that most macrophage-tropic HIV variants studied use CCR5. In contrast, non-macrophage-tropic variants may use CCR5, CXCR4, or both (Goodenow and Collman 2006).

Among CCR5-using viral variants, efficiency of CD4 use is a major entry determinant of macrophage tropism. Macrophages express less CD4 on their cell surface than CD4⁺ T cells, and most macrophage-tropic viral variants are capable of infecting cells expressing little CD4. The viral Env determinants of coreceptor usage (CCR5 versus CXCR4) map in large part although not exclusively to the third hypervariable (V3) domain (Dragic 2001). Among R5 variants, the viral Env determinates that regulate the efficiency of CD4 use and thus macrophage tropism are more varied and include changes in the first and second variable loops (V1/V2) as well as loss of glycosylation in the CD4-binding site and second constant domain (C2) (Duncan and Sattentau 2011).

Of note, different tissue macrophages vary in their expression of CCR5 and thus permissiveness to HIV-1 infection. For example, vaginal mucosal macrophages are susceptible to infection *in vitro* and in the rhesus macaque/SIV model *in vivo*, whereas small intestinal mucosal macrophages are reported to lack entry coreceptors and resist infection (Hladik and McElrath 2008).

Endocytosis in Macrophage Entry

HIV entry occurs predominantly at the plasma membrane surface, in contrast to viruses that enter via endocytic pathways. However, some models suggest that HIV entry into macrophages (and some cell lines) may involve endocytic pathways, although its relative importance remains to be fully defined (Wilén et al. 2012).

Immunological Correlates of Macrophage Tropism

Macrophage-tropic HIV-1 strains are generally more sensitive to neutralization by HIV⁺ serum and monoclonal antibodies than non-macrophage-tropic variants. At least one explanation for this is that these variants exhibit increased

constitutive exposure of regions within Env, such as the coreceptor interacting domains, that enable viral entry in the presence of reduced CD4 levels. These regions are highly vulnerable targets of neutralizing antibodies. In contrast, variants that are not macrophage tropic normally shield such epitopes until entry is triggered by CD4 (Bhattacharya et al. 2003). Macrophage-tropic variants are able to tolerate this enhanced sensitivity to neutralization because they typically reside in immune-privileged compartments like the CNS, emerge in late-stage disease, or possibly avoid antibodies by infecting new target cells via cell-to-cell spread.

Anatomic Compartmentalization of Macrophage Infection and Macrophage Tropism

The neutralization sensitivity of macrophage-tropic viral variants has implications reflected in observations of HIV infection *in vivo*. While infected macrophages can be found in the gut, vaginal tract, and lungs, infected macrophages and highly macrophage-tropic viral variants are most abundant in the CNS. In patients with AIDS-associated neurological disorders, microglia and perivascular macrophages are the principal infected cells and viral reservoir in the CNS, rather than T cells. The propensity of macrophage-tropic HIV-1 to preferentially emerge in the CNS is likely due to both the abundance of myeloid cells and paucity of T cells in this compartment and the relative shelter from peripheral immune pressure that allows the low-CD4-using, neutralization-sensitive variants to exist (Bhattacharya et al. 2003).

Viral RNA can be detected in the cerebrospinal fluid in most HIV-infected individuals who are not on antiretroviral treatment (ART) (Churchill and Nath 2013), and as many as 40–70% of untreated individuals experience some form of HIV-associated neurological disorder (Yadav and Collman 2009). However, productive infection of macrophages in the CNS may be less common, as infected CNS-resident cells have been detected in approximately 20% of patients when examined during autopsy (Churchill and Nath 2013). Based on decay rate following ART initiation,

CSF virus appears to be derived from a combination of short-lived and long-lived cells, with the former likely reflecting virus originating from blood T cells and the latter likely originating from brain macrophages and more dominant in individuals with more severe neurological disease.

While non-CNS HIV-1 variants are typically poorly macrophage tropic, macrophage-tropic variants may be more often isolated from the blood and lymph nodes of late-stage and highly immunodeficient AIDS patients. Like the immune-privileged CNS, emergence of macrophage-tropic viral variants in late-stage disease with advanced T-cell loss may also reflect a setting in which there are limiting numbers of T-cell targets combined with diminished immune pressure. Consistent with this notion, studies in the rhesus macaque model infected with SIV or SHIV (SIV/HIV chimeras) show that macrophages may be an important, or even principal, reservoir in very-late-stage disease when nearly all detectable CD4⁺ T cells have been depleted or following experimental CD4⁺ T-cell depletion (Blankson et al. 2002).

Postentry Early Events in Replication Cycle

Following fusion and entry, the viral genome is reverse transcribed from RNA into DNA and transported into the nucleus within a structure known as the pre-integration complex, comprised largely of viral capsid proteins.

Cellular Restriction Factors

Mammalian cells have a variety of intrinsic proteins that have, as major (if not exclusive) functions, the ability to restrict incoming retroviruses, including among them lentiviruses such as HIV and SIV. In cases where virus successfully establishes infection in target cells, it either has evolved to avoid these restriction factors or has acquired specific genes to impede their function. These restriction factors are typically interferon stimulated, contributing to innate

immunity augmenting intrinsic cellular resistance to infection.

A cellular restriction factor that has a unique role in macrophages is SAMHD1, which impedes HIV-1 reverse transcription by hydrolyzing deoxynucleosides in the cytoplasm. As nondividing cells, macrophages have lower pools compared to T cells of deoxynucleoside precursors required for reverse transcription of viral RNA into DNA (Gavegnano et al. 2012). HIV-2 and most SIVs encode a *vpx* gene, whose gene product interferes with SAMHD1 and enhances viral permissiveness in macrophages. In some SIVs lacking *vpx*, a similar function is ascribed to Vpr, but HIV-1 lacks both the *vpx* gene and any as-yet-identified gene with equivalent function. Nevertheless, HIV-1 does infect macrophages in vitro and in vivo, and macrophage-tropic Env allows productive infection of macrophages in vitro, despite the absence of Vpx, relatively high levels of SAMHD1 and low levels of nucleotides in the cytoplasm. This suggests that SAMHD1 restriction of HIV-1 modulates but is not sufficient to fully block infection of macrophages.

The APOBEC3 family of proteins restricts virus replication by multiple mechanisms, the most well defined of which is cytosine deamination during reverse transcription that leads to G-to-A hypermutation, unless countered in the virus-producer cell by the virally encoded Vif protein. While APOBEC3 proteins are not known to differentially restrict HIV infection in T cells versus macrophages, it has been suggested that APOBEC3 expression in immature blood monocytes contributes to their resistance to infection relative to differentiated macrophages, but this line of evidence is not yet conclusive. TRIM5 α and related members of the TRIM family are intrinsic cellular resistance factors that target the capsid of incoming viruses for degradation. While simian TRIM proteins block efficient HIV-1 infection, HIV-1 has evolved to avoid targeting by human TRIM proteins. TRIM proteins are expressed in both macrophages and T cells, and some evidences suggest that different members of the large TRIM are differentially expressed in

macrophages compared to T cells, although they likely function similarly in the different cell types (Bergamaschi and Pancino 2010).

Antiviral restriction factors place HIV and SIV under continuous evolutionary pressure to avoid triggering innate host immune signaling while maximizing replication and target cell tropism. For example, the cellular DNase TREX1 degrades cytoplasmic viral DNA, but rather than acting as an antiviral mechanism, this process actually enhances HIV infection because the virus hijacks the TREX1 system in order to prevent self-integration and reduce the risk of triggering an interferon response, particularly in T cells. In contrast, viral genomes persist for a longer time in myeloid cells and thus are more prone to detection by innate immune sensors. One current hypothesis is that the inability of HIV-1 to antagonize myeloid-specific restriction factors leads to reduced infection of macrophages and dendritic cells and, consequently, only weak triggering of the interferon response in these cells. In contrast, HIV-2 and various SIVs, which possess Vpx and Vpr proteins that overcome restriction in these cells, may then trigger a robust interferon response that in turn reduces viral replication across multiple cell types (Yan and Lieberman 2011). However, despite this intriguing and plausible hypothesis, there is as yet no evidence that in vivo macrophage infection is greater in SIV compared to HIV or that the viruses elicit distinct interferon responses in vivo (Mashiba and Collins 2013).

Nuclear Migration and Integration

Macrophages differ from activated CD4⁺ T-cell targets of infection in that they are terminally differentiated nondividing cells. In contrast to other retroviruses, which are unable to enter the nucleus of nondividing cells, lentiviruses including HIV have the ability to efficiently enter the nucleus of nondividing cells and establish ► [integration](#). The viral Vpr protein plays a major role in enabling nuclear migration in nondividing cells, interacting with cellular proteins to allow nuclear entry without disruption of the nuclear envelope, and thus is an important factor for efficient

macrophage infection. In addition to Vpr, the viral matrix, capsid, and integrase proteins also contribute to efficient nuclear migration in nondividing cells. On the other hand, Vpr plays an important role during infection of proliferating activated CD4⁺ T cells by inducing cell cycle arrest, a function that is not operative in nondividing macrophages (Kogan and Rappaport 2011).

HIV-1 has a distinct predilection for integration into genes and especially into transcriptionally active genes. This is a feature common to both macrophages and CD4⁺ T cells. One difference between cell types, however, is that while integration is considered essential for efficient viral gene expression in CD4⁺ T cells, some models suggest that low levels of transcription from unintegrated viral DNA may occur in macrophages, driven by Vpr and enabled by the non-proliferating nature of macrophages that allows persistence of unintegrated genomes (Le Douce et al. 2010).

Viral Expression: Transcription, RNA Processing, and Translation

Key factors that determine whether an integrated virus is productively expressed or latent include the complement of cellular transcription factors present and state of cellular activation. In macrophages in particular, the relatively quiescent activation state compared to activated CD4⁺ T cells and distinct transcription factor patterns are associated with generally lower levels of viral gene expression. Compared to CD4⁺ T cells, macrophages possess higher ratios of repressive forms of transcription factors such as C/EBP β , Sp3, and OKT18. In addition, macrophage-specific chromatin conformations and recruitment of histone deacetylases to the HIV-1 promoter may also contribute to relative transcriptional quiescence. Together, these factors contribute to overall lower viral expression in macrophages compared to T cells and may also contribute to the potential for long-term survival of infected cells via decreased direct cytopathogenicity or immune recognition in vivo (Le Douce et al. 2010).

Viral Assembly and Release

Following structural gene expression, the virus is assembled and is released from the cell, either as cell-free virus at the plasma membrane or, perhaps especially in the case of macrophages, via cell-to-cell transfer through viral synapses. Differences in viral assembly and release between macrophages and T cells are a dynamic area of research.

Budding Versus Exocytosis

In macrophages, mature HIV-1 virions have been observed in large vesicular structures within cells. There is long-standing debate as to whether this reflects budding into intracellular multivesicular body-like structures or into deep invaginated pockets of the cell membrane. Thus, it is also unclear whether HIV-1 is released from macrophages following fusion of these multivesicular bodies with the plasma membrane (exocytosis), or if the virus buds from the cell surface as it does other target cells, only into these invaginated pockets (Carter and Ehrlich 2008). In either model, the nature of the budding site is believed to provide a mechanism shielding virions from immune surveillance.

Tetherin

The host cell restriction factor tetherin inhibits release from the cell surface of HIV-1 virions (as well as many other enveloped viruses). The HIV-1 viral protein Vpu counteracts tetherin by promoting its degradation, enabling release of virus from the cell surface. Macrophages express high levels of tetherin relative to primary T cells. Release of virus carrying mutant Vpu is more attenuated in macrophages *in vitro* than in CD4 lymphocytes, which have lower levels of tetherin. This implies that tetherin may play a particularly significant role in viral restriction in macrophages relative to other target cell types (Dube et al. 2010).

Cytopathicity and Reservoir Function

Macrophages are relatively resistant to the cytopathic effects of HIV-1 infection *in vitro* and are

able to produce virus for prolonged periods of time, in contrast to productively infected CD4+ T cells that are rapidly killed following infection. The *in vivo* half-life of tissue macrophages is estimated to be approximately 2 weeks in healthy individuals. *In vitro*, the lifespan of macrophages is not reduced by HIV infection, and current dogma that the same is true *in vivo* (Blankson et al. 2002), although this remains an active area of research (Cassol et al. 2006; Kuroda 2010).

In Vivo Reservoir

Infected individuals receiving antiretroviral therapy, even those with viral loads below the limit of detection for many years, experience viral rebound after termination of therapy. In addition, even when antiretroviral therapy reduces plasma viremia below the threshold of clinical assays, most patients maintain very low levels of plasma virus that can be detected with ultrasensitive methods. The source of both persistent low-level plasma virus on treatment and rebound virus when therapy is stopped is a key area of current investigation. While productively infected activated CD4+ T cells survive for only a short time *in vitro* and *in vivo*, resting CD4+ T cells that are latently infected are one important reservoir for long-term persistence. In addition, because of their resistance to viral cytopathicity and long natural lifespan, macrophages have the potential to serve as another long-lived HIV-1 reservoir in patients treated with antiretroviral therapy (Le Douce et al. 2010). Because macrophages penetrate and reside deep within tissues, they may be particularly shielded from antiretroviral drugs due to their anatomic localization.

Brain macrophages are an especially important current focus of interest as a long-term reservoir, given the low tissue-specific bioavailability of many antiretroviral drugs in the CNS. Although infected macrophages have been found in many tissues, they are the predominant infected cell type in the brain. In addition to their role in viral persistence, they are the source of viral expression and inflammation contributing to HIV-1-associated neurological disorders, even in infected individuals with undetectable peripheral viral loads on long-term antiretroviral therapy

(Yadav and Collman 2009). Furthermore, CNS viral load exhibits slower decay kinetics than does plasma viral load following antiretroviral therapy, although viral RNA is generally undetectable in the CNS in long-term-treated patients (Blankson et al. 2002).

Consequences of HIV-1 Infection for Macrophage Function

In contrast to the clear evidence that HIV-induced CD4+ T-cell loss and dysfunction lead to immune dysfunction and AIDS, it is not clear whether there is substantial macrophage immune dysfunction induced by HIV infection that directly contributes to immunodeficiency. On the other hand, monocyte-macrophage activation induced by HIV-1 binding and/or infection, or by other consequences of infection such as gastrointestinal tract mucosal damage and chronic microbial translocation, clearly contributes to disease pathogenesis. Generalized immune activation is a central feature of HIV pathogenesis, and levels of the monocyte activation marker soluble CD14 are strongly linked to disease. In HIV-infected people, blood monocytes show changes in transcriptomic profiles, and in SIV infection monocyte turnover is linked to rapid disease progression (Kuroda 2010).

Unlike most neurotropic or neurovirulent viruses, HIV does not directly infect neurons, and HIV-associated neurological disorders result from indirect effects mediated by glial cells, primarily infected or activated brain macrophages and microglia. Viral Env binding to the CD4/chemokine coreceptor complex triggers intracellular signaling cascades, which lead to release of cytokines, chemokines, and other mediators that augment inflammation. In addition, productive macrophage infection leads to release of neurotoxic cytokines and small-molecule mediators that injure neurons. Thus, HIV-induced activation and infection of macrophages are responsible for the indirect neuronal injury seen in HIV-associated neurological disorders, which persist albeit at lesser degrees of severity even in people on antiretroviral therapy (Yadav and Collman 2009).

Conclusion

HIV-1 infects macrophages both in vitro and in vivo. Several aspects of HIV-1 infection in macrophages distinguish infection of these cells from other HIV-1 target cells. HIV-1 viruses that infect macrophages are primarily CCR5 tropic and generally neutralization sensitive given their adaptation to low levels of CD4 expressed on these cells. A number of host restriction factors and other cellular proteins dictate unique aspects of HIV-1 entry, reverse transcription, nuclear migration, and egress in macrophages relative to HIV-1 infection of CD4 T cells. HIV-1 infection of macrophages in the CNS has a well-defined role in AIDS-associated neurological disorders and an likely albeit less clear role in other tissues. Importantly, macrophages may be a reservoir of persistent infection in patients treated with antiretroviral therapy. Defining the specific role of macrophages in disease progression, understanding mechanisms of macrophage HIV-1 infection, controlling HIV-1-triggered macrophage immune activation, and finding ways to successfully eliminate persistent macrophage reservoirs will be central to developing successful therapies to treat and perhaps block HIV-1 infection.

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Male Condoms

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Definition

The male condom is a barrier method for the prevention of HIV and sexually transmitted infections (see entries “► [Immunopathogenesis of HIV Coinfections](#),” “► [Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#),”

“► [Immunopathogenesis of HIV Coinfections](#)”) and for the prevention of pregnancy (see entries “► [Pregnant Women: Care and Treatment](#),” “► [Preventing Mother-to-Child Transmission of HIV-1](#),” and “► [Behavioral Aspects of HIV Mother-to-Child Transmission](#)”). When placed over a man’s erect penis, it is designed to cover the penis during sexual intercourse and physically block ejaculatory fluids, sperm cells, and infectious agents from entering the sexual partner’s body. The male condom is designed to cover both the glans and shaft of the penis and may have a reservoir tip to provide space for the ejaculate. For effective use, the condom must be placed over the penis before genital contact. Modern condoms are manufactured from a variety of materials including natural latex, synthetic materials such as polyurethane and polyisoprene, and natural membranes including sheep intestine (“lambskin”).

Introduction

Male condoms appear to have been the first barrier contraceptive designed for male use. Evidence from ancient artwork and the written record suggests that condoms may have been used for contraception thousands of years ago: a 12,000-year-old example of cave art discovered in France depicts a man and woman engaging in sexual intercourse with the penis covered (Collier 2007). In addition, over 3,000 years ago in Egypt, written records describe men wearing a sheath covering only the glans of the penis, made of oiled animal intestines or bladders. It is possible that these sheaths were intended for use during sexual intercourse to prevent pregnancy or infection. However, the earliest unambiguous description of a condom to be used specifically during sexual intercourse, developed for protection against syphilis, was made in the 1500s by the Italian anatomist and physician Gabriello Fallopio (Youssef 1993).

Currently, the male condom is a popular form of birth control and protection from HIV and other sexually transmitted infections. Condoms are safe, easy to use, and widely available. Condoms can increase sexual pleasure for some couples by

prolonging an erection and preventing premature ejaculation. Additionally, containment of semen within the condom may decrease the messiness of sexual intercourse. However, the male condom also has several disadvantages. Many men report a decrease in sensitivity and sensation during sexual intercourse with condom use. In addition, placement of a condom on the erect penis may interrupt sexual intercourse.

Many of the possible disadvantages of condom use may be overcome through technical advances and behavioral shifts. For example, one brand of condom has been introduced with a design innovation meant to address complaints about reduced sensitivity and sensation. Instead of the typical tubular form, the condom is manufactured with more room at the tip of the condom so that it fits snug at the base of the penis, but loose around the glans. This design is intended to provide more sensation during sexual intercourse by allowing the loose material to move more, providing more friction. As a second example, some safe-sex interventions (see entries “► Behavioral Science Highlights of Evidence and Research,” “► HIV Prevention and Women,” “► HIV Prevention for Serodiscordant Couples,” “► HIV Prevention in Youth” “► HIV Prevention for MSM,” “► HIV-1 Transmission: Influence of Bodily Secretions,” “► HIV-2 Transmission,” “► Prevention of Alcohol-related HIV Risk Behavior,” and “► Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk”) have specifically addressed complaints about interrupting sexual intercourse to place a condom (Shain et al. 2004). If the receptive partner or both partners erotically incorporate the placement of the condom into sexual foreplay, then the perception of condom use as interrupting sexual activity may be reduced or eliminated.

Effectiveness of Condoms in Preventing Pregnancy and Sexually Transmitted Infections

It is important to distinguish between the efficacy of the male condom, which refers to the protection that the male condom affords when used perfectly,

and the effectiveness of the condom, which refers to the protection actually afforded by the condoms when used in the real world. The effectiveness of male condoms in preventing pregnancy and sexually transmitted infections is dependent on correct and consistent use. For correct use of the condom, several guidelines must be followed. First, the condom must be placed on the penis before genital contact begins. Pre-ejaculatory fluid may contain viable sperm cells and infectious agents; in addition, penile skin may harbor mucosal viruses such as the ► [human papillomavirus \(HPV\)](#). Second, the condom must remain intact and covering the penis throughout sexual intercourse. If the condom breaks, the sexual partners should cease genital contact, and a new condom should be placed on the penis before resumption of genital contact. Third, after ejaculation, the condom should be held at the base and the penis should be withdrawn while it is still erect, to prevent leakage or spillage of semen. A new condom should be used for each act of sexual intercourse.

With correct and consistent use, the male condom has been shown to prevent pregnancy, HIV, and many other sexually transmitted infections with high efficacy. Laboratory studies have clearly shown that the latex condom is an impervious barrier to sperm cells and sexually transmitted bacteria and viruses, including HIV. Clinical studies have shown that the condom protects the user and the partner against sexually transmitted infections, including HIV, gonorrhea, chlamydia, ureaplasma, trichomoniasis, and herpes simplex virus. The data on protection from HIV is more comprehensive than for other sexually transmitted infections. For HIV, studies of serodiscordant couples (see entry “► HIV Prevention for Serodiscordant Couples”) followed over time have shown that latex condoms are highly effective in protecting against HIV: in two large studies of serodiscordant couples, researchers found that between 0 and 2% of consistent condom users seroconverted over a 2-year period, versus 10–12% of couples who were not using condoms consistently (Saracco et al. 1993; de Vincenzi 1994).

The effectiveness of condoms in preventing human papillomavirus infections (HPV) is

reduced in comparison with other sexually transmitted infections. This is thought to be because the human papillomavirus may be resident on the skin in areas not covered by the condom. Research on human papillomavirus infection in men has shown that HPV can be detected in a variety of anatomic sites including the glans of the penis and the shaft of the penis and on other areas of the skin surrounding the genitals.

Latex Condoms

Most modern condoms are manufactured from latex, due to the material's strength, elastic properties, and impermeability to sperm, viruses, and bacteria. Latex condoms can be stretched to over 800% before breaking and have been shown to far exceed the minimum strength required for effective use. During the manufacturing process, latex condoms are tested for holes using an electric current. Latex condoms deteriorate when exposed to ultraviolet light and therefore should be packaged in foil or with opaque packaging on both sides. Material degradation over time is a concern with extended storage of condoms. Extended storage before distribution is less likely to occur in highly developed countries; however, longer supply chains may exist in some countries with the greatest burden of HIV/AIDS. Extended storage and unpredictable distribution of condoms, notably in developing countries with tropical or desert climates, may increase the rate of condom deterioration. Nevertheless, research on condom testing after extended storage has demonstrated that stored condoms may still be uniform in strength and therefore suitable for use.

The characteristics of latex have made it the most popular material for condom manufacture. However, a significant drawback of this material is that the latex is damaged by oil-based lubricants such as petroleum jelly, mineral oil, and other substances. These oils, and lubricants based on these oils, result in a loss of elasticity in the latex, leading to condom breakage as well as slippage during sexual intercourse. Therefore, if lubricants are used with latex condoms, water-based lubricants or other non-oil-based lubricants should be used to avoid material degradation.

Approximately 1–5% of the general population around the world is sensitive to latex, with increased prevalences of latex allergy among highly exposed occupational groups, including health care workers and rubber industry workers. Latex allergic reactions make the use of latex condoms problematic for these individuals. Condoms manufactured from different materials are available, including natural membranes and non-latex synthetic materials such as polyurethane and polyisoprene.

Natural Membrane Condoms

Natural membrane or “lambskin” condoms, typically manufactured from sheep intestine, are one alternative for people with sensitivity to latex. Natural membrane condoms offer protection against pregnancy similar to the protection offered by the latex condom. However, the porous nature of the intestinal membrane results in reduced protection against many sexually transmitted infections. While sperm cells are too large to pass through the pores of the membrane, the pores have been shown to be large enough to allow the passage of viruses including HIV, herpes, and HPV. The natural membrane used in the manufacture of these condoms is considerably stronger, but less elastic than latex, and reportedly transmits more body heat and a more natural sensation during sexual intercourse. Natural membrane condoms are significantly more expensive than latex condoms.

Synthetic Condoms

Condoms are currently manufactured using several synthetic materials as alternatives to latex or natural membranes. Polyurethane condoms are thinner than latex condoms, allowing greater sensitivity and more transmittal of body heat. They are odorless and colorless, reportedly twice as strong as latex, and unlike latex condoms, they can be used with oil-based lubricants. In comparison with latex condoms, polyurethane condoms are less sensitive to ultraviolet light and

temperature and are therefore more resistant to deterioration in storage. However, polyurethane condoms may be noisy during sexual intercourse and may require more lubricant than latex condoms. Laboratory tests have found polyurethane condoms to be as effective as latex condoms in preventing the transmission of sperm and sexually transmitted infections including HIV. However, polyurethane condoms may be less elastic than latex condoms, resulting in a looser fit, higher potential for breakage and slippage, and therefore potentially reduced real-world efficacy against pregnancy and sexually transmitted infections.

Other synthetic materials used in the manufacture of condoms include AT-10 resin and polyisoprene. Polyisoprene condoms cannot be used with an oil-based lubricant but are softer and more elastic than polyurethane condoms.

Spermicidal Lubricants

Surveys have shown that a majority of condom users prefer lubricated condoms. Lubricants may contain spermicidal agents or antimicrobial agents, potentially increasing the efficacy of condoms for pregnancy prevention as well as prevention of sexually transmitted infections. One type of spermicidal lubricant, nonoxynol-9 (N-9), is a surfactant that causes damage to sperm membranes. Results of HIV studies using N-9 have provided evidence suggesting that N-9 may increase the risk of HIV infection. One prominent study found approximately 50% more HIV infections among sex workers using N-9 versus placebo. If N-9 increases the risk of HIV, this effect may be due to increased vaginal irritation and mucosal inflammation (see entry “► [Inflammatory Cytokines](#)”), potentially decreasing the body’s defense against HIV. For example, increased mucosal inflammation may result in recruitment of HIV-target immune cells to the sites of virus entry. Concerns about the possible adverse effects of N-9 have led to condom manufacturers including alternative lubricants in condoms.

Conclusion

The basic form and function of the male condom has been in place for at least 450 years and perhaps much longer. Modern materials and manufacturing technology have improved the male condom and reduced the cost to the point that it is a widespread and effective public health tool for the prevention of HIV, other sexually transmitted disease, and pregnancy in developed and undeveloped countries around the world. When used correctly and consistently, it is a highly effective tool to prevent the sexual transmission of HIV.

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Malignancies in Children with HIV Infection

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Definitions

Children with HIV infection have an increased incidence of malignancies. A number of them are similar to those tumors that are associated with HIV infection in adults, such as Kaposi

sarcoma (KS), aggressive non-Hodgkin lymphoma (NHL), and ► [cervical cancer](#). Additionally, HIV-infected children have a higher risk than older patients of developing leiomyomas and leiomyosarcomas associated with ► [Epstein-Barr virus](#). The overall incidence of malignancies in HIV-infected children is smaller than in HIV-infected adults, but children's relative risk is higher because the incidence of cancer is usually low in this age group. HIV-associated cancers in children are now a health problem especially in Africa, because of the high incidence of pediatric HIV infection and the high prevalence of infections with oncogenic viruses responsible for many of these tumors.

Changes in the Incidence of Childhood Cancers Associated with the HIV Epidemic

In the adult population subgroup infected with HIV, the lifetime incidence of cancer has been found to be as high as 40% (Flint et al. 2009). Certain tumors, such as Kaposi sarcoma (KS), or non-Hodgkin lymphomas (NHL) are much more frequently encountered in the adult HIV-positive population subgroup and for this reason have been identified as AIDS-defining cancers. Invasive ► [cervical cancer](#) is also considered AIDS-defining. However, besides these three, numerous other malignancies are found more frequently in HIV-infected adults: the list includes ► [Hodgkin lymphoma](#), anal cancer, lung cancer, liver cancer, nonmelanocytic skin cancer, myeloma, leukemia, and conjunctival tumors (Engels et al. 2008).

Similarly, the HIV epidemic in children (0–14 years) was associated with an increase in the incidence of certain cancers, especially non-Hodgkin lymphomas, soft tissue tumors, cancer of the uterine cervix in adolescent girls, as well as thyroid and pulmonary neoplasms. It is estimated that about 2% of children with HIV will develop a cancer as their AIDS-defining disease, as compared to an incidence of malignant disease in the general pediatric population of around 0.0013% (Mueller 1999). The highest incidence increase with AIDS in the United States and Europe was

observed for NHL, followed by leiomyomas and leiomyosarcomas, associated with ► [Epstein-Barr virus \(EBV\)](#) infection. Kaposi sarcoma remained, in these regions, rare by comparison with adults living with HIV. It should be noted that while the incidence of NHL in children with AIDS in the United States is substantially lower than in adults, the standardized incidence ratio of NHL is higher in children because the background rate in the general population is substantially lower.

With the development of tests to screen blood products for HIV and the use of antiviral drugs to prevent maternal-child transmission during birth, the incidence of HIV infection in children has fallen dramatically in the United States and Europe, and it is now very unusual to see HIV-associated tumors in children. By contrast, the incidence of HIV infection remains high in Africa and other resource-limited regions, and HIV-associated malignancies are an important clinical problem. In African children, the incidence of Kaposi sarcoma and, to a lesser extent, that of ► [Burkitt lymphoma \(BL\)](#) soared after the onset of the AIDS epidemic. This has been attributed to the fact that in Central Africa, as far as Cameroun in the west and Malawi in the south, KS was already endemic, with incidence rates greater than 6/1,000, before the advent of AIDS. In these regions, the prevalence of infection with the causative agent of KS, ► [human herpesvirus-8 \(HHV-8\)](#), also known as [Kaposi sarcoma-associated herpesvirus \(KSHV\)](#) is relatively high in children. At the same time, high rates of BL of 4 to 5/1,000 existed in parts of Uganda and Tanzania before the AIDS epidemic; in other parts of Central Africa the rates were lower: 0.2/1,000 in males and 0.04/1,000 in females. Even the smaller rates of BL appear huge when compared with European standardized incidence rates, which, during the AIDS epidemic, did not rise over 3.94/million. The ways in which the AIDS epidemic exacerbated the endemic rates of these cancers will be discussed in the next section.

There are no epidemiologic data on HIV-associated tumors for the whole African continent and in fact, collecting more complete and accurate epidemiologic data would help substantially by informing further research which could

lead to clinical advances in this area. This said, statistics that are available from various countries exemplify the changes in the incidence of cancers in HIV-positive children. In Uganda it was found that the probability of acquiring KS was 95 times higher in children with HIV while the probability of developing BL was 7.5 times higher, but no increase in risk was seen for other cancers (Newton et al. 2001). In Zambia, beside the clear increase in the incidence of KS, several other cancers were noted more frequently after the onset of the AIDS epidemic: non-Hodgkin lymphoma, nasopharyngeal carcinoma, rhabdomyosarcoma, and retinoblastoma (Chintu et al. 1995). In South Africa, the probability of developing BL was found to be over 46 times higher in children living with HIV than in the general pediatric population (Stefan et al. 2011).

Oncogenesis in HIV Infection

The list of malignancies that appear more frequently in people infected with HIV is extensive. However, only a few cancers are highly prevalent in this population group: KS, non-Hodgkin lymphomas (NHL), and invasive cancer of the uterine cervix. They are considered to be AIDS-defining conditions, together with infections like tuberculosis, candidiasis, *Pneumocystis jiroveci* pneumonia, and others. It is significant that with the exception of a large subset of NHL, the above cancers are also, almost always, caused by infections. KS is the result of infection with human herpesvirus 8 ▶ [HHV-8/KSHV](#). Burkitt's lymphoma and other AIDS-associated lymphomas (primary central nervous system lymphomas, diffuse large cell lymphomas, ▶ [Hodgkin lymphoma](#), and primary effusion lymphomas) as well as leiomyomas and leiomyosarcomas are caused mainly by ▶ [EBV](#). Cancer of the uterine cervix is caused by high-risk strains of ▶ [human papilloma virus \(HPV\)](#) (Flint et al. 2009).

Smooth muscle tumors – leiomyoma and leiomyosarcoma – are also encountered in children with HIV; in one study in the United States, they represented the second most common tumor (Mueller 1999). The presence of EBV, in latent

form, in these tumors was signaled initially by McClain et al. in 1995. Jenson et al. (1997) have observed a high presence of the virus in these neoplasms, averaging 4.5 viral genomes per cell. Additionally, the monoclonality of EBV within a given tumor is interpreted as evidence of its involvement in the oncogenetic process. It remains, however, unclear how the abnormal cell division is induced by EBV. It was suggested that certain viral proteins, which continue to be produced by the virus in its latent state, may interfere with the mTOR system, which regulates cell division (Purgina et al. 2011).

It is generally accepted that HIV does not have any intrinsic oncogenic activity, but instead promotes the oncogenesis induced by the viruses mentioned above. This effect is caused, on the one hand, by an inefficient immune surveillance against both oncogenic viral agents and the tumor cells they may produce. On the other hand, the HIV-associated chronic hyperactivity of the immune system mediated by cytokines, stimulates the immune cells' proliferation, which in turn enhances the replication of oncogenic viruses within those cells. Secondly, the cytokines promote the growth of blood vessels in the tumor tissue. The inefficient immune surveillance may also explain why other cancers, not known to be associated with infectious agents, appear slightly more frequently in HIV-infected children (Flint et al. 2009).

The Influence of the HIV Infection on the Clinical Presentation of Malignancies in Children

The immunodeficiency in AIDS might result, in theory, in faster progression of the malignant disease. This could be expressed by more advanced or widespread cancers at the first presentation, a poorer response to treatment and a reduced survival rate. It is also possible that the symptoms and signs of the cancer would be altered by the immune suppression, as the immune response is often contributing to generating the signs and symptoms of a disease. There is a dearth of information in the literature on these aspects, due to the relative rarity of cancer in children coupled with a

relative rarity of HIV in this age group in resource-rich regions, with the resulting difficulty of finding enough cases to comply with the power requirements of the studies. Africa offers a unique opportunity of comparing the clinical presentation of KS and BL, which remain endemic in HIV-negative children, alongside to the epidemic form, which is associated with AIDS.

With regard to KS, there is evidence pointing to a more disseminated disease at presentation in HIV-infected versus noninfected children, including more frequent visceral involvement. There is more frequent lymph node involvement than skin involvement by KS in children with HIV (Gantt et al. 2010). The epidemic has been associated with an increase of the incidence of KS in females, who are also comparatively more often infected with HIV at birth than males are.

In BL, the most frequent localization of the tumor is facial, irrespective of the HIV status (71.4% in HIV-positive, 76.6% in HIV-negative); however, children with retroviral infection have significantly more frequent liver and thoracic involvement, as well as lymphadenopathy. As a consequence, there are significantly more children presenting in advanced stage (stage D) in the HIV-positive subgroup compared with the HIV-negative (37.15 vs. 20.3%, respectively), with the corresponding effect on the survival rates (Orem et al. 2009).

In conclusion, KS and BL, whose endemic variants coexist, in Africa, with their epidemic variants associated with AIDS, offer an opportunity to assess the effect of HIV infection on the clinical manifestations and course of cancers. Retrospective studies of these two diseases, in children with and without HIV, point to a faster progression of the malignancies in the presence of immune deficiency, with more disseminated forms involving more often thoracic or abdominal organs. The consequence of this negative influence is a poorer prognosis.

The literature on smooth muscle tumors and HIV was reviewed by Purgina et al. (2011). Out of the 64 cases published worldwide, only 19 were under 10 years of age and a further 3 were aged between 10 and 20 years. They found that the most frequent localization (but

without a breakdown on age groups) was the central nervous system, followed by the lung and gastrointestinal tract. Other sites involved were bones, serosa and genito-urinary tract. In one-third of cases the tumors were either multiple or recurrent. The clinical progression appeared to be slow, with most of the deaths reported being due to other causes than tumor growth or dissemination.

Particularities of the Management of Malignant Disease in Children with HIV and Cancer

While studies conducted in NHL before the development of HAART suggested that AIDS-associated NHL was best treated with low-dose chemotherapy regimens, there is increasing evidence that standard cytostatic or radiotherapy protocols are preferred instead of low-dose protocols. Cancer chemotherapy is associated with a severe immune suppression due to the destruction of leucocytes. In children with a healthy immune system, the leucopaenia induced by cytostatics is transient and will redress itself during the intervals between therapeutic doses. They are, however, susceptible to severe infections during the neutropenic spells. In contrast, prior to the development of HAART, children with an already compromised immunity by HIV would often not recover but would develop a progressive neutropaenia during the course of their treatment (Chanock and Pizzo 1995). They were thus even more prone to developing infection during cancer chemotherapy. The answer to this complication, however, does not consist in using a low-dose cytostatic protocol but rather in efficient infection prevention by controlling the child's environment, by applying strict hygiene measures and using well-planned prophylactic antibiotherapy, guided by the oncology unit's cumulative antibiogram and by the knowledge of the infectious agents that are usually being encountered in AIDS (Chanock and Pizzo 1995). Excessive neutropenia is less of a problem in patients who are receiving HAART, but can still pose difficulties in patients who have very low nadir CD4 counts.

There is evidence that peripheral stem cell transplantation, which assists with the reconstitution of the immune system, can be of use in the treatment of children with HIV-associated malignancies. Should a bone marrow transplant be indicated, the child may possibly benefit from receiving HIV-resistant (naturally resistant or genetically modified) bone marrow cells, in an attempt to cure the retroviral infection (Krishnan and Forman 2010). This is an area of ongoing research.

Most specialists with experience in the field now feel that radiotherapy should be given in standard doses (and not in reduced doses) irrespective of the HIV status of the patient. There is evidence that in HIV-positive children the toxicity of irradiation is higher, mainly for the mucosa of the digestive tract. Further, radiotherapy was clearly shown to reduce the CD4+ cell counts, both in HIV-negative and positive patients. This reduction is persistent over several years. There are no published data yet to document the extent of the potential increase in risk for opportunistic infections in HIV positive patients who receive irradiation. While there is no evidence to support the initiation of highly active antiretroviral therapy (HAART) at the onset of radiotherapy or before it in such cases, the use of prophylactic antibiotics is common.

Leiomyomas and leiomyosarcomas are most often treated by surgical resection. They are not sensitive to chemotherapy, but radiotherapy has been used in conjunction with surgery (Purgina et al. 2011).

There is convincing evidence that HAART has a beneficial role in the management of AIDS malignancies. Antiretroviral therapy reduces the HIV viral load and allows for restoration of the depleted CD4+ lymphocytes and, consequently, the immunity of the patient improves. Kaposi sarcoma can undergo complete or partial remission after initiation of HAART even without other specific therapy. This has been best studied in adults but can also occur in children (Niehues et al. 1999), to the extent where it appears logical to consider HAART as a first line of treatment in childhood KS, while cytotoxic drugs constitute the second line or agents to be used in patients with extensive disease. Caution should be used, however, in conducting antiretroviral therapy on

patient with KS, as a substantial increase in the volume of the lesions may occur after a few weeks or months of treatment due to the immune reconstitution inflammatory syndrome (IRIS). In this condition, the reconstitution of the CD4+ cell number stimulates the immune system and leads to additional inflammatory reaction around the cancerous lesions. Depending on the localization of these lesions (for instance close to the airways), the IRIS may be fatal; the addition of chemotherapy may control the process.

The effect of antiretroviral treatment in NHL, including BL, is not as well defined, but it is thought to be beneficial: HAART can potentially make patients better tolerate standard-dose chemotherapy and thus improve considerably the prognosis of the patients. An analysis of 87 patients treated for NHL in the same institution found that only 14.3% of the subjects not receiving HAART achieved complete response to chemotherapy, compared with 57.6% of those taking antiretrovirals ($p \leq 0.0001$). The mean survival time was similarly extended from 4.8 to 14 months respectively ($p = 0.01$) (Cornejo-Juárez et al. 2008). This was not, however, a randomized trial and it is possible that HAART administered prior to treatment also affected the type and extent of lymphoma over and above any effect seen during treatment.

While there is an increase of cervical intraepithelial lesions incidence in people infected with HIV, and while cervical cancer is an AIDS-defining disease, the evidence accumulated so far does not find a clear effect of HAART on the incidence of cervical pre-cancerous lesions or on the course of the cervical cancer once it develops, with or without treatment (Adler 2010). Also, HAART is not, by itself, effective treatment for non-AIDS-defining cancers. These appear in individuals who are infected with HIV but are not necessarily immunocompromised and may not have an indication for HAART. These cancers remain relatively rare in children, although their incidence increased slightly.

There are too few cases of use of HAART in smooth muscle tumors, but the data accumulated so far point to a stabilizing effect on the course of the malignancy (Purgina et al. 2011).

Finally, the use of HAART in conjunction with cancer chemotherapy calls for careful planning and close monitoring, due to possible drug interactions, as both cytotoxic agents and antivirals may be metabolized by the same hepatic enzymes (cytochrome P450 group), with consequent increase or decrease in the actions of some of the agents involved (Mounier et al. 2009). More research is needed to define such interactions.

Tuberculosis, malaria, and malnutrition are frequently seen in children with malignancies and AIDS, especially in low-income countries. Irrespective of the potential influence of the HIV epidemic, studies have reported a high incidence of tuberculosis in children with cancer (Stefan et al. 2008). The association of tuberculosis, HIV, and malignant tumors in children creates difficulties in diagnosis and impacts on the survival rate.

There is substantial evidence that the co-operation of malaria and Epstein-Barr virus is important in the pathogenesis of BL; however, the mechanisms involved are still unclear. For instance, it has been shown that during malarial infection the replication of EBV is enhanced, and the treatment of malaria is significantly reducing the EBV viral load (Donati et al. 2006). It is hypothesized that HIV contributes to the pathogenesis by inducing immunodeficiency, but these interactions are incompletely understood at this time.

Malnutrition in children with cancer is frequently seen and not necessarily related to AIDS. During cytotoxic therapy, malnourished children have a higher rate of profound neutropaenia, resulting in a higher risk of severe infections and death (Israëls et al. 2009). A thorough evaluation of the nutritional status of children with cancer on admission – even more so if they are infected with HIV – and a nutrition plan established by a dietician with experience in cancer management would be very useful in these cases.

Conclusion

In conclusion, although HIV does not cause cancer by itself, it strongly facilitates the oncogenetic action of other viruses: HHV-8, EBV, and HPV. This explains the significant increase of the

incidence of malignancies known to be induced by the above agents. Other cancers were also observed more frequently in children with HIV, probably due to inefficient immune surveillance against malignant cells. The infection with HIV worsens the prognosis of cancers. A contributor to this is the relatively more severe and prolonged neutropenia during the treatment with cytotoxic drugs, which opens the gate to potentially lethal infections. There is evidence that HAART aids in the recovery from neutropenia, thus facilitating the administration of standard chemotherapy protocols. Coexistent tuberculosis, malaria, and malnutrition, much more prevalent in low-income countries, add to the complexity of cancer management in children with HIV.

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Management of AIDS-Related Kaposi's Sarcoma

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Definition

Kaposi's sarcoma (KS) (► [Overview: Aids-Related and Non-aids-Related Cancers](#); ► [Cancers Related to HIV](#)) is a multifocal angiogenic neoplasm whose development requires systemic infection with a human herpesvirus, known as human herpesvirus-

8 (HHV-8) or the KS-associated herpesvirus (KSHV) (► [Kaposi's Sarcoma-Associated Herpesvirus](#)). KSHV infection is required for the development of this neoplasm, but KS occurs in only a fraction of KSHV-infected individuals. Although KS lesions may arise in the absence of overt immunodeficiency, defects in endogenous viral control mechanisms increase the likelihood that KS will develop (► [Epidemiology of AIDS-Related Malignancies](#)). KS is thus more frequently observed among individuals with underlying conditions, such as HIV infection, which impair antiviral immune responses, and is considered an AIDS-defining condition when it occurs in an HIV-infected person. In formulating a management strategy for KS in HIV-infected individuals, two main aspects of the disease need to be considered: (1) the extent of dissemination of KS and the extent to which it impairs the functional status and quality of life of the affected individual and (2) the degree to which HIV infection is suppressed and the options available for improving HIV control and immune function.

Introduction

KSHV, like other human herpesviruses, persists in host cells in a latent form. Available treatments are not able to eradicate human herpesvirus reservoirs, so any therapeutic approaches – either directed at KSHV or at the resulting KS lesions – must currently be considered palliative. There are, however, treatments that can be successfully utilized to control KS tumors either via direct effects on tumor cell growth or indirectly, by stimulating host anti-tumor and antiviral defenses, and in some instances it is possible to eradicate all discernable disease. In AIDS-associated KS, combining both therapeutic approaches is often the best way to manage tumor-associated morbidity.

Evaluation of the Patient

Diagnostic Tests

A definitive diagnosis of KS requires biopsy and pathological examination of a suspected lesion.

Although an experienced clinician may diagnose KS presumptively based on the appearance of typical red or violaceous skin or mucous membrane plaques, patches, or nodules, other pigmented vascular lesions (e.g., bacillary angiomatosis, pyogenic granuloma) may look similar to KS, particularly in darker-skinned individuals, so biopsy is indicated in all cases of suspected KS. Immunohistochemistry using antibodies to KSHV-associated proteins (e.g., latency-associated nuclear antigen, LANA-1) and/or antibodies to endothelial cell proteins (e.g., CD-31) can help confirm the diagnosis of KS and is particularly useful for determining the nature of vascular lesions showing atypical histopathology (O'Donnell et al. 2010).

Documenting the extent of disease (► [Presentation and Pathogenesis of Kaposi's Sarcoma](#)) is required for KS staging and monitoring the response to treatment. The extent of KS varies widely; some individuals may present with a few, inconspicuous skin lesions and show minimal or no progression over time, whereas others may show widespread skin and/or visceral disease and rapid development of new lesions. Evaluation includes a medical history; review of systems (including functional impairments related to the presence of edema, oral lesions, ulcerated skin lesions, pulmonary and/or gastrointestinal lesions) and careful examination of the entire skin surface, including the feet, the external genitalia, the scalp, and the ears; documentation of lesional ulceration; examination of the oral cavity; assessment of lymph node enlargement and edema; and a rectal examination, including testing of a stool specimen for the presence of occult blood. KS-associated edema is typically non-pitting; edema is most common on the lower extremities but may involve the genitals, the periorbital area (with resulting visual impairment), the upper extremities, and the trunk. A chest x-ray should be performed in all patients to evaluate for parenchymal lesions or effusions. Computerized tomography (CT) or magnetic resonance imaging (MRI) of the chest may provide better definition of parenchymal KS lesions. In some cases where pulmonary symptoms are prominent but the results of radiographic studies

are normal or equivocal, bronchoscopy may disclose endobronchial KS lesions. Bronchoscopy may also be helpful in diagnostically challenging situations where pulmonary KS and opportunistic infections coexist or when imaging of the lung is suggestive of KS in a patient who does not have other evidences for KS. Patients should be questioned about the presence of gastrointestinal symptoms (pain, dysphagia, symptoms of obstruction or bleeding). If positive, or if the stool shows the presence of occult blood, upper and/or lower gastrointestinal endoscopy may demonstrate typical KS lesions. Such lesions may be submucosal and difficult to biopsy, but some lesions, particularly superficial or ulcerated lesions, may show KS on histopathologic examination. Routine gastrointestinal endoscopy in the absence of gastrointestinal signs or symptoms is not indicated. As a rule, contrast-enhanced plain radiographs or scans are not useful in evaluating and managing gastrointestinal KS.

Unless lymph node enlargement is particularly prominent or asymmetric, biopsy of lymph nodes to diagnose KS is not indicated, as the finding of incidental KS in lymph nodes has no bearing on prognosis. Even in the presence of proven lymph node KS, regional lymph node dissection is not indicated. Similarly, wide surgical excision of cutaneous KS lesions is not indicated as it does not prevent local or distant KS spread, and surgical excision should be reserved for diagnostic purposes or for the rare isolated lesion that is either cosmetically disfiguring or causing local symptoms (e.g., an isolated, pedunculated lesion of the foot that is bleeding).

Staging

Several staging classifications have been proposed for KS in the presence and absence of HIV infection. The classification used most commonly to describe HIV-infected individuals was described by the AIDS Clinical Trials Group in 1989 (Table 1) and subsequently validated as a predictor of survival based on data obtained in the "pre-HAART" era between 1989 and 1995 (Krown et al. 1997). The staging system, often referred to as the TIS (for tumor/immune system/systemic illness) or ACTG classification,

Management of AIDS-Related Kaposi's Sarcoma, Table 1 Kaposi sarcoma staging classification

	Good risk (0) (all of the following)	Poor risk (1) (any of the following)
Tumor (T)	Confined to skin and/or lymph nodes and/or minimal oral disease ^a	Tumor-associated edema or ulceration Extensive oral KS Gastrointestinal KS KS in other non-nodal viscera
Immune system (I)	CD4 cells $\geq 200/\mu\text{L}$	CD4 cells $< 200/\mu\text{L}$
Systemic illness (S)	No history of opportunistic infection (OI) or thrush	History of OI and/or thrush
	No "B" symptoms ^b	"B" symptoms present
	Performance status ≥ 70 (Karnofsky)	Performance status < 70 Other HIV-related illness (e.g., neurological disease, lymphoma)

From: Krown et al. (1989). Reprinted with permission. ©1989. American Society of Clinical Oncology. All rights reserved

^aMinimal oral disease is non-nodular KS confined to the palate

^b"B" symptoms are unexplained fever, night sweats, $>10\%$ involuntary weight loss, or diarrhea persisting more than 2 weeks

categorizes the disease as "good risk" (denoted by subscript 0) or "poor risk" (denoted by subscript 1) based on tumor extent, CD4 count as a measure of immune system function, and the presence or absence of systemic manifestations of HIV infection. A subsequent analysis of the utility of the TIS classification in predicting survival in the "post-HAART" era (Nasti et al. 2003) showed that the presence of poor-risk tumor features, particularly the presence of pulmonary KS, in combination with poor-risk HIV-related symptoms (i.e., low performance status, history of other AIDS-defining illness, unexplained night sweats, fever, weight loss, diarrhea) (T_1S_1), defined an especially poor-risk group of patients with a significantly shorter survival than those patients with good-risk tumor features and/or absence of poor-risk HIV-related symptoms (T_0S_0 , T_1S_0 , or T_0S_1).

Response Assessment

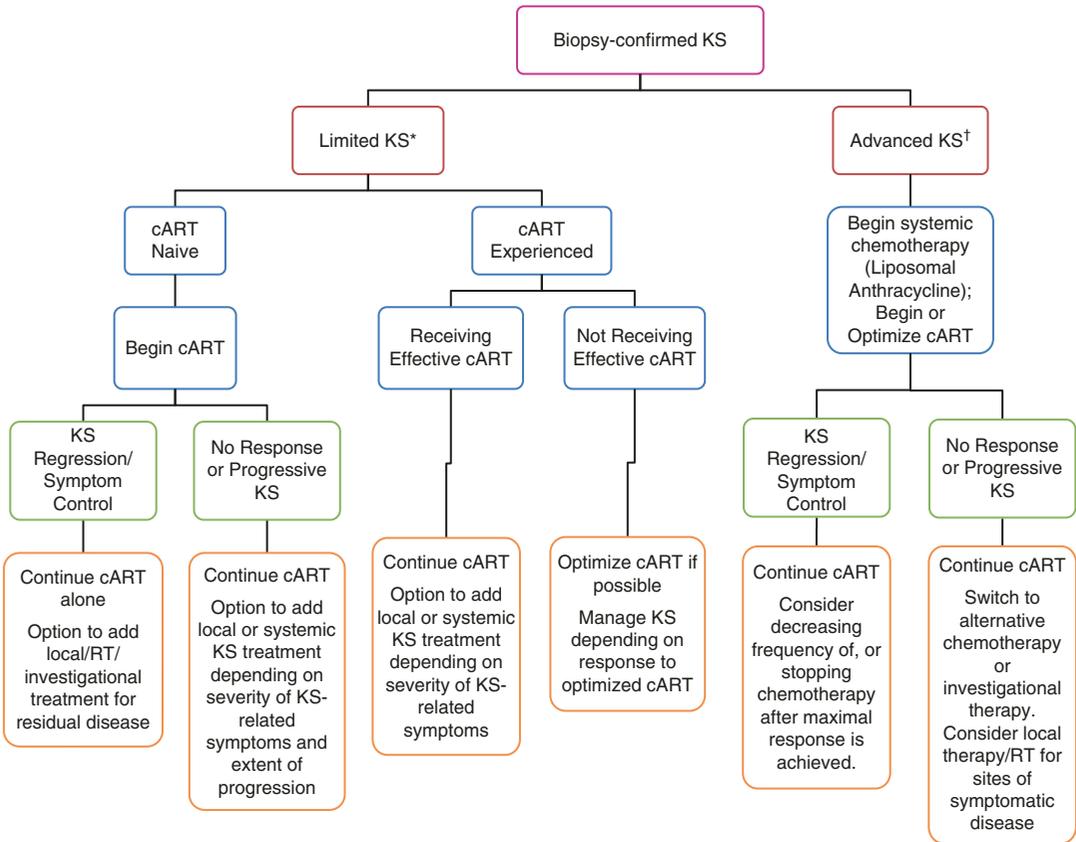
Evaluating the efficacy of treatments for KS presents unique challenges. Cutaneous KS lesions are often multiple, closely spaced or confluent, and irregularly shaped and show varying degrees of nodularity, making accurate assessments of tumor size difficult. Lung lesions are often irregular and poorly defined. Gastrointestinal lesions often require invasive procedures, such as endoscopy, for detection and evaluation, and assessments of lesion numbers and size are only semiquantitative. Tools that attempt to assess

patient benefit with respect to relief of KS-related symptoms (e.g., pain, edema, respiratory symptoms) have been developed but thus far have not been validated as surrogates for objective response or survival. In fact, the results of some studies have suggested that even among individuals not meeting the criteria for objective tumor response, chemotherapy may provide clinical benefits with respect to KS-related symptoms, although symptomatic improvement may also reflect the long-term benefits of coadministered cART (Cianfrocca et al. 2010).

Therapeutic Options

Developing an Overall Treatment Strategy

The extent and rate of progression of KS lesions (► [Presentation and Pathogenesis of Kaposi's Sarcoma](#)) is quite variable, as is the severity of tumor-related symptoms, organ dysfunction, and quality of life. The presence of KS may, by itself, be life threatening (e.g., when there is extensive lung involvement or gastrointestinal bleeding). More commonly, however, KS contributes to morbidity by way of physical manifestations (e.g., by causing pain, difficulty walking, or predisposing to cellulitis of edematous extremities) or by the emotional distress and social stigma occasioned by the presence of highly visible or disfiguring lesions. In some cases, KS may be



Management of AIDS-Related Kaposi's Sarcoma, Fig. 1 KS therapeutic strategy decision tree. *Limited KS is KS confined to the skin and/or lymph nodes and/or non-nodular palatal KS, not causing significant disfigurement or interfering with function and without symptomatic tumor-associated lymphedema, tumor ulceration,

pulmonary KS, or other symptomatic visceral KS. †Advanced KS includes cutaneous KS that is ulcerated, disfiguring, or rapidly progressive; oral KS that is nodular or involves structures other than the palate; symptomatic tumor-associated lymphedema; any pulmonary KS; symptomatic or bulky KS in other visceral sites

an incidental finding that contributes to neither mortality nor morbidity. In individuals with HIV-associated KS, there is also variation in the severity of immunosuppression, the degree to which HIV replication is controlled, and the presence and severity of HIV-related symptoms and other comorbid conditions, including opportunistic infections, wasting, cytopenias, and HIV-related or HIV-unrelated organ (e.g., heart, kidney, liver, lung) dysfunction that may influence the available therapeutic options and overall prognosis. Thus, treatment for AIDS-associated KS must be individualized according to the specific disease features in a given individual. In addition, because there is no treatment for KS

that can be said to be curative, and because a variety of potential management options with different side effects and therapeutic potential may be available for a given patient, the patient's personal goals must be considered in devising a course of treatment. A basic overall schema for developing a therapeutic approach for AIDS-associated KS is outlined in Fig. 1.

HIV Management

The widespread introduction in 1996 of combination antiretroviral therapy (cART) capable of inducing long-term suppression of HIV replication and restoration of immune function was associated with a marked reduction in the incidence of

opportunistic diseases including KS. In addition to its well-established role in KS prevention, cART, often referred to as highly active antiretroviral therapy (HAART) (► [Antiretroviral Medications, Adult Care and Treatment](#)), is considered a critical component of the effective management of HIV-infected patients with an established KS diagnosis. In some cases, particularly when KS is diagnosed in antiretroviral-naïve patients or when it occurs in the setting of suboptimal HIV suppression and immune reconstitution by ART, initiating effective cART or optimizing the cART regimen may, by itself, lead to KS regression. The decision of whether to attempt to treat KS in such individuals with cART alone, or to use cART together with other KS-directed treatments (e.g., chemotherapy), depends on how widely disseminated the KS lesions are and the degree to which they impair function and quality of life. HIV-infected patients diagnosed with or suspected to have KS should be evaluated by an oncologist, preferably one familiar with KS evaluation and management options, very early in the course of the disease, to facilitate treatment planning and follow-up. There is general consensus that attempts to manage KS with cART alone should be confined to individuals with limited, asymptomatic, or minimally symptomatic KS that does not involve visceral organs. Among patients with advanced KS, there is evidence that the combination of cART with cytotoxic chemotherapy results in a higher rate of KS regression and a longer response duration than that afforded by cytotoxic chemotherapy alone (Bower et al. 1999).

Several mechanisms may be involved in cART-induced KS control, but their relative contributions are unknown. These include overall improvements in immune function leading to generation of specific anti-KS responses and inhibition of HIV-1 proteins, angiogenic growth factors, and cytokines (► [Inflammatory Cytokines](#)) that are thought to be important in KS development and progression (Stebbing et al. 2003). In addition, there is *in vitro* evidence that several HIV protease inhibitors may interfere with tumor growth and angiogenesis through mechanisms that are independent of their effects on HIV

infection. One protease inhibitor, nelfinavir, has also been shown to directly inhibit KSHV replication *in vitro* (Gantt et al. 2011). However, the extent, if any, to which HIV protease inhibitors provide benefit to KS patients over and beyond their effect on HIV is not yet known.

Local Treatments

Several methods that aim to control individual KS lesions by the topical application or intralesional injection of antineoplastic agents may be considered for the management of selected patients with limited, non-bulky, slowly progressive cutaneous KS. Local treatments avoid many of the side effects associated with systemically administered therapy and often provide acceptable cosmetic results, sometimes after single or short-term treatment courses. However, these approaches may be associated with local inflammatory skin reactions, pain, and changes in skin pigmentation, prolonged treatment or re-treatment may be required, and there is no expectation that local lesion control will impede the development of new KS lesions in untreated areas.

Of the available local treatment options for KS, only alitretinoin 0.1% gel, applied to cutaneous KS lesions two to four times daily, has been specifically approved by the US Food and Drug Administration (FDA) for this purpose. Although improvement of treated lesions has been observed as early as 2 weeks after starting alitretinoin, much longer treatment courses may be required and treatment durations of up to 96 weeks have been described. Other commonly used local approaches include liquid nitrogen cryotherapy and intralesional injections of vinblastine. The results of several other intralesional treatments have been described in small clinical trials, including recombinant interferon alfa, granulocyte-macrophage colony-stimulating factor and platelet factor 4, and human chorionic gonadotropin, but none of these has been widely adopted in clinical practice. Photodynamic therapy, in which photosensitization of KS lesions is achieved using topical or systemically administered photosensitizers and tumor cell killing is achieved by subsequent irradiation with light from a laser, has also been reported to induce local KS lesion regression, as

has the topical administration of imiquimod 5% cream.

Radiation Therapy

KS lesions are highly radiosensitive. Radiation therapy (RT) has most often been used to treat cutaneous or oral KS lesions but has also been used rarely in the past to palliate complications of advanced, visceral KS, including bleeding gastrointestinal lesions and extensive pulmonary lesions. Although small-field, single-fraction or multi-fraction irradiation with superficial electron beams has been used as a local approach to treat individual KS skin lesions, photon beam RT is more commonly used in the management of more widespread disease such as extensively involved lower extremities, with or without associated lymphedema. Although often effective in achieving local lesion control, patients frequently develop long-term cutaneous changes including a woody appearance and pigmentation changes, and as with other local approaches to therapy, there is no expectation that control of individually treated lesions impedes the development of new KS lesions in untreated areas. An increased risk of mucosal radiation toxicity was described in the era before the introduction of cART, but there is scant recent data on the subject.

With the introduction of cART and the subsequently reduced incidence of advanced KS, the need for RT has declined. In addition, since other systemic treatments, including chemotherapy, are well tolerated and highly effective for treating widespread KS, RT is now generally used only as palliative treatment in individuals with KS that is limited in extent but causing local symptoms (Housri et al. 2010) or in those in whom other treatment alternatives have failed or are not appropriate because of poor performance status or comorbidities.

Systemic Chemotherapy

Systemic chemotherapy is indicated for the management of advanced KS. Advanced KS can be broadly defined as encompassing widespread, symptomatic, and/or rapidly progressive cutaneous KS, including (but not limited to) fungating and/or ulcerated lesions and lesions that cause

pain or disfigurement; extensive, nodular oral KS; symptomatic KS-associated lymphedema; pulmonary KS, including endobronchial or parenchymal lesions, and tumor-associated pleural effusion; and symptomatic gastrointestinal KS, causing pain, obstruction, and/or bleeding. The successful and safe treatment of KS in the HIV-infected patient requires cognizance of and careful monitoring for the multiple overlapping toxicities and potential drug-drug interactions between chemotherapy drugs, antiretroviral agents, and drugs used to treat other HIV-related complications and close cooperation between the primary HIV care provider and the oncologist (Rudek et al. 2011).

KS is highly sensitive to multiple chemotherapeutic agents, many of which were shown to induce regression of classic KS and/or African endemic KS long before AIDS-associated KS was first described. However, only three chemotherapeutic agents have been approved by the United States FDA specifically for use in AIDS-associated KS. These include two liposomal anthracyclines (pegylated liposomal doxorubicin and liposomal daunorubicin) and paclitaxel (Krown 2008). Most oncologists experienced with treating AIDS-associated KS have favored pegylated liposomal doxorubicin as first-line chemotherapy based on observed rates of tumor regression and symptom palliation, good tolerance over long treatment courses, and convenient treatment schedule (one dose every 3 weeks). Paclitaxel is also highly effective (Krown 2008), but is more frequently associated with peripheral neuropathy, asthenia, and alopecia than liposomal anthracyclines and is primarily used as second-line therapy after anthracycline failure or anthracycline-related toxicity.

Although not specifically FDA approved for KS treatment, activity against AIDS-associated KS has been reported for various other chemotherapy drugs used alone (bleomycin, docetaxel, doxorubicin, epirubicin, etoposide, gemcitabine, vinblastine, vincristine, and vinorelbine) or in combination (e.g., the ABV regimen in which doxorubicin (Adriamycin) is combined with bleomycin and vincristine, bleomycin and vincristine without doxorubicin, alternating cycles of

vincristine and vinblastine). Of these agents, only etoposide has shown anti-KS activity when administered by the oral route. The relative efficacy of these drugs in KS is difficult to estimate because they were studied primarily in the pre-cART era in uncontrolled, nonrandomized trials that employed different methods to document the extent of disease and in which response definitions were sometimes ambiguous and inconsistently applied.

The optimal duration of chemotherapy has not been well defined. In advanced KS, durable tumor control usually is dependent not only on the achievement of cytoreduction with chemotherapy but also on the extent to which immunocompetence is restored with antiretroviral therapy. Prior to the availability of cART, significant tumor regression was often observed after chemotherapy for AIDS-associated KS, but the duration of remission was usually short after chemotherapy was stopped. Once effective cART became available, however, it was often possible to discontinue chemotherapy after achieving KS regression without the rapid regrowth of the KS lesions. In some cases, tumor regression continues after chemotherapy is discontinued. These observations support the concept that cytotoxic chemotherapy is not expected, by itself, to cure KS and support the practice of stopping or decreasing the frequency of chemotherapy after stabilization of the lesions, control of tumor-related symptoms, and improvement in quality of life, rather than continuing cytotoxic treatment indefinitely and on a pre-defined schedule until complete tumor regression is achieved. Although KS may subsequently progress after stopping chemotherapy, it is not uncommon for tumors to regress again when chemotherapy (often with the same drug that initially induced tumor regression) is reinstated; this suggests that tumor progression does not result from the acquisition of mutations that confer chemotherapy resistance.

Interferon Alfa

Recombinant interferon alfa (IFN- α) preparations were the first agents specifically tested and FDA approved for the treatment of AIDS-associated KS in the 1980s, on the basis of studies performed

prior to the availability of cART (Krown 2007). Although infrequently used to treat KS for the past decade or more, primarily because of its side effects, the need for frequent parenteral administration, and the introduction of other, easier to administer, and more highly effective agents like liposomal doxorubicin, the sometimes remarkable activity of IFN- α against KS is noteworthy and probably reflects the net result of its multiple potential effects on this neoplasm, which include inhibitory effects on viruses (including HIV and KSHV) and on cell growth and function, including abnormal angiogenesis. Many of these potential mechanisms of IFN action are being investigated in studies of investigational agents that more narrowly target proteins and signaling pathways that are overexpressed or activated in KS.

Investigational Approaches

The growing knowledge of KS pathobiology has provided multiple opportunities to evaluate rational targeted therapies using novel agents. Several preliminary studies have shown the potential promise of this approach. Among the agents for which there is preliminary evidence for anti-KS activity are drugs such as imatinib that interfere with tyrosine kinase-mediated transmembrane receptor signals for angiogenic growth factors that are overexpressed in KS lesions (Koon et al. 2005); mTOR inhibitors, such as rapamycin (sirolimus), that target the constitutively activated Akt/mTOR signaling pathway in KS (Krown et al. 2012); and interleukin-12, a cytokine that enhances type 1 immunity, mediates antiangiogenic effects, and downregulates a constitutively active G-protein-coupled receptor that is encoded by KSHV (Yarchoan et al. 2007). These and other agents, such as those that can reverse KSHV latency and induce apoptosis of infected cells and immunomodulators such as thalidomide and its derivatives, require further formal testing.

Diagnosis and Management of KS-IRIS

Starting cART in the setting of advanced HIV infection has been associated, in some cases, with an apparently paradoxical appearance or worsening of various opportunistic diseases,

including KS, during immune system recovery. This process, known as immune reconstitution inflammatory syndrome (IRIS), has been reported to occur in a variable proportion of patients with KS. The frequency with which KS-IRIS develops is the subject of some debate and may vary in different patient populations. Estimates for the frequency of KS-IRIS have been much higher, for example, in sub-Saharan Africa than in the USA. One difficulty in interpreting the literature on the frequency of KS-IRIS is the lack of a consistent, predefined case definition (Letang et al. 2012). Reports of KS-IRIS have not used standard, rigorously defined criteria for KS progression, and the time interval after initiation of cART during which progression occurred and the degree to which CD4 counts must have increased and to which HIV viral load must have been suppressed has not been uniform.

The optimal management of patients suspected to have KS-IRIS has not been well studied. As a general rule, cART should be continued, and the patient should be evaluated for the presence of opportunistic infections that may have precipitated worsening of KS. Institution of nonsteroidal anti-inflammatory agents may be considered, but there is general consensus that the use of corticosteroids should be avoided whenever possible because they may exacerbate KS progression. The decision to institute or modify systemic chemotherapy in patients with suspected KS-IRIS needs to be individualized. In some cases, KS has been reported to stabilize and eventually regress with continued cART treatment, without addition or modification of KS-specific therapy. In other cases, however, KS may rapidly progress with symptomatic edema and life-threatening involvement of vital visceral organs. In such individuals, prompt institution of systemic chemotherapy may be lifesaving.

KS Management in Resource-Limited Settings: Special Considerations

The foregoing remarks on KS management apply to the experience in well-resourced settings where both cART and large numbers of cancer therapeutic agents have been widely available for many years, rates of KSHV infection and KS tumors are

relatively low, and many patients present with KS of limited extent. By contrast, the vast majority of AIDS-associated KS cases worldwide occur in low-resource settings, primarily in sub-Saharan Africa (► [Sub-Saharan Africa, Specific Characteristics of HIV/AIDS Epidemic](#); ► [HIV Cancers in the Developing World](#)), where rates of KSHV infection are much higher than in the USA and Europe, access to cART is more restricted, the diagnostic and therapeutic armamentaria and the options for supportive care for treatment-related complications are constrained, and comorbid conditions, such as tuberculosis, are common. These factors may affect the choice and tolerance of KS therapy. Moreover, in such settings, KS often is diagnosed at an advanced tumor stage.

Although much has been published on the epidemiology of KS in HIV-infected adults and children in sub-Saharan Africa, there have been few prospective studies to assess KS management strategies in this setting. Some initial reports suggest that chemotherapy with agents commonly available in this setting (i.e., bleomycin and vincristine, with or without concomitant, non-liposomal doxorubicin) provides benefit to patients receiving concomitant cART (Borok et al. 2010; Mosam et al. 2012). Studies to compare the ability of different treatments to induce KS regression, the impact of therapy on survival and quality of life (including effects on KS-associated signs and symptoms, the frequency of drug-related toxicities, and effects on HIV control), prognostic factors, ease of administration of therapy (which may have an impact on therapeutic adherence), and cost-effectiveness are needed to address these important management issues.

Conclusion

KS is the most common HIV-associated malignancy worldwide. In high-resource settings, its incidence has declined since the widespread availability of cART, but KS still represents a therapeutic challenge in a subset of individuals, including some with well-controlled HIV infection. A variety of strategies that include cART, either alone or in combination with other

therapeutic modalities, are available for the management of such patients and in many cases can control lesion growth and KS-related symptoms. However, currently available treatments must be considered palliative, as none have been shown to eradicate KSHV, the causal virus required for KS development. In lower-resource settings, in particular sub-Saharan Africa, the incidence of HIV-associated KS remains high. Although many of the same considerations that figure in developing an approach to the management of KS in well-resourced settings apply to management decisions in lower-resource areas, the availability of therapeutic agents and supportive care is more limited, the incidence of other comorbid illnesses is higher, and the tumor itself may be intrinsically more aggressive in African patients, so the optimal therapeutic approach may be different in this setting.

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Mass Media and HIV Prevention

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Definition

Mass media. “A medium of communication (as newspapers, radio, or television) that is designed to reach the mass of the people” (Merriam Webster’s Collegiate Dictionary 1993, p. 715).

Mass media interventions use various mediums of communication – such as television, radio, newspapers, and websites – to deliver HIV prevention messages to large numbers of people with the intention of educating, changing attitudes, or influencing behavior. Ideally, mass media interventions in the prevention of HIV are thoughtfully designed and built upon formative research and sound media and behavior change theory. Mass media interventions are messages or interactive interventions delivered through multiple media formats. Mass media interventions may be implemented or delivered by different sources (commercial, nonprofit, or governmental). Specific messages and desired effects can vary significantly between different campaigns. Mass media interventions in HIV prevention have been proven to be valuable and effective.

This brief entry discusses the use of mass media in HIV preventive interventions. Mass media are briefly defined and delineated. The entry reviews the advantages of using mass media and describes ways to use mass media effectively in HIV prevention. Evaluations of mass media HIV prevention interventions are reviewed, and the entry closes with future implications for mass media in preventing HIV infection.

Why Mass Media?

Mass media interventions can reach many people at a low rate of cost per person reached and can be cost-effective in preventing new HIV infections even among populations that are relatively low risk for HIV (Cohen et al. 2005). Mass media also have the potential to reach many different groups including people that may not be reached through conventional HIV prevention interventions. For instance, in China, knowledge about HIV has been slow to circulate through interpersonal channels, so mass media are one way to reach people who otherwise may not have any/accurate knowledge about HIV (Li et al. 2009).

Types of Media in HIV Prevention Campaigns

Determining what type of media is appropriate to use in a mass media HIV prevention campaign depends upon the location and target of the specific campaign. Campaigns can include more traditional forms of mass media such as print materials, billboards, and television or radio ads, or newer forms of mass media such as Internet advertising, smartphone applications (apps), or video games. At times, novel media interventions have been used to reach otherwise difficult-to-reach populations or to work within budgetary constraints. Examples of novel media campaigns include: a television soap opera portraying a family with HIV designed to increase HIV knowledge in the Ivory Coast (Shapiro et al. 2003), an audio drama broadcast over loudspeakers in Thailand (Elkins et al. 1997), and live plays performed in communities in Sri Lanka (McGill and Joseph 1996). In the era of Internet and smartphones, social media sites such as Facebook or Twitter are becoming targets for mass media campaigns, as are integrated smartphone apps. For instance, a program promoting testing for sexually transmitted infections (STIs) included the use of multiple social media sites to encourage individuals to seek STI testing as well as short message service (SMS or text messages) to inform them of STI testing

sites near their current location (Friedman et al. 2014). Mass media campaigns which use multiple forms of media are recommended due to the likelihood of increased exposure and increased effects of the messages; however, the cost of media campaigns may prohibit this multiple delivery method (Bertrand and Anhang 2006). An example of a campaign using multiple forms of mass media is a recent program targeting increased safe sex among men who have sex with men in Scotland, which utilized a campaign website, targeted online advertising, a smartphone app, as well as print materials such as posters and leaflets (Flowers et al. 2013).

Designing Effective Campaigns

For mass media campaigns to be effective in preventing HIV, they must be effectively designed. Effective mass media campaigns require careful planning, including the following steps (as delineated by Noar 2006): (1) research prior to forming the campaign to increase knowledge about the target audience, (2) rooting the campaign in sound theoretical concepts (e.g., behavior change theory), (3) defining a specific target audience, (4) designing a message that is specific to that target audience, (5) using media that is most likely to reach the target audience, and (6) evaluating the media campaign. Understanding the target audience is a very important component of designing media campaigns; formative research such as focus groups or analysis of trends within a community can help contribute to understanding the target audience and aid in intervention design.

While targeting one specific group is often the strategy of mass media campaigns, some particularly ingenious campaigns have targeted multiple groups with messages that evoke different meanings depending on the consumer's group membership by using "strategic ambiguity," wherein components of the message may remain ambiguous to target multiple groups simultaneously (DeJong et al. 2001). For example, a message may be ambiguous enough about race or gender to allow the viewer to believe that he or she is

watching someone within the message who is of a race or gender that is similar to his or her own race or gender. Creating mass media messages that target multiple groups allows the campaign to be delivered to an increased target audience, thereby reducing the cost of the media per targeted viewer.

Special care must be taken in targeting specific populations for HIV prevention, as an iatrogenic effect (an unintended harm caused by the message) could occur if the mass media consumer believes that he or she is not part of the targeted population and therefore is not at risk for HIV (Palmgreen et al. 2008). Alternatively, targeted prevention efforts may inaccurately stereotype the populations being targeted (Palmgreen et al. 2008). For instance, early mass media campaigns often inaccurately portrayed injection drug users as African American (Dejong et al. 2001), promoting an inaccurate association between this specific racial group and injection drug use. Similarly, members of a group may reject HIV prevention messages if they feel that their group is being unfairly stigmatized.

Initial comprehensive evaluations of mass media HIV prevention campaigns indicated a need for improvement in evaluation of the campaigns and increased theoretical rationale for campaigns (Myhre et al. 2000). Early HIV prevention mass media campaigns in the USA (created and/or broadcast from 1987 to 1994) were often poorly designed and were problematic due to shortcomings that included failure to target high-risk groups, failure to promote HIV testing, and failure to address barriers to safe sex (Dejong et al. 2001). More recently, mass media campaigns appear to have improved as they often have included elements of effective campaign design (Noar et al. 2009) or have been created through careful evaluation processes (as in Noar et al. 2014).

Implementation of Mass Media Campaigns

Mass media campaigns can be implemented by several sources, including government, not for profit or community-based organizations or for

profit businesses. Media time may be donated by a commercial media venue or may be paid for by a government or other vested organization. Alternatively, businesses that stand to profit from HIV prevention (for instance, condom manufacturing companies) may combine HIV prevention messages with advertisements for their product. Some organizations have experimented with innovative and comprehensive mass media dissemination of HIV prevention messages. The Kaiser Family Foundation, for instance, engaged media partners (such as popular television networks) to donate television time for public service announcements and to integrate HIV prevention messages into the storylines of popular television shows (Hoff et al. 2009). This kind of comprehensive approach seamlessly integrates HIV prevention messages into entertainment programming, which is reinforced by multiple media sources.

Mass Media Messages in HIV Prevention

HIV prevention messages in mass media can have several different goals. Messages may aim to educate individuals about HIV or HIV risk or encourage individuals to engage in a specific behavior (e.g., risk reduction, HIV testing). Research on the target population is vital to effectively developing and delivering a message. For example, one mass media campaign targeting African American adolescents designed messages to target beliefs in this community that condom use made sexual intercourse less pleasurable (Romer et al. 2009). Romer et al. (2009) targeted this belief by promoting the idea that intercourse with a condom can be more pleasurable because there is less associated concern about safety and STIs and found differences in this core belief among adolescents exposed to these targeted messages. Identifying core beliefs which may be barriers and/or assets to HIV prevention efforts allows the message content to be individualized to the target community and can result in a culturally relevant message. Targeted messages should take into account the characteristics of the community being targeted. For instance, within communities with high HIV rates, prevention campaigns may

encourage use of antiretroviral medications in order to lower rates of disease transmission, while in communities with low HIV rates, this would be an ineffective and inappropriate intervention. Customizing prevention messages to the needs of target audiences is vital in creating effective mass media campaigns.

Effectiveness of Mass Media Campaigns

Mass media campaigns have been shown to be effective in HIV prevention, including the following outcomes: increased knowledge and reduced HIV/AIDS-related stigma (Li et al. 2009), increased rates of voluntary HIV testing (Vidanapathirana et al. 2005), and increased rates of safer sex (Agha 2003). In some cases, mass media campaigns have been shown to be increasingly effective with increased exposure to the campaigns, thereby exhibiting a dose–response relationship, (Agha 2003). Mass media campaigns have also been shown to primarily effect short-term (rather than long-term) behavior change (Vidanapathirana et al. 2005) and thus should be considered part of an ongoing prevention effort rather than a one-time intervention. Taken together, these findings implicate the utility of using mass media campaigns in an ongoing basis.

Future Directions in HIV Mass Media Campaigns

Media usage patterns are continually changing as new technologies become available and go in or out of vogue. Newer trends away from traditional television watching and toward Internet-delivered programming have already and will continue to increase the need for adaptive and creative ways to reach target audiences. Internet television in its various forms, including video sharing websites, is one way to deliver HIV prevention messages. Sharing media through and advertising on social networking sites are other ways to deliver HIV prevention messages to targeted communities. Video games or smartphone apps can be

interactive ways to encourage behaviors that would prevent HIV. Newer mass media programs can include billboards or other media that direct consumers to use their smartphones to scan barcodes, which direct them to other media on their phone or direct them to websites to play HIV prevention games. As technologies continue to develop, novel means of using mass media will continue. Future research could identify successful components (i.e., by using dismantling) of mass media interventions so that these specific components could be used to target specific communities in culturally appropriate ways.

Conclusion

Mass media can be an affordable and effective tool in HIV prevention efforts. It can also be used in novel ways to reach otherwise difficult-to-reach populations. Mass media HIV prevention efforts will continue to adapt to new technologies to reach contemporary audiences.

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Maturation Inhibitor

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Definition

Maturation inhibitors are a class of HIV antiretroviral agents currently in development. They act at the viral maturation step, which involves cleavage of the structural protein “Gag,” resulting in the development of an infective viral particle. These agents aim to stop viral maturation by inhibiting Gag cleavage. While not currently in clinical use, they represent a promising new therapeutic class for HIV infection.

Introduction

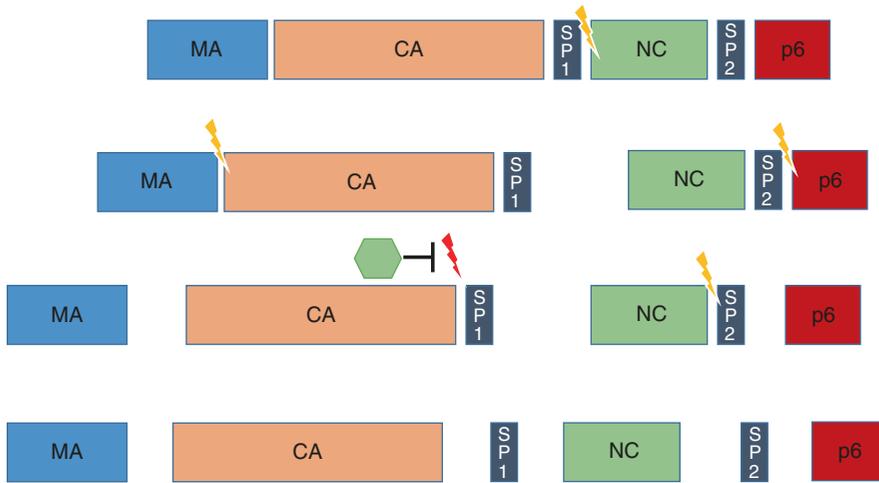
Combination antiretroviral agents with distinct mechanisms have become the mainstay of therapy for HIV infection. A variety of drug classes are available for use, as discussed elsewhere in this text, targeting viral enzymes including reverse transcriptase (RT), protease (PR), and integrase (IN). All currently recommended antiretroviral regimens use a combination of the above classes to provide therapeutic effect (Panel on Antiretroviral Guidelines for Adults and Adolescents 2014; Gunthard et al. 2014). The rationale for a combination approach to therapy is largely based on the ability of HIV-1 to rapidly acquire drug resistance when exposed to single agents (Simon et al. 2006; Temesgen et al. 2006).

Additional drug classes have been developed and are continuously being sought, with examples including inhibition of viral entry by targeting the gp41 envelope protein or by targeting the CCR5 coreceptor (Haqqani and Tilton 2013). Both of the above drug classes are in limited use, often reserved for therapy when resistance has developed to first-line or subsequent regimens (Haqqani and Tilton 2013; Llibre et al. 2015).

A recently discovered approach to HIV therapy, maturation inhibitors target proteolytic cleavage of the precursor Gag polyprotein (Pr55^{Gag}). The precursor Gag polyprotein is one of the three polyproteins that are eventually cleaved into the composite proteins making the structure of the complete virion, the other two being the precursors to Gag-Pro-Pol (Pr160^{Gag-Pol}) and to Env (Swanstrom and Wills 1997). As an initial step in assembly, the Gag and Gag-Pro-Pol polyproteins migrate to the host cell plasma membrane, where they undergo multimerization, and facilitate assembly of the immature virion via protein-RNA interactions, protein-protein interactions, and interaction with host motor proteins and cellular transport machinery (Sundquist and Krausslich 2012). Ultimately, Gag is necessary for a number of important steps in virion assembly: initiation of the budding process, control of virion size, and directing and packaging the components of the developing virion (Swanstrom and Wills 1997).

As they arrive at the plasma membrane, both the Gag and Gag-Pro-Pol precursors undergo cleavage by viral protease into their component proteins (Adamson and Freed 2007). The Pro-Pol component of Gag-Pro-Pol is cleaved into the viral reverse transcriptase, protease, and integrase. The Gag component is cleaved into a set of structurally important proteins: matrix protein (MA), capsid (CA), nucleocapsid (NC), the p6 protein, and the spacer peptides SP1 and SP2 (Sundquist and Krausslich 2012). This cleavage induces structural changes in the developing virion, resulting in assembly of the conical capsid lattice needed for a mature virus (Ganser-Pornillos et al. 2008). Gag processing is highly ordered. Differential rates of cleavage by the PR protein result in a specific sequence to processing: First, the precursor protein is separated into the NC-SP2-p6 and MA-CA-SP1 fragments. Next, PR cleaves the MA-CA site and the SP2-p6 site and then finally the NC-SP2 site and the CA-SP1 site (Adamson and Freed 2007; Erickson-Viitanen et al. 1989; Krausslich et al. 1988; Wiegers et al. 1998) (Fig. 1).

It is this final step, the cleavage between CA and SP1, that is the target of the drug class



Maturation Inhibitor, Fig. 1 The Gag polyprotein with stepwise cleavage by protease. The cleavage between CA and SP1 is blocked by the action of bevirimat

described here. This class of drug has been termed “maturation inhibitors.”

Maturation Inhibitors

Bevirimat

The story of maturation inhibitors begins with 3-*O*-(3',3'-dimethylsuccinyl)-betulinic acid, later named bevirimat (BVM), which was studied for clinical use between 2003 and 2010.

Initially developed as PA-457, the compound was derived from betulinic acid based on a mechanism-blind screening assay. After development of the compound, it was determined that the mechanism was disruption of the PR-mediated cleavage of the Gag precursor protein (Li et al. 2003; Kanamoto et al. 2001; Zhou et al. 2003). Specifically, bevirimat acts near the cleavage site between CA and SP1, preventing the conversion of the CA-SP1 precursor to the mature CA protein (Li et al. 2006). Even a transient, subtle disruption in the highly regulated mechanism of Gag cleavage can be sufficient, as uncleaved CA-SP1 present during assembly can interfere markedly with maturation (Checkley et al. 2010; Muller et al. 2009). The presence of bevirimat has been shown to stabilize the immature, noninfective capsid lattice, rather than allowing the formation

of the conical capsid necessary for infectivity (Keller et al. 2011).

The conservation of the CA-SP1 cleavage site sequence is essential to the action of bevirimat: viruses with divergent CA-SP1 sequences such as SIV and HIV-2 did not show response to the drug (Zhou et al. 2003). Further work identified specific amino acid polymorphisms resulting in decreased antiviral activity of the drug (Zhou et al. 2006; Adamson et al. 2006; Lu et al. 2011).

Phase 1 and phase 2a studies of bevirimat showed promise, with safety and efficacy in small numbers of patients studied without apparent development of resistance (Smith et al. 2007; Yebra and Holguin 2008; Martin et al. 2007a, b). Further studies showed safe coadministration with protease inhibitors without apparent interaction (Martin et al. 2008). However, phase 2b clinical trials found that about half the patients given bevirimat did not show clinical response despite adequate levels because of CA-SP1 site polymorphisms predisposing to resistance (McCallister et al. 2008; Van Baelen et al. 2009; Adamson et al. 2010). The prevalence of naturally occurring polymorphisms conferring reduced susceptibility or resistance to bevirimat was found to be unacceptably high: between a third and half of treatment-naïve viruses tested (Seclen et al. 2010; Verheyen et al. 2010; Margot et al. 2010).

Development on bevirimat was stopped in 2010. However, interest in the Gag processing pathway as a therapeutic target remains, and new investigational medications in this class continue to be studied.

MPC-9055

A second maturation inhibitor named MPC-9055 had also started development in 2009. It too showed similar limitations with the development of polymorphisms conferring resistance, and development was halted in 2010 (Baichwal et al. 2009).

BMS-955176

As of this writing, a second-generation maturation inhibitor is currently under development. Known as BMS-955176, the phase 2a clinical data were presented in 2015. Like bevirimat, BMS-955176 also targets the cleavage of the CA-SP1 precursor but unlike its predecessor seems to offer improved binding to Gag polymorphs. Monotherapy for 10 days was well tolerated and resulted in dose-dependent declines in HIV viral load, with a median reduction of 1.64 log₁₀ copies/ml. The study showed similar declines in HIV-1 viral load among wild-type Gag proteins and proteins with baseline polymorphisms that may have conferred resistance to bevirimat (Hwang et al. 2015). Another study presented on the same year showed activity and safety of the drug in combination with atazanavir and ritonavir (Hwang et al. 2015). Further studies to assess the role of BMS-955176 remain underway.

Conclusions

While the strides in controlling HIV through antiretroviral therapy have been massive, the continued problem of evolving drug resistance shows the need for continued development of new, safe, and effective antiviral agents. Our improving knowledge of the HIV life cycle makes novel therapeutic targets a possibility. The HIV Gag polyprotein, essential for virus assembly and

maturation, provides one such target. While drugs targeting Gag processing are not yet ready for clinical use, our preliminary understanding of this mechanism leads to hope that they will one day be an additional armament in the battle against the HIV epidemic.

Cross-References

- ▶ [HIV Life Cycle: Overview](#)
- ▶ [HIV-1 Maturation](#)
- ▶ [Treatment Failure and Resistance](#)

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Medication Adherence and HIV-Associated Neurocognitive Disorders (HAND)

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Definition/Introduction

Among persons living with HIV infection, adherence to antiretroviral therapy (ART) and other prescribed medications is the critical factor associated with reductions in morbidity and mortality. As a result, HIV is often considered a chronic illness. There have been several advancements in ART medication development, formulation, and administration that have increased the ease of ART adherence as compared to the early years of the epidemic. Specifically, side effect profiles have improved medication tolerability, and there are newer combination medications that allow for most regimens to be between one to three pills per day. Along with the view that HIV has become a chronic illness, however, is the observation that the number of chronic comorbidities among those with HIV has grown. Thus, pill burden remains high among HIV-infected persons as compared to the general population, although many of the medications are for the treatment of HIV-related conditions (e.g., neuropathy, chronic pain) and associated comorbidities (e.g., increased rates of cardiovascular disease). A recent meta-analysis showed that only 62% of HIV+ individuals are $\geq 90\%$ adherent to their ART (Ortego et al. 2011).

Several factors have been consistently shown to be associated with nonadherence to ART and include mood disorders, substance abuse, and

lack of social support, among others. Pertinent to this chapter, neurocognitive impairment is also a strong predictor of ART adherence. Even in this era of effective ART, HIV-associated neurocognitive disorders (HAND) persist in up to half of HIV+ individuals (Heaton et al. 2010). Critical to the diagnostic criteria for HAND is whether neurocognitive impairment impacts everyday functioning and the severity of the daily functioning problems. Interestingly, there may be a bidirectional relationship between cognition and medication adherence, that is, poor neurocognition may lead to medication nonadherence and subsequently worse medication adherence leads to less controlled HIV disease, which confers risk for worse neurocognitive functioning. Although effective interventions have been developed for improving ART adherence, few interventions consider how neurocognition may complicate treatment implementation.

Given the prevalence of HAND and the importance of medication adherence, this chapter will (1) broadly review HAND and its association with daily functioning, (2) discuss medication adherence among HIV-infected persons, (3) assess the relationship between HAND and medication adherence, and (4) discuss HAND remediation efforts. It is important to note that we will be using a broad definition of medication adherence to include all medications and not exclusively ART; however, the great majority of studies relating HAND to medication adherence have focused fully on adherence to ART.

What Is HAND?

In the current era of ART, the profile of HAND is typically one of mild-to-moderate neurocognitive impairment with either the absence (asymptomatic neurocognitive impairment, ANI) or presence of daily functioning difficulties (mild neurocognitive disorder, MND), rather than the more severe presentation of HAND (i.e., HIV-associated dementia, HAD). The profile of ANI and MND is somewhat variable given that HIV infection is associated with damage to both cortical and subcortical structures (fronto-striato-

thalamic circuits appear particularly susceptible). As a result, the most commonly observed neurocognitive deficits are in learning (e.g., ability to encode new information over repeated exposures), episodic memory (e.g., ability to retain both verbal and nonverbal information over a delay, e.g., at least 30 min), executive functions (e.g., a broad term associated with frontal lobe functioning, posited to include problem solving, abstraction, planning, cognitive flexibility, and inhibition), and working memory (e.g., ability to maintain and manipulate information “online”) (Heaton et al. 2011). Certain neurocognitive functions tend to be unaffected, such as naming abilities, simple attention, and somatosensory functions, which distinguishes ANI and MND from more severe dementing conditions.

Of ecological relevance to the present chapter, such HIV-related neurocognitive impairment is reliably associated with everyday functioning declines in this population. Although neurocognitive deficits are largely “mild” in nature (meaning that performances typically fall just below one standard deviation below the expected mean of healthy comparison participants), up to 60% of HIV+ individuals with HAND demonstrate difficulties on functional outcomes. Most commonly, the effects of HIV-associated neurocognitive impairment are observed on instrumental activities of daily living and other more complex daily living tasks (e.g., financial management, driving, unemployment) rather than basic activities of daily living (e.g., dressing and bathing). Impairments on basic activities of daily living remain largely intact and are more strongly related to physical symptoms due to advanced HIV disease progression (Blackstone et al. 2013a).

Medication Adherence in HIV-Infected Persons

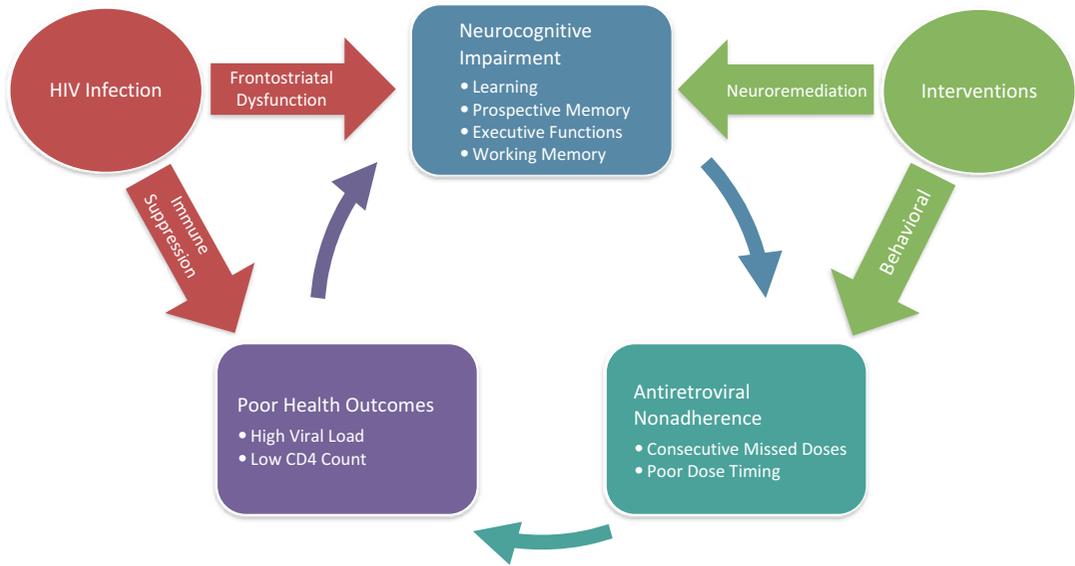
Without effective ART treatment, HIV infection leads to a progressive failure of the body’s immune system. Once an individual is infected with HIV, the virus fuses to the surface of the host cell (e.g., CD4 lymphocyte), which permits HIV to enter the cell. Once inside, HIV releases an

enzyme called reverse transcriptase to convert its genetic material (i.e., HIV RNA) into HIV DNA. HIV then produces another enzyme called integrase that allows HIV DNA to be integrated with the cell DNA. HIV then uses the machinery of the host cell to create long chains of HIV proteins that are the building blocks for more HIV. An HIV enzyme called protease cuts up the long chains of HIV proteins, and the smaller HIV proteins combine with HIV RNA to form a new virus, which then bud from the host cell. The life cycle of HIV is important to consider because antiretroviral medications used for the management of HIV infection act to interrupt the HIV replication cycle.

Antiretroviral medications used in the treatment of HIV infection protect the immune system by blocking HIV at different stages of the HIV replication cycle. The initial antiretroviral medications were nucleoside reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase from converting HIV RNA into HIV DNA. Subsequently, nonnucleoside reverse transcriptase inhibitors (NNRTIs), which bind and inhibit reverse transcriptase, and the viral protease inhibitors (PIs), which block protease, were introduced. Agents targeting other phases of the HIV life cycle, including inhibition of the fusion peptide chemokine receptor 5 (CCR5) and viral integrase, have recently become part of “the cocktail” of drugs available for the treatment of HIV.

Combination ART, which combines two drugs that target reverse transcriptase (i.e., NRTIs and/or NNRTIs) with drugs that target the viral protease, is now the standard treatment approach. At the beginning of modern ART, regimens were notable for frequent dosing (i.e., high “pill burden”) and significant adverse effects, which negatively affected ART adherence. Current combination ART regimes and drug formulations reduce pill burden and dosing complexity as well as adverse effects, all of which provide greater opportunities for successful ART adherence. Consequently, combination ART regimens have reduced disease progression and mortality substantially compared to regimens using agents targeting only one aspect of the HIV life cycle (Fig. 1).

Adherence to ART is key to deriving therapeutic benefit. Although ART has led to



Medication Adherence and HIV-Associated Neurocognitive Disorders (HAND), Fig. 1 A model illustrating the hypothesized mechanisms by which HIV infection detrimentally impacts antiretroviral (ART) adherence and neurocognitive functioning, as well as potential

points for intervention. Of note, several other factors are known to confer risk for suboptimal adherence to ART and to impact performance on neurocognitive tasks (e.g., mood disorders and substance abuse); however, these factors are not the primary focus of this chapter

improvements in clinical outcomes, antiretrovirals need to be taken with consistency to work effectively. Although extremely high ART adherence (i.e., greater than 95% adherent) was initially considered necessary to optimize viral suppression, poor ART adherence does not necessarily lead to a complete lack of therapeutic benefit. Recent research indicates that patterns of adherence have different impact on the risk of detectable HIV RNA levels. Specifically, missing days of medication consecutively may have a greater impact on the efficacy of ART than missing the same amount of time in a nonconsecutive manner (Genberg et al. 2012). Furthermore, the efficacy of some combination ART regimens may be less affected by patterns of adherence due to longer half-lives of agents (e.g., NNRTIs and boosted PIs). Although perfect adherence to ART may not be necessary to achieve positive clinical outcomes, limited ART adherence may lead to poor virologic control and death and create treatment-resistant HIV strains.

Despite the abundance of empirical evidence supporting the therapeutic benefit of ART adherence, the most valid and reliable measure of

medication adherence is a topic of ongoing deliberation. Selection of the best strategy for assessing medication adherence requires consideration of the advantages and disadvantages of the various assessment tools. Multiple methodologies have been employed to assess medication adherence, which include direct and indirect measures. Direct methods may involve the collection of biological assays of active drug, metabolite, or other markers in bodily fluids to confirm active drug ingestion. Indirect methods, which do not directly measure the presence of the drug in an individual, include self-report, clinician assessment, medical chart review, clinic attendance, behavioral observation (e.g., directly observed therapy, pill count, pharmacy refill records, medication event monitoring system), and therapeutic impact (e.g., HIV RNA viral load, CD4 lymphocyte count, Centers for Disease Control-defined stage of disease progression, and mortality). Each assessment method has advantages and disadvantages to consider, including financial and logistical cost, as well as issues of psychometric and epidemiologic validity and reliability.

Intersection of HAND, Adherence, and ART

Neurocognitive Impairment and Adherence in HIV

Numerous studies have shown that worse neurocognitive functioning is associated with poorer medication adherence. Specifically, HAND has been found to confer up to a 2.5-fold increased risk for nonadherence (e.g., Hinkin et al. 2004). Broadly, cross-sectional studies have shown that better higher-order executive functions, as well as learning and working memory abilities, are particularly important for optimal medication adherence among HIV-infected persons.

Longitudinal studies have also examined the temporal relationship between ART adherence and HAND. In one such study, it was found that among neurocognitively impaired persons with HIV infection, ART adherence decreased significantly on weekend days as compared to weekdays. The authors hypothesized that the weekend-related adherence decrease was driven by a lack of structured schedules on the weekends compared to the weekdays. Importantly, there appears to be a reciprocal relationship between HAND and adherence such that as one worsens, the other worsens (Ettenhofer et al. 2010). A recent longitudinal analysis showed that over a 6-month period, individuals with declines in neurocognitive performance were more likely to evidence worsening adherence. Worse learning, memory, and executive dysfunction predicted poorer adherence; not surprisingly, on the other hand, higher levels of adherence were associated with more intact broad frontostriatal abilities, including better processing speed, attention, executive functions, and motor functioning.

More research, however, needs to be conducted, particularly with regard to relationships between neurocognitive function, daily adherence (e.g., the proportion of doses taken on a given day), and duration and frequency of frank interruptions in treatment. It is important for such research to include representation of both genders and vulnerable populations that face difficulties in consistently sustaining access to treatment (e.g., seriously mentally ill, drug abusing, homeless, or

incarcerated populations, and those in resource-limited settings). Cysique and Brew (2009) list some of the remaining challenges for those living with HAND.

One neurocognitive ability that may be especially germane to medication adherence in among people with HIV infection is prospective memory (i.e., “remembering to remember”). Given that one of the necessary neurocognitive components of successful adherence is remembering to take one’s medication at the designated time, the role of prospective memory in medication-taking behaviors is particularly face-valid and ecologically relevant. Additionally, prospective memory draws upon prefrontal and hippocampal neural systems and necessitates aspects of both episodic memory (e.g., retrospective recall) and executive functioning skills (e.g., self-monitoring), all of which are susceptible to disruption following HIV infection. In fact, HIV+ individuals evidence large effect size decrements in prospective memory performance compared to their seronegative counterparts (i.e., Cohen’s $d = -0.92$).

Not surprisingly, deficits in prospective memory are linked to worse self-reported ART management skills (e.g., “I am less efficient at adhering to my regimen than I used to be”), independent of other important factors known to predict medication management (e.g., mood, attitudes toward medications, relationship with medical providers, and worse performance on traditional neurocognitive batteries) and beyond frequency of implementation of behavioral ART adherence strategies (e.g., using an alarm). Additionally, global prospective memory abilities demonstrate medium effect sizes with ART adherence (i.e., Woods et al. 2009), and appear to be particularly driven by time-based prospective memory abilities (i.e., remembering to complete a future intention at a particular time), which exhibit large effect sizes with ART adherence (i.e., Cohen’s $d = 0.60$; Woods et al. 2009). In fact, Poquette et al. (2013) recently demonstrated that it may be HIV-related difficulties in executing intentions across longer delays (i.e., 15 min) but not shorter delays (i.e., 2 min) that are most strongly tied with ART nonadherence. Counterintuitively, increased implementation of memory-based adherence strategies

is associated with poorer event-based prospective memory abilities and worse ART adherence, as measured by the medication event monitoring system (Blackstone et al. 2013b). Although the mechanisms behind this finding are not yet clear, these results may indicate that although individuals with HIV-associated prospective memory deficits tend to report using more memory-based adherence strategies, such strategies are not completely successful at bolstering ART adherence in the context of prospective memory deficits.

ART Efficacy

Given the persistence of HAND in the context of ART adherence, research is under way to develop more effective antiretroviral agents capable of penetrating the central nervous system (CNS). Antiretroviral agents vary in their ability to penetrate the blood-brain barrier and enter the CNS, depending on their protein binding, molecular size, and lipophilicity. This variability has been quantified by a metric termed the CNS penetration effectiveness (CPE) ranking system. Better penetrating (i.e., higher CPE) regimens, with presumed better CNS effectiveness, have been shown to better suppress viral loads in the cerebrospinal fluid. Longitudinal investigations have documented that patients with HAND demonstrate better improvement with initiation of higher CPE regimens; however, results of cross-sectional studies have been discrepant. These cross-sectional results may reflect the possibility that higher-CPE regimens were selected to treat more medically ill patients, who have a higher likelihood of neurocognitive impairment. As the HIV-infected population continues to age, it will be important to determine if certain ART agents require dosing modifications based on increased blood-brain permeability experienced late in life.

In addition to the issue of CNS penetration, treatment initiation may influence neurocognitive outcomes. Both low CD4+ nadir and the inadequate suppression of peripheral viral load are consistently associated with worse neurocognitive performance in the presence of adequate ART adherence. Given that neurocognitive impairment may be detectable shortly after infection with

HIV, initiation of ART before damage to the immune system occurs, and viral control is lost and is recommended to improve neurocognitive outcomes. A recent report documented similar rates of neurocognitive impairment between early-treated HIV patients and uninfected persons, suggesting that initiation of ART shortly after infection may protect the CNS, thereby preserving neurocognitive function (Crum-Cianflone et al. 2013). Earlier initiation of ART may prevent individuals from obtaining a low nadir CD4+ lymphocyte count, thereby eliminating a risk factor for poor neurocognitive outcomes. Additionally, earlier initiation of ART may allow individuals to benefit from the effects of ART on neurocognitive function at an earlier stage of HIV disease (Winston and Vera 2014).

ART Neurotoxicity

Although some research indicates that ART regimens with relatively high CPE ranking result in better neurocognitive outcomes, the toxicity of various antiretroviral compounds to neurons in the CNS has not been well described. ART neurotoxicity is plausible, based on our knowledge of its adverse systemic effects on the peripheral nervous system and evidence from studies using magnetic resonance spectroscopy of the brain. A recent *in vitro* study using measures of neuronal dysfunction sensitive to the earliest forms of HIV-associated damage found a wide range of neurotoxic potencies (e.g., CNS damage involving beading, simplification of the dendritic processes, and neuronal shrinkage) among antiretroviral agents in current clinical use, with no additive effects observed for antiretroviral combinations. As ART continues to be developed, protection of the brain and elimination of the CNS viral reservoir are high priorities in the treatment of HIV.

The interplay between potential neurotoxic effects and ART intolerability plays an important role in ART adherence. ART intolerability is a significant driving factor for treatment switches and interruptions. In addition to peripheral CNS side effects (e.g., gastrointestinal intolerance and skin rashes), neurotoxic effects may prompt changes to patients' ART regimens, potentially

at the expense of switching to a combination ART regimen with a lower CNS penetration. However, based on current evidence, the benefits conferred by ART in protecting the CNS likely outweigh risk of any chronic neurotoxic effects.

Remediation

Despite over 20 years of literature exploring the detrimental effects of HIV infection on the CNS system resulting in neurocognitive deficits and, subsequently, medication nonadherence, very little is known regarding the remediation of such impairments. Given the established relationship between HAND and ART nonadherence, neuro-rehabilitation approaches to improve HAND may additionally result in improved adherence. To date, there have been several pharmacological and cognitive/behavioral approaches to remediate HAND with mixed to promising results.

Some non-ART adjunctive pharmacological approaches to manage HAND, including lithium and serotonin reuptake inhibitors (i.e., citalopram, sertraline, or trazodone), were associated with restoration of global neurocognitive functioning and decreased detectable CSF viral load among small samples of HIV+ individuals (e.g., $n = 21$; Letendre et al. 2006). However, other neuro-enhancing drugs (e.g., methylphenidate) demonstrated limited success, with negligible improvement relative to placebo, poor generalizability in larger longitudinal cohorts, or effects limited to psychological symptoms (e.g., depression). Of note, the intrinsic problem with use of pharmacological approaches to improve HAND is the cyclical relationship between neurocognitive impairment and medication adherence among HIV+ individuals, that is, those individuals who are most impaired (and are therefore in greatest need of treatment) are also the least likely to be able to successfully adhere to the treatment regimen, potentially resulting in limited success of such approaches.

Given the inherent real-world difficulties with pharmacological techniques, alternatives, such as cognitive and behavioral strategies, have begun to be explored. For instance, several computerized

cognitive remediation techniques have shown proof-of-concept feasibility and acceptability among HIV+ individuals and show encouraging results (e.g., increased speed of information processing abilities). However, these preliminary studies are still limited in their conclusions due to poor specificity (e.g., did not use HAND as inclusion criteria), lack of control comparisons, lack of long-term follow-up, and small sample sizes. On the other hand, experimental approaches to improve HAND are also under way, including self-generation techniques (e.g., asking participants to actively generate information, rather than passively listen, in order to deepen encoding and long-term memory) and cuing approaches (e.g., providing category cuing to improve verbal fluency), which have shown positive results among HIV+ individuals in the laboratory. Additionally, cognitive-behavioral techniques, such as compensatory mechanisms (e.g., alarms), which target everyday functioning directly, may be useful for HAND rehabilitation. For example, older HIV+ adults who reported regularly using compensatory strategies in daily life were more likely to successfully perform a naturalistic prospective memory task (i.e., remembering to call the examiner) compared to non-strategy users. Future research is needed to expand our understanding of neurorehabilitation approaches to HAND at both the cognitive mechanistic and the behavioral levels and determine how these improvements impact ART adherence. Although not yet tested from an interventional standpoint, physical activity has been associated with less neurocognitive impairment and may provide another alternative to HAND amelioration.

Importantly, an alternative approach to HAND remediation as a mechanism to improve ART adherence is to simply target adherence behaviors directly. Incorporation of technological approaches, such as text messaging, as means of “real-time” behavioral intervention is on the rise and shows promise for improved ART adherence, but these technological interventions are in the early stages of development (Finitsis et al. 2014). Given the increasing ubiquity of cell phones, mobile health interventions have the potential to decrease traditional treatment barriers, such as transportation,

insurance, and physical limitations, allowing providers to reach difficult-to-track populations. Further exploration of such “real-time” behavioral interventions to improve ART adherence are therefore warranted as our understanding of HAND and adherence moves forward.

Conclusion

Both HAND and ART adherence difficulties persist even in the current ART era that has evolved HIV into a chronic illness. Certain medication classes are more impervious to nonadherence, and consecutive missed doses appear to be worse for HIV outcomes, as compared to sporadic single-missed doses. In addition, while there is evidence that those medications with the greatest ability to cross into the CNS may reduce HAND symptoms, there is also some evidence on neurotoxicity. Thus, a balance needs to be struck between getting drugs into the nervous system and modulating levels of these drugs to avoid neurotoxicity. Notably, ART adherence and HAND are intertwined in a complex, bidirectional interplay, such that worse adherence may lead to greater immunosuppression and risk for HAND. Alternatively, those with existing neurocognitive impairments may be at greater risk for worse adherence and subsequent disease progression. Interventions designed to improve HAND and those designed to improve adherence must recognize the importance of the complex, bidirectional interplay of ART adherence and HAND for successful patient outcomes.

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Merkel Cell Carcinoma and Other HIV-Associated Skin Cancers

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Definition

Cutaneous neoplasms are cancers that originate from the skin and are the most common malignancies in the United States. Patients with HIV and other immunocompromised diseases have not only a greater risk of developing common skin cancers such as basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma but also more unusual cutaneous tumors such as Merkel cell carcinoma (MCC), Kaposi sarcoma (KS), and certain lymphomas.

Merkel cell carcinoma (MCC) is a rare and aggressive cutaneous neoplasm of neuroendocrine origin, most commonly associated with increased ultraviolet radiation. A recently described polyomavirus (MCPyV) has been found present in the majority of MCC suggesting an oncogenic viral role in tumorigenesis. Immunosuppression may also contribute to MCC development given the tumor's increased incidence in patients with HIV and hematologic malignancies and in those who have undergone solid organ transplantation.

Merkel Cell Carcinoma

Introduction

Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine cutaneous tumor. MCC was first described in 1972 by Toker as "trabecular carcinoma" due to its histologic latticelike structure (Izickson and Zeitouni 2011). With the discovery of the tumor's neuroendocrine origin, it was renamed Merkel cell carcinoma. Merkel cells are the only skin cells with neuroendocrine function and are specialized touch receptors found in the basal layer of the epidermis. However, despite similarities in name, morphology, and ultrastructural

appearance between Merkel cells and MCC, there is lack of evidence to support a direct relationship between Merkel cells and the development of Merkel cell carcinoma. In fact, recent evidence suggests that MCC may instead arise from less differentiated stem cells in the skin (Izickson and Zeitouni 2011).

Given its aggressive nature, the timely diagnosis of MCC is necessary, and management is often approached in a multidisciplinary fashion (Izickson and Zeitouni 2011).

Epidemiology

Parallel to other rare cutaneous malignancies, the incidence of MCC is low but is rapidly increasing. Currently, there are approximately 1,500 newly diagnosed cases each year in the United States. The incidence has tripled from 0.15 cases per 100,000 in 1986 to 0.44 cases per 100,000 in 2001 and is now estimated at 0.50/100,000 (Heath et al. 2008; Wieland et al. 2011; Fields et al. 2011a). Interestingly, the incidence of MCC now exceeds that of cutaneous T-cell lymphoma but is still 100 times as rare as melanoma.

The risk of development of MCC in patients with HIV is about 13 times that of the normal population (Heath et al. 2008). In one study there was an increased risk of MCC in HIV patients (OR 2.65), and a substantial risk in AIDS patients (SIR 11) was also documented (Lanoy et al. 2010). In the HIV population, there is an earlier age of disease onset compared to immunocompetent persons (Izickson et al. 2011). The risk of MCC is also increased in other immunosuppressed populations and, for reasons that are not yet clear, appears to be particularly increased in patients with chronic lymphocytic leukemia (Heath et al. 2008; Paulson et al. 2013). Currently, there is no documented correlation between the CD4 count and the onset of MCC (Izickson et al. 2011). MCC may occur before or after a diagnosis of AIDS. There is a slight male predominance seen in both the general population and those with HIV. In immunocompetent patients, MCC occurs more commonly in fair-skinned Caucasian individuals. MCC however can arise in sun-protected non-head-and-neck areas in HIV patients, suggesting that ultraviolet

radiation may play less of a role in this population. The median age of onset in immunocompetent patients is 69 years old, while in the HIV population, it was found to be 49 years old (Izickson et al. 2011). Time to diagnosis from HIV to MCC was 9.5 years in one study, which is longer than reported for other non-AIDS-defining malignancies (Izickson et al. 2011).

Pathogenesis

The exact etiology of MCC is unknown and is most likely multifactorial. The immune system clearly plays a role as demonstrated by the increased risk of development of MCC in patients with HIV or other immunodeficient conditions. In one study of 195 cases of MCC, 7.8% had some form of immunosuppression, including bone marrow transplantation, HIV, organ transplantation, or leukemia/lymphoma (Heath et al. 2008). Furthermore, it was reported that 14.5% of 1,024 persons with MCC were receiving or had received an immunosuppressant agent (Qian Zhan et al. 2009).

Chronic ultraviolet radiation contributes to the etiology as demonstrated by the high incidence of development of MCC on the head/neck and sun-exposed areas. Reportedly, there is a 100-fold increase in occurrence of MCC in patients who received UVA for psoriasis (Izickson and Zeitouni 2011). Concurrences of MCC with SCC and BCCs have also been reported (Izickson and Zeitouni 2011).

In 2008, Feng and colleagues from the University of Pittsburgh discovered a novel polyomavirus, which they called Merkel cell polyomavirus (MCPyV). Of 437 MCC tumors, 72% demonstrated positivity for MCPyV, and in some reports, ~80% of tumors were positive (Izickson and Zeitouni 2011). In a recent study, using monoclonal antibody to large T antigen, MCPyV was found in 97% of MCC (Rodig et al. 2012). The MCPyV harbors a genomic mutation that allows it to integrate in infected cells; this occurs prior to the clonal expansion of MCC. MCPyV expresses both large and small T antigen as well as viral structural proteins in cells. In over 2,000 tissue samples of other malignant and premalignant tissue analyzed for the MCPyV, only a subset of

small cell lung carcinoma samples showed abundant positivity. This finding suggests that at least one other neuroendocrine tumor besides MCC is associated with this virus (Fukumoto et al. 2013). MCPyV genomes have frequently been found in normal skin and other body sites and fluids, such as tonsillar tissue and respiratory secretions. In MCCs, however, the levels of MCPyV are over 60 times higher than the highest values of other tissues (Koljonen 2010).

In HIV-positive men, there is an increased prevalence of cutaneous MCPyV. Also, the MCPyV viral DNA load is significantly higher in patients with poorly controlled HIV infection (Wieland et al. 2011). In a recent study, MCPyV DNA was detected more frequently in the sera of HIV patients than in the sera of non-HIV patients (Fukumoto et al. 2013), suggesting that viremia is associated with host immunity.

The exact role of MCPyV in the pathogenesis of MCC is actively being researched.

Clinical Presentation

MCC typically presents as a solitary, rapidly growing, non-tender erythematous/pink nodule located frequently on the head and neck region (20% periorbital), followed by the trunk and extremities. Ulceration may be present. Most tumors show rapid growth within the first 3 months, which is the average time from initial presentation to diagnosis. At time of consultation, the size of the primary tumor is usually less than 1 cm in ~20%, between 1 and 2 cm in 40%, and greater than 2 cm in 35% (Qian Zhan et al. 2009). The median size is between 1.8 and 2.1 cm at the time of diagnosis (Koljonen 2010).

Given the nonspecific nature of MCCs, it can often be confused with benign conditions, such as cysts, lipomas, dermatofibromas, or vascular lesions. It may also be confused with other malignant tumors, such as nonmelanoma skin cancer, lymphoma, or sarcoma. The acronym AEIOU was developed to aid in the clinical diagnosis of MCC and stands for Asymptomatic/lack of tenderness, Expanding rapidly, Immune suppression, Older than 50 years old, and Ultraviolet-exposed site on person with fair skin. Heath et al. found that 89% of 195 cases demonstrated three or more of

these features (Heath et al. 2008). MCCs can frequently be associated with other types of cutaneous carcinomas on actinically damaged skin (Izikson and Zeitouni 2011; Qian Zhan et al. 2009). Metastatic MCC may present cutaneously as a 1–3 cm skin-colored or red nodules or indurated subcutaneous tumor that grows rapidly over 2–4 weeks (Izikson and Zeitouni 2011; Zeitouni et al. 2000). If metastases occur, the most common sites are the skin, lymph nodes, liver, lung, and bone.

Histopathology

Histology and immunohistochemistry help to confirm the clinical diagnosis. Pathologically, the tumor consists of sheets of dermal small round blue cells with scant cytoplasm and medium-sized nuclei. Mitoses are abundant and chromatin is typically in a salt and pepper pattern. It is an ill-defined tumor which frequently extends into subcutaneous fat and/or muscle. Necrosis, vascular invasion, and perineural invasion are common features. Most MCC stain positive with cytokeratin 20 (CK 20), neuron-specific enolase, epithelial membrane antigen neurofilaments, CAM 5.2, and synaptophysin. CK 20 stains in a characteristic paranuclear dot pattern which is virtually pathognomonic for MCC (Izikson and Zeitouni 2011). MCC also stains positive for MCV large T antigen.

There are three main histologic types: intermediate (80%), trabecular (10%), and small cell type (10%). The small cell pattern is difficult to distinguish from other blue cell carcinomas, particularly small cell lung cancer. Differentiation may be made based on thyroid transcription factor-1 and CK7 positivity in small cell lung carcinoma compared to negative staining for MCC (Merkel Cell Carcinoma 2010). Furthermore, it is not uncommon to diagnose a collision tumor histologically, consisting of a BCC or SCC in combination with a MCC (Izikson and Zeitouni 2011; Qian Zhan et al. 2009).

Staging

In 2010, the American Joint Committee on Cancer (AJCC) published the new MCC consensus staging system (Table 1) that replaced several

previous systems (Merkel Cell Carcinoma 2010). The current system, which incorporates an assessment of tumor (T), lymph nodes (N), and metastases (M), is based on prognostic analysis of 5,823 MCC cases from the National Cancer Data Base (Lemos et al. 2010). Both primary tumor site and pathological nodal status are now incorporated in staging and prognosis of MCC patients (Lemos et al. 2010).

Management

A multidisciplinary approach is often necessary for the treatment of MCC and may include radiological imaging, surgery, radiation therapy, and chemotherapy. The use of radiographic imaging such as magnetic resonance imaging (MRI), CT scan, and PET scan may be indicated for staging patients. PET scan has been shown to be more sensitive and as specific as CT scan for nodal evaluation. In a retrospective study PET/CT resulted in an increase in stage in 16% of cases at baseline and identified metastatic disease (bone–bone marrow) better than CT at follow-up (Hawryluk et al. 2012).

Treatment of the primary tumor is surgical, with either wide local excision or Mohs micrographic surgery. Wide local excision with 1–2 cm margins to investing fascia of muscle or pericranium is generally needed. Mohs micrographic surgery (MMS) has been shown to be effective in achieving tumor-free margin control and may be especially useful where tissue sparing is critical (Izikson and Zeitouni 2011).

At presentation, 27–30% of patients will have clinical positive nodal disease (Lemos et al. 2010). Management of regional disease includes lymph node dissection and/or radiation therapy and possible chemotherapy. Patients with clinically negative nodes can be offered sentinel lymph node biopsy (SLNB), elective lymph node dissection, radiotherapy, or observation. The rate of positive SLNB is between 19% and 38% (Fields et al. 2011a) and is recommended for most Stage I patients. Routine use of immunohistochemistry on SLN is highly suggested in order to more accurately identify micrometastases. SLNB has been shown as important for staging, prognosis, and for determining therapy; however,

Merkel Cell Carcinoma and Other HIV-Associated Skin Cancers, Table 1 TNM criteria and stage groupings of the new AJCC staging system for Merkel cell carcinoma

T	N	M	Survival
Tx , primary tumor cannot be assessed	Nx , regional nodes cannot be assessed	Mx , distant metastasis cannot be assessed	
T0 , no primary tumor	N0 , no regional node metastasis ^a	M0 , no distant metastasis	
Tis , in situ primary tumor	cN0 , nodes not clinically detectable ^a	M1 , distant metastasis ^c	
T1 , primary tumor ≤2 cm	cN1 , nodes clinically detectable ^a	M1a , distant skin, distant subcutaneous tissues or distant lymph nodes	
T2 , primary tumor >2 but ≤5 cm	pN0 , nodes negative by pathologic exam	M1b , lung	
T3 , primary tumor >5 cm	pNx , nodes not examined pathologically N1a , micrometastasis ^b	M1c , all other visceral sites	
T4 , primary tumor invades bone, muscle, fascia, or cartilage	N1b , macrometastasis ^c		
	N2 , in-transit metastasis ^d		
Stage		Stage Grouping	
0	Tis	N0	M0
IA	T1	pN0	M0
IB	T1	cN0	M0
IIA	T2/T3	pN0	M0
IIB	T2/T3	cN0	M0
IIC	T4	N0	M0
IIIA	Any T	N1a	M0
IIIB	Any T	N1b/N2	M0
IV	Any T	Any N	M1

^a“N0” denotes negative nodes by clinical, pathologic, or both types of exam. Clinical detection of nodal disease may be via inspection, palpation, and/or imaging; cN0 is used only for patients who did not undergo pathologic node staging

^bMicrometastases are diagnosed after sentinel or elective lymphadenectomy

^cMacrometastases are defined as clinically detectable nodal metastases confirmed pathologically by biopsy or therapeutic lymphadenectomy

^dIn-transit metastasis is a tumor distinct from the primary lesion and located either (1) between the primary lesion and the draining regional lymph nodes or (2) distal to the primary lesion

^eBecause there are no data to suggest a significant effect of M categories on survival in MCC, M1a–M1c are included in the same stage grouping

its impact on recurrence or survival remains unknown.

MCC is a radiosensitive tumor with both adjuvant and definite radiation therapy (RT) playing a role in its management. Numerous studies, mostly retrospective, have investigated the use of adjuvant radiation therapy and have found that it may improve locoregional disease (Rush et al. 2011). It has an unclear effect on distant recurrences or disease-specific survival. There are few studies assessing the use of RT alone for microscopic

disease found on SLNB, but it appears that RT may offer similar local control as compared to complete lymph dissection (Fields et al. 2011a). Definitive radiation therapy has been shown to achieve good in-field control rates and can be offered to patients with inoperable disease or used in selected cases of residual disease post-surgery (Rush et al. 2011).

Systemic chemotherapy is recommended for patients with distant metastatic disease either at presentation (7%) or at relapse time following

therapy. No standard chemotherapy regimen has been established for treating metastatic MCC, but generally platinum-based combination therapy or etoposide has been used. Chemotherapy may carry substantial toxicity risk. Overall, two-thirds of patients may respond to chemotherapy, but response rates are short and recurrences are quite frequent. Chemotherapy has not been shown to increase survival (Izickson and Zeitouni 2011; Qian Zhan et al. 2009). Due to its similarities to small cell lung carcinoma, the same chemotherapy regimens are used for MCC, including anti-metabolites, bleomycin, cyclophosphamide, anthracyclines, and platinum derivatives (Izickson and Zeitouni 2011; Qian Zhan et al. 2009).

Chemotherapy can be used for locally advanced disease and recurrence, as well as for therapy of *in-transit* metastases with isolated limb perfusion or limb infusion methods (Zeitouni et al. 2011). *In-transit* metastases refer to metastases located in subcutaneous or intradermal tissue and deep lymphatics, manifesting as multiple subcutaneous nodules. *In-transit* metastases usually occur after surgical resection of the primary tumor. Isolated limb perfusion or infusion allows for high-dose chemotherapy, such as melphalan, to be administered to an extremity while reducing systemic toxicities (Zeitouni et al. 2011). By isolating the extremity, the dosage of chemotherapy may be increased leading to more effective tumor targeting and may prevent amputation (Zeitouni et al. 2011).

In HIV patients, studies on the effect of antiretroviral therapy on MCC progression are lacking. Because of the association of MCC with immunosuppression, it is possible that initiation or adjustment of antiviral therapy could be beneficial in selected patients, although it is not clear that it would be effective once the tumor has developed (Wieland et al. 2011). Reducing iatrogenic immunosuppression should be attempted when clinically feasible.

In summary, the recommended treatment of MCC:

- Surgical resection with either wide local excision with 1–2 cm margins or MMS of the primary tumor

- Consideration of sentinel lymph node biopsy for patients with clinically negative nodal disease; regional lymph node dissection and/or radiation therapy for those with positive nodal disease
- Adjuvant radiation therapy for local or regional control in lymph node-positive cases, locally advanced disease, or local recurrence following surgery
- Definitive radiation therapy for inoperable cases
- Consideration of systemic chemotherapy for selected locally advanced disease, recurrence, and distant metastases
- Consideration of antiretroviral therapy in HIV patients
- Consideration of decreasing iatrogenic immunosuppression

Prognosis

MCC is associated with both a high morbidity and mortality rate. Following initial therapy, patients may develop local recurrence (25–30%), regional disease (52–59%), or distant metastatic disease (30–36%). The median time to recurrence is about 8 months, with 90% of recurrences developing within 2–3 years of diagnosis. Lemos et al. found a 5-year overall survival of 40% and relative survival of 54% (Lemos et al. 2010). According to the new staging system, primary tumor size (≤ 2 , > 2 cm) and pathological nodal status both affect prognosis (Lemos et al. 2010). Estimation of 5-year survival varies from 18% to 64% depending on stage of disease. Immunosuppressed patients have a poorer MCC-specific survival (Paulson et al. 2013).

In a recent study, microscopic nodal status was not associated with recurrence or survival; rather clinically positive nodes increased the risk of death, and lymphovascular invasion was strongly associated with disease-specific mortality (Fields et al. 2011b). Patients with occult primary tumor (Stage IIIB) appear to have a more favorable outcome than patients with known primary tumor of similar stage (Lemos et al. 2010). In one study, there was no correlation between Breslow thickness of the primary biopsied MCC and clinical stage of disease or survival

(Izikson et al. 2012), while another study found that increasing tumor thickness was associated with poor disease survival (Lim et al. 2012).

Tumors on the lip carry a worse prognosis, and location may be an independent prognostic factor in head and neck MCC. In regard to HIV patients with MCC, the average survival after MCC diagnosis in a recent study was 18 months (Izikson et al. 2011).

Follow-Up

Patients should be monitored closely with full skin exams and lymph node evaluation every 1–3 months for the first year, then every 3–6 months for the second year, and then followed on a yearly basis. Patients should also be encouraged to perform monthly complete skin exams. Imaging with PET/CT may be valuable as a restaging tool.

Key Points for Persons with MCC and HIV

- MCC is more commonly seen on non-sun-exposed areas compared to immunocompetent patients.
- Increased risk of development of MCC in HIV patients when compared to immunocompetent population.
- Age of diagnosis approximately 20 years earlier; average age of 49 years compared to 69 years for immunocompetent patients.
- Average length of time between diagnosis of HIV and development of MCC: 9.5 years.
- No clear relationship between CD4 count and MCC development and/or survival rates established.
- Need for aggressive management, possible antiretroviral therapy.
- Survival rates decreased when compared to immunocompetent patients.

Kaposi Sarcoma (KS) (See Separate Section on Kaposi Sarcoma)

Kaposi sarcoma was the most common skin cancer seen in HIV-infected patients during the first years of the AIDS epidemic. With the introduction of HAART, the incidence of KS has greatly

decreased but there is still a substantially increased risk (OR 21.58) (Lanoy et al. 2009). In the United States, it is still the second most common tumor in HIV-infected patients after lymphoma. Its incidence varies considerably in different regions, and in some countries in Africa, it is the most common tumor overall. (Crum-Cianflone et al. 2009; Jessop 2006).

Squamous and Basal Cell Carcinomas

Squamous cell carcinoma (SCC) is a type of skin cancer derived from the squamous epithelial cell of the epidermis, whereas basal cell carcinoma (BCC) is derived from the basal cell layer of the epidermis. They are the most common types of skin cancer, with approximately three million cases of BCC and 700,000 cases of SCC diagnosed yearly (Skin Cancer Foundation). While they are both associated with chronic actinic damage, SCC also can be associated with the human papillomavirus (see ► [Human Papillomavirus \(HPV\)](#)). The rate of development of non-melanoma skin cancers in the HIV population is about three to five times that of the general population (Rieger et al. 2008). Further, they tend to develop at a younger age in the HIV population and are less frequently located on sun-protected areas when compared to the general population. In the HIV population, SCCs tend to be more aggressive in terms of recurrence and risk of metastases, whereas basal cell carcinomas have not been shown to behave differently when compared to the general population (Rieger et al. 2008). Lastly, due to the increased incidence of high-risk HPV in the HIV population, SCCs in the anogenital, mucosal, and digital areas are more prevalent.

Clinically, SCC appears as erythematous keratotic papules or plaques which may be eroded. They are most commonly seen on the sun-exposed areas including the head, neck, dorsal arms, and upper chest in the immunocompetent population. They are associated with chronic daily sun exposure. Basal cell carcinomas are described as pearly papules or nodules with telangiectasias on sun-exposed areas. They are associated with intermittent sunburns and sun exposure.

Histopathology can confirm the suspected clinical diagnosis of BCC/SCC. Treatment approaches for these carcinomas include surgical excision, Mohs micrographic surgery, topical immunomodulators, cryotherapy, electrical dissection and curettage, photodynamic therapy, chemotherapy, and radiation therapy.

Lymphomas (See Other Sections on Lymphomas)

Non-Hodgkin B-cell lymphoma (NHL) is more prevalent in the HIV population (OR 2.41), particularly in patients with <200 CD4 cells/mm³ (Fields et al. 2011b; Rieger et al. 2008) and is considered an AIDS-defining illness (Silverberg et al. 2011). Cutaneous T-cell lymphoma (CTCL) and Sezary syndrome may also be increased in the HIV population. Various forms of B-cell NHL may involve the skin. Clinically, cutaneous NHL often presents as erythematous, indurated nodules that may ulcerate. A high percentage of cases of B-cell NHL are associated with an Epstein-Barr virus, especially in HIV individuals; the percentage involvement varies among different forms of lymphoma (Rieger et al. 2008). CTCL presents as erythematous scaly plaques in sun-protected areas. However, in HIV, these plaques may be seen in atypical locations and present at an earlier age of onset compared to the general population (Rieger et al. 2008). The incidence of B-cell NHL has decreased in HIV patients since the advent of HAART therapy.

Melanoma

Melanoma is an aggressive malignant tumor of melanocytes. There are approximately 120,000–150,000 new cases diagnosed worldwide annually (Skin Cancer Foundation). Clinically, melanoma presents as an irregular dark brown to black macule, papule, plaque, or nodule. However, melanoma may also present as a red or skin-colored tumor. It most commonly appears on the back of men and the lower extremities of women but may present anywhere where there

are melanocytes including mucosal surfaces and nail beds. When diagnosed early, melanoma is curable. Invasive disease may require surgery, sentinel lymph node biopsy, lymph node dissection, radiation, or chemotherapy. Some epidemiologic studies have found an increased incidence of melanoma in HIV-infected patients, while other studies have not found an increase (Lanoy et al. 2009). However, melanoma was found to be more aggressive in this population, presumably due to the immunosuppression. The disease-free and overall survival rate of HIV persons with melanoma is decreased (Silverberg et al. 2011; Rodrigues et al. 2002).

Conclusion

Patients with HIV are at a high risk for developing a number of cutaneous carcinomas such as MCC, Kaposi sarcoma, lymphoma, BCC, and SCC; melanoma is elevated in some but not other studies. All these tumors may also be more aggressive in HIV-infected patients necessitating timely diagnosis. HIV patients should have regular full-body skin exams in order to assess, promptly diagnose, and treat any skin cancers.

Cross-References

- ▶ Epidemic Kaposi Sarcoma, Pathogenesis and Presentation
- ▶ Kaposi Sarcoma-Associated Herpesvirus (KSHV) or Human Herpesvirus 8 (HHV-8)
- ▶ Management of AIDS-related Kaposi's Sarcoma
- ▶ Diffuse Large B-Cell Lymphoma

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Merkel Cell Polyomavirus (MCV)

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Definition

Merkel cell polyomavirus (MCV) is a recently discovered member of the *Polyomaviridae*, a family of small DNA viruses that replicate in the

nucleus of their host cell. MCV is one of the ten identified polyomaviruses that naturally infect humans and furthermore one of four polyomaviruses that are known to cause severe human disease, predominantly in immunosuppressed or immunodeficient individuals. Of these, MCV is of particular interest since it presently is the only human polyomavirus known to be involved in tumorigenesis. The virus was first identified in 2008 in tissue from ► [Merkel Cell Carcinoma and Other HIV-associated Skin Cancers](#) by high-throughput sequencing (Feng et al. 2008). Considerable evidence suggests that MCV is causally linked to MCC pathogenesis: Viral DNA is monoclonally integrated into the genome of the tumor cells in up to 90% of all MCV cases, and the integrated MCV genomes furthermore harbor signature mutations that selectively abrogate viral replication while preserving cell cycle deregulating functions of the virus (Chang and Moore 2012). Nonetheless, the development of MCC is doubtless a very rare complication of MCV infection, given that MCV is highly prevalent in the general population, with 44–80% of adults displaying serum reactivity against viral antigens. What cells represent the natural reservoir of MCV infection in healthy individuals, whether the virus is potentially linked to human diseases other than MCC, and how precisely MCV infection contributes to cellular transformation during MCC pathogenesis are unresolved issues that are the subject of current research efforts.

Introduction

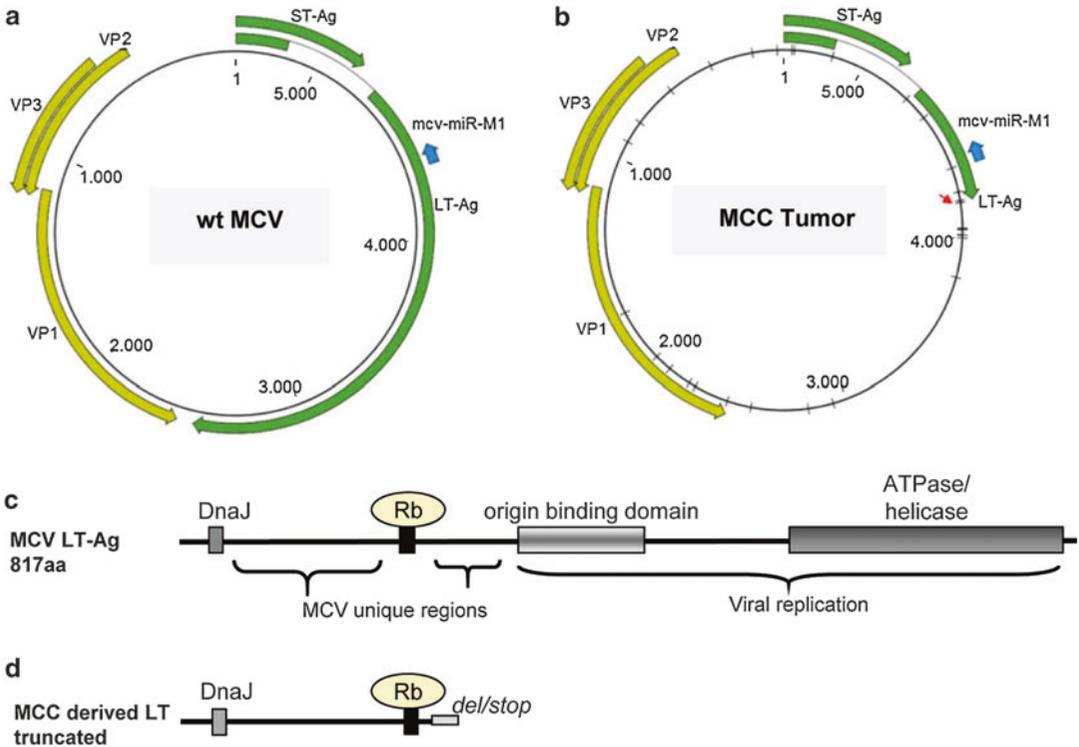
Merkel cell carcinoma (MCC) is a rare but highly aggressive skin cancer which predominantly arises in elderly and immunosuppressed patients. The risk to develop MCC is 10- to 15-fold increased in patients undergoing solid organ transplantation (SOT) and HIV patients. This, together with occasional reports of spontaneous regression of MCC after reconstitution of the immune system, strongly suggests an infectious etiology of the disease (Schrama et al. 2012). In 2008, high-throughput sequencing of RNA derived from MCC tissue successfully identified a novel

human polyomavirus (Feng et al. 2008). Subsequent studies from different laboratories worldwide confirmed the frequent detection of viral DNA in MCC; hence the virus was named Merkel cell polyomavirus (MCV) (Chang and Moore 2012). Due to the considerable molecular and serological evidence that argues for a causative link between MCV and MCC pathogenesis (see section “[MCV Epidemiology](#)” below), MCV has been classified by the WHO International Agency for Research on Cancer (IARC) as a group 2A carcinogen (Chang and Moore 2012).

Polyomaviruses and other small DNA viruses (adenoviruses and papillomaviruses) have long been known to induce malignant transformation of cells *in vitro* or *in vivo*. This ability is closely linked to the replication strategy of these viruses: Upon entry in the host cell nucleus, they rapidly express early genes that mediate aberrant S-phase entry and inhibit apoptosis, e.g., by inhibiting pRb and p53. These manipulations serve to create an environment supportive of massive replication of viral DNA and subsequent production of viral progeny, a process that usually results in the death of the host cell, or its clearance by the immune system. However, if viral DNA replication is blocked (e.g., due to the infection of a nonpermissive species or cell type) and if the viral genome additionally integrates in the cellular genome and hence is stably propagated upon host cell division, constitutive low-level expression of early viral gene products may promote cellular transformation. Many polyomaviruses can cause tumors under experimental conditions, e.g., when introduced into tissues or animal species in which the virus normally does not replicate. However, in their natural host, polyomaviruses only very rarely induce tumors. Indeed, MCV is one of only two members of the family (the other being African green monkey polyomavirus) that has been reported to contribute to neoplastic disease of its natural host.

MCV Genome Organization

MCV is a non-enveloped virus with a circular DNA of 5,386 bp. Its genome shows the typical



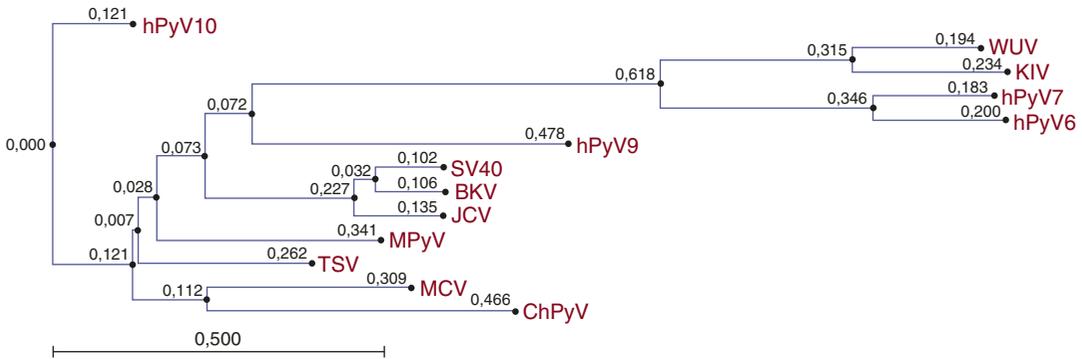
Merkel Cell Polyomavirus (MCV), Fig. 1 MCV genome organization. (a) The genome is divided in three regions: the early gene region illustrated in *green*; the late gene region, counterclockwise to the early transcripts, pictured in *yellow*; and the noncoding control region. MCV is one out of few polyomaviruses encoding for a viral microRNA (MCV miR-M1) (shown in *blue*), located antisense to the LT transcripts. (b) Sequences identified in MCC tissue. LT sequences carry point mutations, frameshift mutations, or small deletions all resulting in

premature stop codon. In the MCC tumor, only the N-terminal region encompassing the retinoblastoma protein-binding region of LT (labeled Rb in the figure) is expressed. The C-terminus containing the origin binding domain as well as the helicase is not expressed. (c+d) Schematic drawing of MCV LT protein domains. (c) MCV LT full-length protein as identified in wild type strains isolated from healthy skin and (d) MCC-derived truncated LT protein

organization of polyomaviruses and can be divided into three regions (Fig. 1): (i) the noncoding control region (NCCR) encompassing the origin of replication as well as the promoters for early and late gene expression, (ii) the early gene region that produces alternatively spliced early transcripts encoding large T antigen (LT), small T antigen (ST), and a 57 kD antigen, and (iii) in reverse orientation relative to the early transcripts, the late genes that encode the structural proteins VP1, VP2, and probably VP3. Whether MCV indeed expresses a VP3 structural protein is still under discussion, given that the amino acid motif Met-Ala-Leu that initiates VP3 translation in other polyomaviruses is not present (An et al.

2012; Chang and Moore 2012). Like certain other polyomaviruses, the MCV genome also harbors a viral microRNA located antisense to the early transcripts. However, it lacks middle T antigen (MT) as seen in mouse polyomavirus and also does not encode an agnoprotein as in SV40.

Phylogenetically, the MCV LT-Ag possesses closest homology to chimpanzee polyomavirus (ChPyV) (Fig. 2). Among the human polyomaviruses (hPyV), MCV is most closely related to trichodysplasia spinulosa-associated virus (TSV), a virus that causes a rare (non-neoplastic) skin disease in immunocompromised individuals. The two remaining polyomaviruses associated



Merkel Cell Polyomavirus (MCV), Fig. 2 Phylogenetic analysis of large T antigen aa sequence from human polyomaviruses (hPyV), SV40, mouse polyomavirus (MPyV), and chimpanzee PyV (ChPyV). Large T antigen sequences from SV40, ChPyV, and ten hPyV were aligned using CLC workbench 6.6.1, and the phylogenetic tree was generated using neighbor-joining method.

Specific viruses studied were BK virus (BKV), NC_001538.1; JC virus (JCV), NC_001699.1; WUV, NC_009539.1; KIV, NC_009238.1; hPyV6, NC_014406.1; hPyV7, NC_014407.1; MCV, JN707599.1; TSV, NC_014361.1; hPyV9, HQ696593.1; hPyV10, JX262162.1; MPyV, NC_001515.1; ChPyV, NC_014743.1; and SV40, NC_001669.1

with major diseases in humans are JC virus and BK virus. These two viruses cause a usually fatal demyelinating or severe renal disease, respectively, and are more closely related to the simian virus 40 (SV40), the prototypical member of the polyomavirus family, than to MCV. BKV and SV40 LT-Ag share more than 75% amino acid (aa) sequence similarity, whereas the LT-Ags of BKV and MCV are only 55% homologous.

MCV Replication and Persistence

A fully permissive *in vitro* replication system for MCV does not yet exist, a fact which severely hampers studies of the viral life cycle. The absence of such a system may be explained by the fact that polyomaviruses often exhibit a highly restricted tissue tropism and only replicate in specific cell types. At present, the authentic cell type in which MCV replicates *in vivo* is unknown. This is true for most human polyomavirus; in fact, the full viral life cycle can so far be only studied for two human polyomaviruses, and in both cases a particular cell system is required (primary human renal proximal tubule epithelial cells (RPTE) for BK virus, and human glial cells for JC virus). In contrast, simian virus 40 can successfully be cultured in monkey cell lines (Vero, CV1).

Consequently, most information about the polyomavirus life cycle is derived from the study of SV40 replication (An et al. 2012).

Attempts to recapitulate the MCV life cycle by transfection of episomal DNA into cultured cells have so far not identified a cell type that allows the efficient production infectious virions. However, the initial phase of the life cycle (receptor binding, entry, and viral DNA replication) can be studied in some established cell lines (Chang and Moore 2012; Neumann et al. 2011; Schowalter et al. 2011). Viral entry is a sequential process that involves attachment of MCV particles to large linear polysaccharides (glycosaminoglycans), and a subsequent post attachment step that requires the presence of sialic acid (Schowalter et al. 2011).

Although not studied in authentic MCV infection, the subsequent steps are likely to be similar to those observed during SV40 infection: after virion uptake by endocytosis, the viral genome is delivered to the nucleus where early gene transcription immediately starts. Polyomaviruses infect quiescent cells and do not encode their own DNA polymerase; they thus need to induce the cell cycle to be able to replicate their DNA. Induction of S phase is achieved by LT-Ag binding to the Rb protein and subsequent inactivation of p53. Late gene transcription is

concomitantly initiated with viral DNA replication, and translation of the structural antigens is followed by virion assembly and egress of viral particles.

Upon transfection of MCV genomes, expression of T antigens and efficient replication of viral episomes have been observed in some, but not all of the tested cell types (Chang and Moore 2012; Neumann et al. 2011), indicating that cell type-dependent postentry blocks to early gene expression or DNA replication may exist. Another block that limits the MCV life cycle appears to be on the level of late gene expression and/or virion assembly, as all cell systems studied so far (including those that permit DNA replication) fail to efficiently produce viral particles. The molecular nature of the mechanisms that hinder viral replication in above models is unknown. Likewise, it remains an open question whether similar mechanisms contribute to a replication block that may precede integration and mutagenesis of MCV genomes during MCC pathogenesis.

MCV and MCC Pathogenesis

Several observations support the notion that MCV infection plays a causative role in MCC tumorigenesis. Firstly, MCV genomes can be detected with high frequency in MCC, with 80–90% of all MCC tumors being positive for MCV DNA. Secondly, the tumor cells express early viral gene products and contain the viral DNA monoclally integrated in the host genome. The fact that integration sites are always identical within a given tumor and its metastases strongly suggests that viral infection must precede tumorigenesis. At the same time, integration sites vary among tumors from different patients, indicating that integration is likely to reflect a selection pressure to maintain viral DNA and expression of early antigens, rather than a proliferative advantage gained by changes in expression or structure of flanking cellular chromatin. Thirdly, all integrated viral genomes harbor signature mutations that are absent from viral episomes residing in tissues in which the virus is thought to replicate (Chang and Moore 2012; Schrama et al. 2012).

Strikingly, although these mutations are diverse among different tumors, they unequivocally occur in the form of point mutations or small deletions that lead to the disruption of the LT-Ag coding region between the sequences that code for the Rb-binding motif and those that encode the origin binding and ATPase/helicase domains (see Fig. 1c, d). Hence, the resulting truncated LT-Ags are unable to support viral DNA replication but retain their ability to bind to and sequester Rb. It is therefore likely that the mutations represent the result of a dual selection pressure: the need to prevent the untimely firing of integrated viral replication origins outside of S phase (which would result in the death of the host cell), while simultaneously preserving cell cycle deregulatory functions of early antigens.

Besides LT-Ag, sT-Ag may also play a critical role during MCC pathogenesis. Indeed, the sT-Ag (which is expressed in MCC tumors and is not affected by the LT-Ag mutations) was found to induce loss of contact inhibition and permit anchorage independent growth of rodent fibroblasts. In contrast to other polyomaviruses (SV40, MPyV), the mechanism by which sT-Ag influences proliferation does not involve PP2A inhibition but appears to act downstream within the Akt-mTOR pathway, ultimately resulting in 4E-BP1 inhibition and stimulation of cap-dependent mRNA translation (Chang and Moore 2012). Experiments using siRNAs to selectively inhibit either sT-Ag expression alone or sT- and LT-Ag expression in MCV-positive MCC cell lines have indeed shown that both early antigens contribute to cell survival (Chang and Moore 2012; Schrama et al. 2012), making them attractive targets for potential future therapeutic approaches.

MCV Epidemiology

Capsid epitope immunoassays demonstrate a high prevalence of MCV (up to 80%) in healthy adults, similar to rates that have been previously described for other human polyomaviruses (Chang and Moore 2012). Fifty percent of children younger than 15 years are seropositive for MCV antibodies, indicating a common infection

that already occurs at young age and that only in very rare cases leads to the development of a tumor. Although antibody titers against viral capsid protein can be detected in the majority of adults, MCC patients with MCV-positive tumors demonstrate higher antibody titers compared to patients with MCV-negative tumors (Chang and Moore 2012). This observation suggests that such patients may be unable to efficiently control MCV replication and supports the notion of a causative role of MCV in MCC. In contrast to seroreactivity against viral capsid proteins, antibodies against the early T antigens are only sparsely detectable in the general population or are only detected at very low titers. However, patients with MCV-positive MCC demonstrate high LT antibody titers. Interestingly, LT antibody titers fluctuate during disease progression with high titers in patients with recurring disease and progressing metastasis and decreased titers in patients in which the tumor did not recur.

In addition to seroprevalence studies, the presence of MCV DNA on the skin of healthy patients has also been analyzed, using swab samples from different body sites and subsequent qualitative as well as quantitative PCR (Wieland et al. 2009). Wild-type MCV genomes that carry no premature stop codons in the early region were successfully isolated from healthy persons, again supporting the hypothesis that MCV is part of the normal skin flora (Schowalter et al. 2010; Wieland et al. 2009). Furthermore, a recent study demonstrated that the amount of viral DNA shedded correlates with serum antibody responsiveness, suggesting that the skin is a major site of MCV replication (Pastrana et al. 2012). Nonetheless, as indicated above, the specific cell type in which MCV may replicate has not been identified yet. MCV may also reside in other tissues, as viral sequences can be detected by PCR in the respiratory tract, saliva, gut, urine, lymphoid tissue, and whole blood from healthy patients. However, compared to the skin viral copy numbers are much lower in these tissues (Chang and Moore 2012). Whether they only represent secondary sites of replication or play other important roles during MCV infection, for example, as long-term reservoirs of infection, is currently unknown.

MCV Infection in HIV Patients

HIV patients show an increased rate of neoplastic diseases, with Kaposi sarcoma (KS) and large B-cell non-Hodgkin lymphomas representing the most common virally induced AIDS-defining cancers. With the widespread use of HAART for more than a decade, the population of HIV-infected patients is increasing, and this population is becoming older. Along with these changes, the number of HIV-infected patients developing non-AIDS-defining cancers is increasing, and the repertoire of these cancers is expanding. MCC is increasingly being appreciated as an HIV-associated cancer. In addition, the sites at which MCC occurs are more diverse in location in AIDS patients compared to HIV-negative patients, and, in HIV-infected patients, it frequently develops on non-sun-exposed body parts. This observation suggests that UV mutagenesis might be lesser important in MCC development in AIDS patients as compared to other patients (Wieland and Kreuter 2011).

Although MCC occurrence in AIDS patients is increased up to 15-fold, studies evaluating the seroprevalence of antibodies against MCV capsid protein have not found a correlation between MCV infection and HIV status or AIDS progression (Tolstov et al. 2011). However, MCV-specific PCR performed with forehead skin swabs of 210 HIV-positive men demonstrated that individuals with poorly controlled HIV infection are more than twice as frequently positive for MCV DNA and also exhibit significantly higher viral loads than a control cohort (Wieland and Kreuter 2011).

MCV in Diseases Other Than MCC

The potential association of MCV with neoplasia other than MCC has been extensively studied in the years since the identification of the virus. Mostly PCR but to some extent also LT immunohistochemistry techniques have been used to detect MCV sequences and/or Ag expression in tumor tissues. So far, MCV has only rarely been detected (and if so, only in low copy numbers) in, e.g., neuroendocrine tumors from different

anatomical sites, mesotheliomas, different skin cancers (BCC, SCC, melanoma, and Kaposi Sarcoma), breast cancer, prostate cancer, and ovarian cancer, suggesting no direct association of MCV with these diseases (Chang and Moore 2012).

Since patients with MCC have an increased risk of developing chronic lymphocytic leukemia (CLL) and vice versa, the association of MCV and CLL was analyzed in particular detail. Two independent studies lead to contradictory results (Chang and Moore 2012): One study detected MCV DNA in 27% of CLL samples and 13% of control samples by PCR, FISH, as well as IHC, whereas another study investigated CLL cases with and without concurrent MCC and has not found MCV sequences or LT-Ag expression in CLL tissues. Based on these findings, it may be reasonable to conclude, at least for the moment, that MCV represents a passenger virus in CLL, perhaps being a result of increased viral replication rates due to decreased immunity. However, at present a contribution of MCV infection to CLL disease cannot be categorically ruled out.

Diagnostic and Prognostic Value of MCV Detection in MCC

Immunohistochemistry (IHC) of MCV antigen expression (LT and sT) is useful to discriminate between virus-positive and virus-negative tumors. IHC staining of MCC tissue with a monoclonal antibody against LT (Cm2B4) has been applied by several studies, and reliable IHC protocols for the diagnostic use have been established. Staining of viral antigens is generally restricted to MCC tumor cells and has not been described for healthy tissue surrounding or interspersed within the tumor. However, some MCV-positive MCC cases that are negative for IHC staining with the Cm2B4 antibody directed against LT-Ag but positive when tested with an antibody that detects sT-Ag have been reported. As sT-Ag expression is detectable in all MCV-positive MCC tumors tested so far, IHC for this viral antigen would be superior to LT-Ag staining. Unfortunately, no antibody against sT antigen is commercially available thus far. Therefore, LT-Ag IHC is routinely

employed to complement the panel of non-viral marker proteins (CK20, CK18, neuron-specific enolase, chromogranin A, synaptophysin) used to diagnose MCC.

The use of LT antigen IHC staining (and/or MCV DNA load) as a prognostic marker is still under discussion, with conflicting reports about MCV status and MCC recurrence. While a Finnish study reported a correlation of LT antigen expression and MCV DNA load with survival rates, with patients with MCV-positive tumors having prolonged survival rates (Sihto et al. 2011), a German study that included MCC cases from Germany and Australia found no direct association between MCV status and MCC recurrence (Schrama et al. 2011).

More recent studies are encouraging with regard to using the immune status of MCC patients as a potential prognostic marker. Several reports indicate that an increased number of CD8⁺ T-cells in MCC tumors are associated with longer disease-free survival. MCV-positive tumors often contain high numbers of infiltrating immune cells. Interestingly, MCV-negative tumors with high numbers of immune cells also have a better disease outcome, so the relationship of these immune cells to a viral antigen is unclear (Paulson et al. 2011; Sihto et al. 2012).

Conclusion

MCV is the first polyomavirus with convincing evidence of an etiological role in human cancer formation. This evidence includes high association (up to 90%) of MCV with the tumor in which the virus was identified, monoclonal genomic integration of viral DNA carrying signature mutations in the cells of primary tumors and subsequent metastases, expression of viral oncoproteins in the tumor tissue, and significantly increased viral titers in patients with disease.

Since the discovery of MCV, significant progress has been made with regard to the understanding of MCV biology and its role in MCC pathogenesis. Even so, the precise mechanisms that lead to virally induced transformation *in vivo* remain to be unraveled. It is to be expected

that future studies of the viral life cycle and its role in cellular transformation will provide not only important information for the diagnosis and prognosis of MCC but may also allow novel therapeutic approaches that directly target viral antigens required for the survival and continued proliferation of the tumor cells.

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MHC Locus Variation

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Definition

The major histocompatibility complex (*MHC*) on the short arm of chromosome 6 contains over 120 expressed genes, 40% of which contribute to immune responses. The classical *HLA* class I (*HLA-A*, *HLA-B*, and *HLA-C*) and class II (*HLA-DR*, *HLA-DQ*, and *HLA-DP*) genes within the *MHC* are the most diversified loci in the human genome. *HLA* genes maintain extensive diversity primarily in the gene segments encoding the peptide-binding domains. The number of *HLA* alleles is in the range of thousands, though only

less than one fifth are defined as common and well-documented (CWD) alleles, which more truly reflect the level of *HLA* polymorphism in populations. It is generally agreed that the extraordinary diversity of *HLA* is generated and maintained by balancing selection through interactions between the host and infectious pathogens.

The products of *HLA* genes are fundamentally important to acquired as well as innate immune responses. The class I loci encode molecules that bind antigenic epitopes usually derived from intracellular pathogens and present them to CD8⁺ T cells, thereby initiating a cytotoxic T-cell response. In addition to their role in acquired immunity, class I HLA molecules also serve as ligands for killer cell immunoglobulin-like receptors (KIR) (“► [KIR Locus Variation](#)”), thereby participating in the regulation of natural killer (NK) cell activity.

HLA and AIDS

HIV/AIDS has been scrutinized extensively for effects conferred by *HLA*, and involvement of *HLA* in HIV disease outcome has been confirmed by a large number of cohort studies. These data have been further underscored in recent years by a series of genome-wide association studies (GWAS) (“Genome-Wide Association Analysis”). Strong evidence points to *HLA* as the most significant single locus in determining outcome after HIV infection.

HLA polymorphism has been associated with over 100 diseases including autoimmune conditions, malignancies, as well as infectious diseases, virtually all of which are multifactorial. Among all infectious diseases studied to date, HIV shows the most clear-cut association with *HLA*. *HLA* polymorphisms may impact AIDS in many aspects including HIV infection/transmission, HIV viral load control, and the rate of progression to AIDS following HIV infection. This chapter will focus on the *HLA* associations that have been consistently detected in multiple cohort studies, including effects of *HLA* zygosity, frequency-dependent selection, and individual *HLA* alleles, along with newer findings including expressional variation of *HLA-C*.

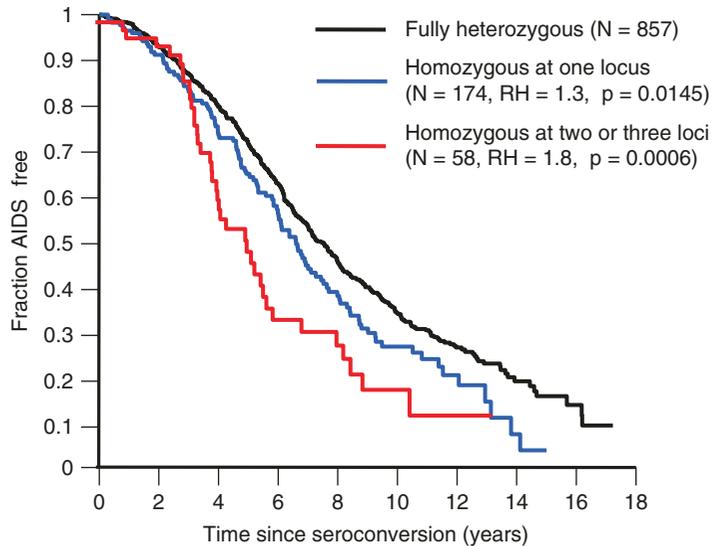
HLA Heterozygote Advantage and Frequency-Dependent Selection

The number of different HLA allotypes expressed on the cell surface is directly related to the size of the repertoire of foreign antigens that can be presented to T cells. An individual who is heterozygous at all three *HLA* class I loci expresses the maximum diversity (six alleles) and is able to present a greater variety of viral peptides than an individual who is partially or fully homozygous. Theoretically, it will take the virus a longer time to accumulate mutations to escape immune surveillance in *HLA* heterozygous individuals relative to homozygous individuals. Under this model, *HLA* homozygous individuals would be expected to progress more rapidly to AIDS than *HLA* heterozygous individuals after HIV infection. Indeed, survival analyses showed a significant association of *HLA* class I zygosity with rate of progression to AIDS outcomes (CD4 < 200, onset of AIDS-defining illness and AIDS-related death; Carrington et al. 1999). Individuals homozygous at any two or all three class I *HLA* loci progressed to AIDS significantly faster than individuals who were heterozygous at all three loci (Fig. 1). Individuals homozygous at only one locus showed an intermediate rate of progression relative to the other two groups. These results demonstrate that (a) all three class I loci, *HLA-A*, *HLA-B*, and *HLA-C*, contribute separately to the zygosity effect since homozygosity at any of the three loci showed more rapid progression to AIDS; (b) the homozygosity effect of any single *HLA* class I locus is enhanced when a second or third locus is also homozygous; and (c) the *HLA* zygosity effect on AIDS progression is not confined to particular populations, as it was detected in cohorts of different ethnic origins.

The effect of *HLA* zygosity on AIDS progression can be explained by recognition of a broader range of HIV-1 peptides by *HLA* heterozygous individuals resulting in a greater number of specific cytotoxic T-cell (CTL) responses against the virus. Other related mechanisms, however, should also be considered. One such mechanism is based on frequency-dependent selection (or rare allele advantage), which argues that the infectious

MHC Locus Variation,

Fig. 1 Kaplan-Meier survival analysis for class I *HLA* zygosity effect on progression to AIDS 1987 (the 1987 CDC definition of AIDS: AIDS-defining illness) in 1,089 seroconverters that include multiple ethnic groups, but mainly European and African Americans (Updated from Carrington et al. 1999)



pathogen is more likely to have adapted to common as compared to rare *HLA* alleles in a given population, such that the rare alleles confer greater protection against the pathogen. An advantage of rare *HLA* supertypes has been reported in HIV disease, where supertypes consisting of the more rare alleles associate with lower HIV viral loads (Trachtenberg et al. 2003). Frequency-dependent selection can be difficult to distinguish from heterozygote advantage, since nearly all homozygotes are composed of common alleles, and the rare alleles are found almost exclusively as heterozygotes. It is possible that both mechanisms are involved in protecting against HIV.

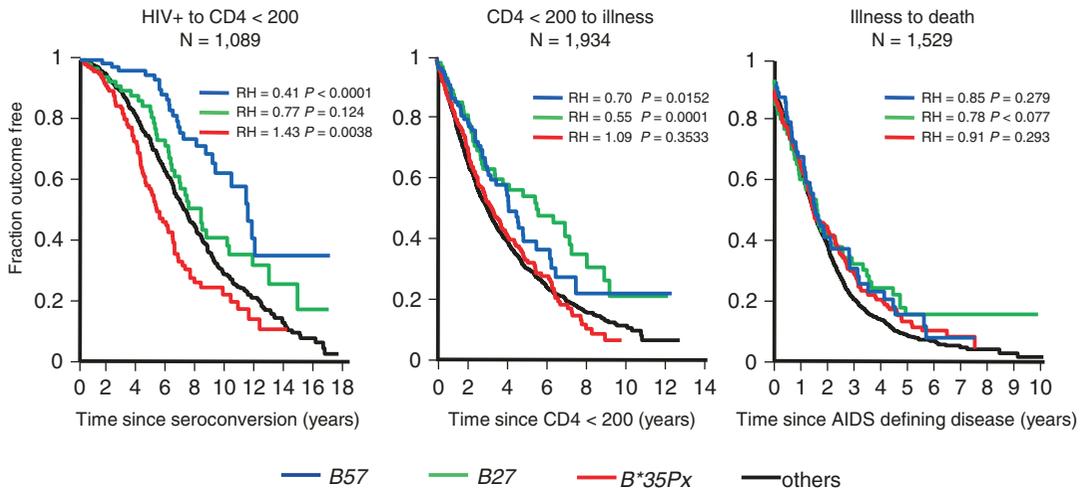
Effect of *HLA* Class I Alleles on HIV/AIDS

Distinct *HLA* allotypes may mediate differential responses to HIV infection and have varied capacities to contain HIV replication. Detection of individual allelic effects helps to identify critical HIV epitopes that are involved in protective immune responses. The most extreme *HLA* allelic effects on HIV/AIDS appear to involve the *HLA-B* locus to a greater extent than *HLA-A* or *HLA-C* (Kiepiela et al. 2004). Three *HLA-B* alleles, *B*57*, *B*27*, and *B*35* (particularly some subtypes of *B*35*), have been consistently associated with the HIV disease outcomes in multiple cohort studies.

*Protective Effect of B*57.* Large cohort studies have shown that *B*57* confers the strongest and most consistent protective effect in terms of viral load control and AIDS progression among all *HLA* variants (Bashirova et al. 2011). There are two major *B*57* subtypes: *B*57:01* in Europeans and *B*57:03* in Africans, which differ by only two amino acids. They share identical peptide-binding motifs and strongly associate with protection from HIV/AIDS as indicated by both the candidate gene approach and GWAS.

A small subset of HIV-infected individuals known as elite controllers (individuals who manage to contain HIV levels to less than 50 copies of the virus per ml of plasma without treatment) (“► **Viremic Non-Progressors**”) are highly enriched for *B*57*, where about 40% carry this protective allele as compared to only 9% in non-controllers, which is about the same frequency that is observed in the general population (Emu et al. 2008). *B*57* confers pronounced protection for time to CD4 < 200 as indicated by survival analysis (Gao et al. 2005; Fig. 2), but most people with the *B*57* allele do eventually develop progressive infection.

The *B*57* allele restricts four immunodominant HIV Gag epitopes, TW10 (TSTLQEQIAWp24₂₄₀₋₂₄₉), KF11 (KAFSPEVIPMEp24₃₀₋₄₀), ISW9 (ISPRTLNAWp24₁₄₇₋₁₅₅), and QW9 (QASQEVKNWp24₃₀₈₋₃₁₆). These



MHC Locus Variation, Fig. 2 Kaplan-Meier survival curves of *HLA-B*57* (blue), *B*27* (green), and *B*35Px* (red) alleles on different stages of AIDS progression from

seroconversion to CD4 < 200, from CD4 < 200 to AIDS-defining illness, and from illness to AIDS-related death

epitopes are relatively conserved, implicating structural constraints on viral mutation that limits ability of the virus to escape from the CTL response. One of the most frequently observed mutations in *B*57*-positive subjects, T242N in TW10, negatively affects viral fitness in vitro and is observed to revert back after transmission to a *B*57*-negative individual (Crawford et al. 2009).

As an allele bearing the Bw4 epitope, a known ligand for KIR3DL1 and potentially KIR3DS1, *B*57* is involved in regulating NK cell function. It has been demonstrated that patients carrying both *B*57* and highly expressed *KIR3DL1* allotypes exhibit a higher level of protection against AIDS progression as compared to patients without this compound genotype combination. These observations suggest that the *B*57* protection against AIDS progression is due at least in part to its interaction with KIR (“► [KIR Locus Variation](#)”) in addition to its role in the adaptive immune system (see “► [KIR Locus Variation](#)”).

Protective Effect of *B*27*. *B*27* allele carriers maintain lower viral loads over the course of infection (Pereyra et al. 2010) and develop AIDS more slowly as compared to non-carriers (Gao et al. 2005). *B*27* molecules restrict immunodominant CTL responses against a conserved Gag epitope, KK10 (KRWILGLNK

p24_{263–272}). Full viral escape typically arises late in infection and precedes a dramatic increase in viremia. This escape requires multiple mutations located within and outside the epitope (Schneidewind et al. 2007). Difficulty to achieve these mutations due to heavy fitness constraints and lack of particularly strong CTL pressure has been proposed to be the mechanism for protection (Gao et al. 2005; Schneidewind et al. 2007). Based on survival analysis, the protective effect is most evident after the CD4 T-cell counts (“► [CD4⁺ T Cell Depletion](#)”) drop below 200 (Fig. 2; Gao et al. 2005).

The predominant subtype of *B*27* in Europeans is *B*27:05*. Both *B*27:04* and *B*27:05* are common subtypes in Asians, whereas *B*27* is only sporadically detected in some other populations, such as Africans. There has been no clear evidence showing that the protective effect of *B*27* is confined to particular subtypes, but this would require very large sample sizes.

Susceptible Effect of *B*35*. *HLA-B*35* is the only *HLA* allelic group showing consistent associations with rapid progression to AIDS (Bashirova et al. 2011). The *B*35* effect on AIDS progression appears to be confined to a certain group of *B*35* subtypes collectively referred to as *B*35Px*, which exhibit distinct peptide-binding preferences. The set of *B*35Px*

molecules (B*35:02, B*35:03, B35:04, B*53:01) preferentially bind peptides with small hydrophobic residue at position 9 as opposed to B*35PY (B*35:01, B*35:08), which favors tyrosine at this position. *B*35Px* shows a consistent association with accelerated AIDS progression across races, whereas *B*35PY* is mostly neutral. The AIDS-associated *B*35:03* differs from the neutral *B*35:01* by only a single amino acid at position 116 on the floor of the peptide-binding groove, but this single replacement appears to have a significant impact on AIDS pathogenesis. Structural differences in the peptide-binding groove were suggested to effect the CTL response to HIV, making the *B*35Px* positive individuals more vulnerable during HIV infection. Similar to the timing of the *B*57* protection, the detrimental effect of *B*35* on progression is limited to time from seroconversion to CD4 < 200 (Fig. 2).

Recent data suggest that the differential *B*35* subtype effects might involve regulation of dendritic cells (DC) (Huang et al. 2009). DCs are major antigen-presenting cells that are crucial in staging CTL responses. DC activity is regulated by a series of inhibitory and activating receptors, one of which is the inhibitory leucocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2). Binding of HLA ligand to LILRB2 expressed on the DC surface results in suppression of DC activity. Interestingly, B*35:03, an AIDS-susceptible B*35Px allotype, binds LILRB2 with greater affinity than does B*35:01, an AIDS-neutral B*35PY allotype, even when these molecules are folded with identical HIV peptides. The greater affinity of B*35:03 for LILRB2 is suggested to inhibit DCs function, which may explain the accelerated AIDS progression.

Other HLA Class I Associations. Additional class I *HLA* associations have been reported in multiple studies. These associations include the protective effect of several *HLA-Bw4* (a public epitope shared by many *HLA-B* and *HLA-A* subtypes, which serves as ligands for KIR) (“► [KIR Locus Variation](#)”) alleles, including *B*13*, *B*51:01*, *B*58:01*, and *B*81:01*, and the deleterious *B*58:02* and *B*45:01*. Some of these alleles have highly confined frequency

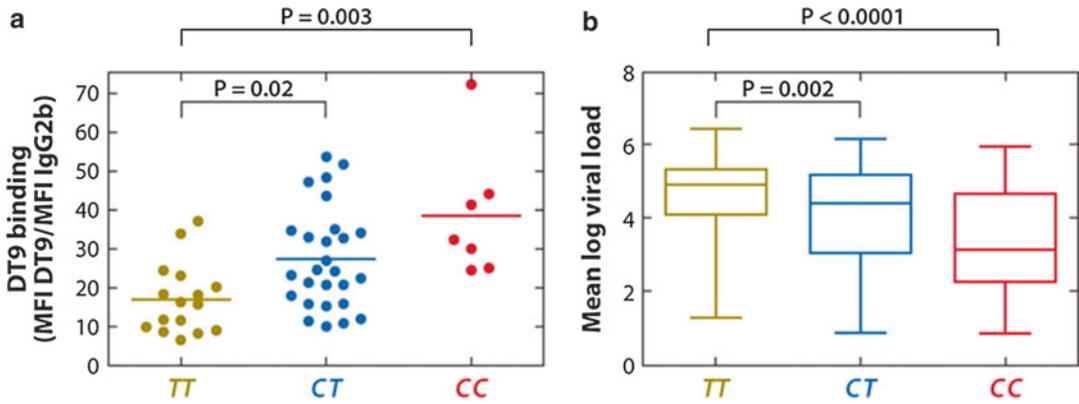
distributions across populations, such as the primarily African *B*81:01* and *B*58:02* alleles, which may explain their somewhat inconsistent associations in different cohort studies. Of particular interest, however, is the identification of *B*13*- and *B*51*-restricted HIV epitopes that result in HIV escape mutants with reduced viral fitness and delayed progression to AIDS (Kawashima et al. 2010; Prado et al. 2009). Since *B*13* and *B*51* are detected in most populations, the associated AIDS protection may have a general interest in terms of vaccine development.

Class II *HLA* and HIV Disease

Although some studies suggest that *HLA* class II allelic variation can influence HIV/AIDS outcomes, the data are not nearly as strong and consistent as that for class I alleles (Bashirova et al. 2011). Some associations with HIV acquisition and level viral load have been reported for the *HLA-DRB* and *HLA-DQB* loci, but the results are not terribly convincing due to the lack of reproducibility and/or small sample sizes. Promiscuity in binding of a given peptide across multiple distinct *HLA* class II allotypes may nullify allele-specific effects to some extent (Kaufmann et al. 2004), but more thorough examinations in larger cohorts with detailed clinical outcomes are necessary before a convincing conclusion can be drawn regarding the influence of *HLA* class II polymorphisms in AIDS pathogenesis.

HLA-C Expression and HIV Control

The limited polymorphism and lower cell surface expression of *HLA-C* relative to *HLA-A* and *HLA-B* have questioned its importance in defense against infectious disease, and no clear allelic effects at this locus have been identified in HIV disease outcomes. *HLA-C* serves as a “professional” ligand for KIR (“► [KIR Locus Variation](#)”), however, highlighting the functional significance of the locus, at least in terms of the innate immune response. The -35 variant



MHC Locus Variation, Fig. 3 The -35 genotype correlates with surface HLA-C expression on T cells (a) and mean log viral load (b). HLA-C expression was detected

using the DT9 monoclonal antibody and compared between pairs of genotypes. The viral load was analyzed in a cohort of 923 HIV-1-infected patients

identified in GWAS (see the section above) associates with HLA-C mRNA and cell surface expression of HLA-C (Bashirova et al. 2011; Fig. 3). The SNP was subsequently shown to be a marker for an insertion/deletion polymorphism within a microRNA (miR) binding site located in the *HLA-C* 3' untranslated region (UTR) (Kulpa and Collins 2011). An insertion variant at this locus allows the binding of hsa-miR-148 to its target site, resulting in relatively low cell surface expression of HLA-C alleles bearing this variant. Alleles with the deletion variant, on the other hand, escape posttranscriptional regulation due to disruption of the miR148a binding site, resulting in relatively higher surface expression. Individuals with the deletion polymorphism (high expression of HLA-C) were shown to progress more slowly to AIDS and control viremia (“► Viremic Non-Progressors”) significantly better than individuals with the insertion polymorphism (low HLA-C expressing alleles). These data implicate high HLA-C expression levels in more effective control of HIV-1, potentially through better antigen presentation to cytotoxic T lymphocytes or stronger NK cell responses to HIV-infected target cells (“► NK Cell Responses to HIV”). In support of this hypothesis, a recent study found that there was an increased frequency of *HLA-C*-associated mutations in HIV proviral DNA among individuals with the genotype that associates with high HLA-C expression (Blais

et al. 2012). These findings suggest that immune pressure on HIV is stronger in subjects with higher HLA-C expression. Linkage disequilibrium with *HLA-B* complicates the genetic analysis of variation within the *HLA-C* locus, however, and further data is necessary to define the role of HLA-C expression levels in HIV disease (Kulpa and Collins 2011).

Conclusions

HLA genes, in particular *HLA* class I, play an important role in determining the level of resistance to outcome after HIV infection. Both the candidate gene approach and GWAS point to *HLA* as the region harboring the most significant AIDS-restriction polymorphisms. Evaluation of the *HLA* influence on resistance to HIV infection has been difficult due to the lack of a precisely quantified measurement of HIV exposure, but analysis of heterosexual couples discordant for infection indicates no clear protective effect of *HLA* in HIV acquisition. Even the most prominent alleles that protect against multiple outcomes after HIV infection, *B*27* and *B*57*, do not associate with reduced risk of HIV infection. Postinfection, however, *HLA* may influence the infectivity of HIV transmitters through its effect on viral load levels. Further, more sharing of *HLA* alleles between HIV+ mothers and their infants does

appear to enhance the risk of infection in the infant.

While the *HLA* class I locus is the single most significant region of the genome in determining outcome after HIV infection, most individuals progress to AIDS in the absence of treatment regardless of their *HLA* type. *B*57* is common among the rare group of patients who control HIV to near undetectable levels off treatment, but most *B*57*-positive patients progress to AIDS as rapidly as those with other *HLA* types. These observations underscore the importance of other variables in determining control of HIV, including environmental and/or viral factors. It is entirely plausible that combinations of genetic variants that synergize in resistance to the virus may explain the discrepancies in viral control among carriers of a given *HLA* allele, such as *B*57*, and these epistatic interactions are not considered in GWAS. A deeper interrogation of genetic effects using a systems biology approach that takes into account the *HLA* loci may be warranted in HIV disease.

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Microbial Translocation

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Definition

Human immunodeficiency virus (HIV) infection is associated with a multifaceted activation of the immune system, which predicts disease progression better than plasma viremia or the number of CD4 T cells in blood. One of the causes of this multifaceted immune activation is microbial translocation, which is associated with the progressive breakdown of the physical and immunological gastrointestinal (GI) barriers. One of the many detriments associated with persistent immune activation is the production of activated CD4 T cells, which serve as preferential targets for the virus in vivo. Ultimately, sufficient numbers of CD4 T cells are depleted, and HIV-infected individuals become susceptible to opportunistic infections, and the disease culminates in acquired immunodeficiency syndrome (AIDS). Ongoing research in both HIV-infected humans and simian immunodeficiency virus (SIV)-infected non-human primate models continues to reveal that a

failure to regulate GI tract immunity and GI-resident microbial communities underlies the persistent immune dysregulation observed in HIV-infected individuals.

Introduction

Human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). Since the initial descriptions of AIDS in 1981, successive generations of antiretroviral (ARV) therapies have led to significant improvements in the quality and life span of infected individuals. Current-era ARV therapy is able to reduce virus replication to undetectable levels and generally to restore circulating CD4+ T-cell counts to near normal levels. However, even individuals treated with ARVs for prolonged periods of time still manifest increased mortality and morbidities compared to healthy, HIV-uninfected persons.

In both ARV-treated and untreated individuals, the strongest correlate of disease progression is chronic and multifaceted activation of the immune system. This includes persistent inflammation and stimulation of innate and adaptive leukocytes. As HIV preferentially replicates within activated CD4+ T cells, this persistently proinflammatory state promotes CD4+ T-cell depletion and contributes to immunodeficiency. Within the GI tract, CD4+ T-cell depletion and damage to the epithelial barrier permit gut-resident microflora and their immunostimulatory structural components to translocate from the GI lumen into the surrounding tissue space. Importantly, although opportunistic infections are a hallmark of AIDS progression, the translocation and dissemination of microbes into systemic circulation is evident throughout HIV infection and has been identified as a significant contributor to immune activation.

Another cause of microbial translocation in HIV-infected individuals appears to result from dysbiosis of the enteric microbiome that is not restored upon ARV initiation. As the commensal microbiome itself is essential for the development and maintenance of the intestinal immune system, persistent dysbiosis induced by HIV infection thus

impedes the recovery of intestinal CD4+ T cells. It is anticipated that continued research into the interplay between HIV, the enteric microbiome, and the human immune system will lead to improved HIV therapeutics.

HIV Infection as a Disease of the Gastrointestinal System

Approximately 10^{14} commensal bacteria colonize the lumen of the human GI tract and exist in a symbiotic relationship with the host (Kamada et al. 2013). Nutrients consumed by the host are utilized by the microflora for survival, and in turn, these microbes aid in nutrient digestion, compete with pathogenic species for nutrients and space, and promote the development of the intestinal immune system. Although commensal microbes are not pathogenic per se, these microflora can opportunistically cause systemic infection in a human host under conditions of genetic or acquired immunodeficiencies. In the absence of disease, several physical and immunological barriers limit the dissemination of both commensal and pathogenic bacteria from the luminal space into the surrounding tissues. These barriers include: (a) a sheet of intestinal epithelial cells that separates luminal contents from the underlying lamina propria of intestinal tissues, (b) a supra-epithelial bioactive layer of mucus that limits microbial colonization at the epithelial barrier, and (c) a cohort of innate and adaptive immune cells within the lamina propria that orchestrate antimicrobial immune responses. The integrity of these barriers is essential for preventing the dissemination or translocation of GI tract bacteria and bacterial products from the luminal space into the intestinal lamina propria.

HIV infection significantly compromises the structural and immunological integrity of the GI tract, resulting in progressive microbial translocation (Brenchley 2013; Marchetti et al. 2013). The GI tract is the largest lymphocyte-containing organ in the body containing an estimated 60% of the body's total lymphocytes. Although progression to AIDS is characterized by the loss of peripheral blood CD4+ T cells to less than

200 cells/ μl , it is well documented in acute HIV infection that the greatest loss of CD4⁺ T cells occurs with the GI tract, with an estimated depletion of 70% of intestinal CD4⁺ T cells. Intestinal CD4⁺ T cells represent the preferential targets for HIV infection by virtue of their activation phenotype, their expression of the CD4 receptor and CCR5 coreceptor, and their expression of the intestinal-homing integrin $\alpha 4\beta 7$, which has been shown to enhance the formation of the virologic synapse.

Despite representing the preferential HIV target population, reported infection frequencies of intestinal CD4⁺ T cells have varied widely and reflect the multiple mechanisms by which CD4⁺ T cells are depleted. Intestinal CD4⁺ T-cell depletion can be mediated by: (a) direct virally induced cell death of infected cells, (b) CD8⁺ T-cell-mediated recognition and cytolysis of HIV-infected cells, (c) activation-induced cell death (AICD), and (d) caspase-1-induced pyroptosis in abortively infected resting CD4⁺ T cells (Brenchley 2013; Doitsh et al. 2013; Marchetti et al. 2013).

Among CD4⁺ T cells within the GI tract, specialized CD4⁺ T cells with unique functionality, known as Th17 cells are preferentially depleted (Bixler and Mattapallil 2013). Th17 cells secrete IL-17 and IL-22, cytokines that can: (a) directly and indirectly promote the recruitment of innate immune cells to mucosal tissues, (b) induce the production of antimicrobial peptides such as S100 and β -defensin by the mucosal epithelial barrier, and (c) promote the proliferation of epithelial cells directly. Although Th17 cells are preferentially depleted, they are not preferentially infected, indicating that Th17 cell development and homeostasis are specifically compromised as a consequence of direct HIV infection. Th17 cell development is dependent upon priming by CD103⁺ dendritic cells (DCs) within the mesenteric lymph nodes and lymphoid follicles within the GI tract. Longitudinal studies in Asian macaque nonhuman primates (who can be progressively infected with SIV) have revealed that SIV infection is associated with a reduction in the total frequency of GI tract-resident CD103⁺ DCs, the net result of which is loss of lymphocytes which express

IL-17 and IL-22. The preferential loss of lymphocytes expressing IL-17 and IL-22 cells is associated with a reciprocal expansion of T cells with other functionalities, such as regulatory T cells or cells expressing proinflammatory cytokines such as TNF α and IFN γ .

The immunological dysfunction within the GI tract is associated with alterations to the actual structural integrity of the GI tract. This so-called HIV-associated enteropathy includes: enterocyte apoptosis, discontinuity of the epithelial barrier, decreased expression of epithelial repair genes, shortened villi, and fibrosis within the lamina propria, lymphoid follicles, and other lymphoid tissue (Brenchley 2013; Marchetti et al. 2013). Direct visualization the intestinal epithelium from biopsies of HIV-infected persons and SIV-infected macaques has revealed that disease progression is associated with the accumulation of breaches along the epithelial barrier. The accumulation of these breaches has been attributed to the widespread apoptosis of epithelial cells during the acute phase of SIV infection, regenerative exhaustion, and the absence of the Th17-directed epithelial repair program. The physical degradation of the mucosal epithelium allows microbes to directly translocate into the lamina propria and then systemically.

Microbial Translocation as a Consequence of HIV Infection

Microbial translocation was first demonstrated by the quantification of the gram-negative cell wall component lipopolysaccharide (LPS) from the plasma of HIV-infected individuals and SIV-infected Asian macaques (Brenchley et al. 2006). In these subjects, plasma LPS increased in tandem with disease progression and correlated directly with markers of innate and adaptive immune activation. Multiple groups have since corroborated and expanded upon these initial findings, demonstrating that a variety of microorganisms and microbial products can be isolated from systemic tissues and that microbial translocation begins at the end of the acute phase of infection. Importantly, although the initiation of ARV therapy is associated with a reduction plasma LPS levels,

plasma LPS remains significantly higher in infected, treated individuals than in uninfected individuals, suggesting that HIV replication precipitates but does not directly mediate microbial translocation and that ARV treatment alone is incapable of completely restoring the physiology of the GI tract.

Recent advances in next-generation sequencing platforms have made the identification of GI tract bacterial communities possible. These studies have revealed that HIV-associated microbial translocation is part of a larger phenomenon known as microbial dysbiosis – an imbalance in the makeup of the microbiome (Brenchley 2013; Marchetti et al. 2013; Vujkovic-Cvijin et al. 2013). This dysbiosis of the microbiome in HIV-infected individuals includes a relative expansion in immunostimulatory protobacterial orders such as Enterobacteriales and Bacteroidales. Dysbiosis also includes enrichment of bacterial genera known to be opportunistic pathogens including *Staphylococcus*, *Pseudomonas*, and *Campylobacter*. Effective ARV therapy does not necessarily promote the restoration of balanced microbial communities in HIV-infected individuals, suggesting that microbial dysbiosis may play a role in the residual inflammation observed in ARV-treated individuals. Microbial dysbiosis has been shown in other diseases associated with microbial translocation including Crohn's disease, ulcerative colitis, and pouchitis, and studies in nonhuman primates have revealed that although SIV infection is associated with an increased incidence of enteropathy, SIV infection itself is not associated with dysbiosis.

Microbial Translocation, Immune Activation, and AIDS Progression

In the absence of chronic or autoimmune disease, immune activation is resolved concurrent with the clearance of a recognized pathogen. With HIV, however, the GI tract is not able to completely heal and microbial translocation persists. Although viral replication contributes to immune activation in HIV-infected persons, immune activation is still evident following

years of ARV therapy, indicating that replication itself does not solely promote immune activation. Instead, microbial translocation and dysbiosis appear to contribute significantly to persistent immune activation.

The microflora and their associated microbial products are particularly immunogenic within the human host, and their accumulation during HIV infection correlates with immune activation and depressed CD4+ T-cell counts (Brenchley et al. 2006; Brenchley 2013; Marchetti et al. 2013). With regards to LPS in particular, several LPS-specific immune mediators are found to be elevated during acute HIV and SIV infection, such as LPS-binding protein (LBP) and soluble and membrane-bound CD14. LPS recognition by these immune mediators complements toll-like receptor 4 (TLR4) signaling to initiate a pro-inflammatory cascade characterized by high levels of IL-6, TNF α , and type I interferons. Although elevated microbial translocation is not limited to LPS or to gram-negative bacteria, the enrichment of disease-associated taxa themselves correlates with elevated levels of plasma markers of inflammation (such as IL-6 and IP-10), increased intestinal indoleamine (IDO), and elevated T-cell activation in chronic HIV infection (Vujkovic-Cvijin et al. 2013).

Indoleamine 2,3 deoxygenase is a tryptophan-metabolizing enzyme expressed by select subsets of activated myeloid cells. Tryptophan metabolism by IDO1 leads to production of catabolites including 3-hydroxyanthranilic acid (3-HAA), which has been shown to limit production of IL-17 by Th17 cells (Bixler and Mattapallil 2013; Brenchley 2013; Vujkovic-Cvijin et al. 2013). Moreover, IDO1 is the rate-limiting enzyme in the tryptophan/kynurenine catabolic pathway, and elevated kynurenine levels in HIV-infected individuals correlates with disease progression. In HIV-infected individuals, elevated IDO1 expression has been detected within myeloid dendritic cells, and interestingly, homologues to additional enzymes within the kynurenine pathway have been identified among disease-associated bacterial taxa within the GI tracts of HIV-infected individuals.

Chronic stimulation of the immune system by microbial products takes a toll on the immune

system. Indeed, monocytes isolated from chronically infected patients are refractory to prototypical LPS stimulation with decreased expression of TNF α and IL1 β (Brenchley et al. 2006). This refractoriness to LPS stimulation *in vitro* is, in turn, correlated with increased PD-1 expression by monocytes themselves and with the production of immunosuppressive IL-10. TLR stimulation of DCs isolated during chronic infection also exhibits a profound reduction in IL-12, IL-6, and TNF α production (Miller and Bhardwaj 2013). Altered TLR responses in dendritic cells have been associated with direct HIV-mediated downregulation of autophagy, which has been suggested to negatively affect antigen presentation. It has been speculated but not formally demonstrated that HIV-mediated autophagy interference or increased inhibitory receptor expression may similarly contribute to defective macrophage phagocytosis.

Microbial Translocation as a Mediator of HIV-Associated Comorbidities

HIV-infected individuals have a greater risk of non-AIDS-associated comorbidities and mortality as compared to uninfected population controls, even if the individual has been treated with ARVs (Brenchley 2013; Deeks et al. 2013; Marchetti et al. 2013). Cardiovascular diseases, neurocognitive dysfunctions, osteoporosis, liver disease, renal complications, and some cancers are more common in these individuals. It is clear that all of these comorbidities are associated with residual inflammation, especially elevated plasma levels of IL-6. There is evidence to suggest that microbial translocation may precipitate or exacerbate the residual inflammation. Indeed, a clinical association exists between microbial products, makers of inflammation, and cardiovascular risk. Consistent with this premise, in HIV-uninfected persons, bacterial endotoxin is a proinflammatory mediator of atherosclerosis. Similarly, plasma levels of soluble CD163, a scavenger receptor expressed by activated monocytes and macrophages, correlate with the presence of noncalcified coronary plaques in HIV-infected

individuals. Also related to cardiovascular disease is hypercoagulability, which is a common phenomenon in HIV-infected individuals. Biomarkers associated with excessive coagulation, particularly levels of D-dimer, are strongly associated with increased morbidity and mortality. Although levels of HIV replication correlate with D-dimer plasma levels in untreated, HIV-infected individuals, ARV-treated individuals still exhibit increased D-dimer levels as compared to control populations, suggesting that residual immune activation from ongoing microbial translocation may drive hypercoagulability. There is some evidence for this in nonhuman primate models of HIV infection, where high levels of virus replication in non-pathogenically SIV-infected monkeys are not associated with increased cardiovascular risks, whereas SIV infection in progressively infected monkeys is associated with increased D-dimer levels and multiorgan thrombus formation.

Antimicrobial Treatments in HIV Infection

A recent appreciation for the contributions of microbial translocation and dysbiosis to AIDS progression has promoted an interest in the development of prophylactic therapies aimed at restoring microbial stasis and antimicrobial immunity. These therapies fall into two broad categories: those aimed at directly repairing antimicrobial immune responses and those aimed at indirectly repairing intestinal immunity by restoring the commensal flora. Among the former, IL-21 cytokine therapy has been considered as a potential intervention for Th17 cell loss (Pallikkuth et al. 2013). IL-21 is essential for the development of Th17 cells and has additionally been demonstrated to promote CD8 $^+$ T-cell and NK cytotoxicity, to induce B-cell maturation and isotype switching, and to promote phagocytosis by macrophages. In acutely SIV-infected rhesus macaques, IL-21 administration has been demonstrated to restore intestinal CD4 $^+$ T-cell frequencies and to reduce plasma LPS into the chronic phase of SIV infection, as compared to untreated controls. Among the IL-21-treated animals, IL-21

therapy was associated with the maintenance of the Th17 population concomitant with the treatment period – the cessation of treatment in these animals was accompanied by the depletion of Th17 cells to the levels observed in control animals, likely due to ongoing virus replication. Although the effects of IL-21 therapy in HIV-infected patients have not been assessed, IL-21 has been demonstrated to be immunogenic and well tolerated in several therapeutic phase II clinical cancer trials.

Treatment with anti-PD1 has been considered as a potential therapeutic to reverse immune exhaustion in HIV-infected individuals and has also been demonstrated to be fairly well tolerated and active in phase II clinical cancer trials. In chronically SIV-infected rhesus macaques, treatment with anti-PD1 in the absence of ARV therapy has indicated that immune exhaustion is reversible (Dyavar Shetty et al. 2012). As compared to SIV-infected control animals, anti-PD1-treated animals displayed improved antiviral and antimicrobial lymphocyte function and decreased immune activation as measured by interferon-stimulated gene expression. Importantly, the improved health in these animals was not associated with a reduction in viral load but was associated with an increase in GI expression of tight-junction-associated genes, a decrease in plasma LPS, and a decrease in the incidence of opportunistic infections. Improved disease prognosis in anti-PD1-treated, chronically SIV-infected non-human primates reflects the paradox that although aberrant immune activation precedes immune exhaustion and correlates with disease progression, antiviral and antimicrobial immune responses are necessary for preventing overt septicemia and rapid disease progression.

Recently, there has been a significant interest in the effects of probiotics in modulating intestinal immunity for the purposes of treating GI-associated immunopathologies. Probiotics consist of single or multiple live bacterial species that have been shown to confer a protective benefit to their host upon consumption (Bron et al. 2012; Kamada et al. 2013). Probiotics benefit their host by competing with pathogenic bacterial species for nutrients and space, inducing the development

of a balanced intestinal immune system, and producing short-chain fatty acids as part of normal metabolic processes. Multiple trials have assessed the effects of probiotics on disease progression in HIV-infected individuals in the form of synbiotics – probiotics administered with “prebiotics” or oligosaccharide mixtures that are thought to promote the growth of probiotic organisms (Brenchley 2013; Marchetti et al. 2013). Although several publications have reported increased CD4 + T-cell counts in response to probiotic treatment, the specific additional benefits induced in these trials have varied widely, confounded by an inconsistent use of probiotic strains, varied administration conditions, and diverse cohort parameters. Of studies that have more rigorously assessed microbial dysbiosis and levels of immune activation in response to probiotic treatment, treatment of both ARV-treated and ARV-naïve HIV-infected individuals with synbiotics has resulted in improved CD4+ T-cell counts, decreased proinflammatory cytokine secretion and sCD14 levels, and improved dysbiosis as compared to placebo controls. Despite the beneficial systemic outcomes observed in these trials, the exact mechanisms by which probiotics promote improved health in HIV-infected individuals remain incompletely defined. In SIV-infected macaques, synbiotic treatment as a supplement to ARVs was recently demonstrated to improve intestinal APC frequency and function, to promote an increase in the functionality of Th17 cells, to decrease fibrosis in GI lymphoid follicles, and to increase GI tract CD4 T-cell reconstitution (Klatt et al. 2013). Although the effects of particular probiotic species and regimens on immune reconstitution during HIV infection warrant further study, the results reported indicate that symbiotic supplementation of ARV therapy may significantly improve the prognosis of HIV-infected individuals.

Conclusion

Progressive HIV infection is associated with the disruption of the physical and immunological GI barriers and the subsequent dissemination of GI

microflora (Brenchley et al. 2006; Brenchley 2013). The outgrowth and translocation of immunostimulatory microflora promote the persistent activation of both innate and adaptive leukocytes, contributing to the inflammatory sequelae characteristic of pathogenic HIV infection (Kamada et al. 2013; Marchetti et al. 2013). Although effective ARV therapy has been shown reduce immune activation, residual inflammation in treated individuals correlates with the continued translocation of microbial products and particularly, with the persistence of disease-associated microflora (Deeks et al. 2013). This lingering microbial dysbiosis inhibits the reestablishment of a competent intestinal immune system, thereby perpetuating inflammation and immunodeficiency (Brenchley et al. 2006; Vujkovic-Cvijin et al. 2013). Recent insights into the contributions of microbial translocation and dysbiosis to HIV disease progression warrant continued research into therapeutics aimed at restoring antimicrobial immunity and commensal stasis.

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Microfinance/Microenterprise Approaches to HIV Prevention

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Definitions

- Microcredit:** Described as an approach that consists of providing credit in relatively small loan amounts through a group lending process and compulsory savings.
- Microfinance:** Involves the provision of credit along with other financial products (i.e., savings accounts), microinsurance (i.e., health, life, and disability), and/or business development education (i.e., literacy training, marketing, and accounting) offered either directly or indirectly.

Microfinance/Microenterprise Approaches to HIV Prevention

Structural factors affecting HIV risk are widely acknowledged; however, research evaluating structural HIV prevention interventions, such as microenterprise, is sparse. In this chapter, microenterprise is defined, a description of its history and potential for HIV prevention is provided, the effects of several microenterprise HIV prevention studies are described, and lastly, recommendations for future programs and studies are provided.

Microenterprise, Microfinance, and Micro-lending

Over the past decade, researchers have examined the role of microenterprise in reducing poverty as well as the risk and burden of HIV among various populations throughout the world. Although distinct, the terms microenterprise, microfinance, and micro-lending all refer to some level of access to resources, either financial and/or social, and are often used interchangeably. Microcredit is often described as providing credit in relatively small loan amounts through a group lending process and compulsory savings. Microfinance involves the provision of credit along with other financial products (i.e., savings accounts), microinsurance (i.e., health, life, and disability), and/or business development education (i.e., literacy training, marketing, and accounting) offered either directly or indirectly. Economists have suggested that participation in microfinance programs that increase the level of access to resources can improve economic well-being by building assets as well as increasing income, thereby decreasing economic vulnerability among the world's poorest people (Leatherman et al. 2012). This chapter will use the term "microenterprise" to encompass the range of these financial services that are targeted to low-income individuals, including credit, savings, insurance, money transfers, and trainings.

Microenterprise, Poverty, and HIV/AIDS

While prior research has established the positive effect of microenterprise schemes on economic outcomes, researchers have just recently started to examine their impact on more distal yet related outcomes, such as health (Sherman et al. 2010). Poverty is a common feature of HIV vulnerability throughout the world, and the link between poverty and HIV risk is tied intimately to the socio-economic context in which HIV occurs (Kim et al. 2007).

As HIV/AIDS enters its fourth decade, there has been a shift in HIV prevention from solely focusing on individual risk behaviors to the environments or structures in which HIV transmission occurs. Such interventions emphasize more on "upstream" factors, or social determinants of health (Williams et al. 2008), such as poverty, in ways that have the potential to produce more effective community responses to public health problems, such as HIV/AIDS (Kim et al. 2008).

By providing access to resources and/or money to those that are economically vulnerable and largely disempowered segments of society who are often times at greatest risk of HIV, microenterprise can play a particularly unique and important role within HIV/AIDS prevention. More specifically, because poverty has been linked with increased risk of HIV, HIV progression, and increased mortality, particularly among women (Kim et al. 2008; Mbirimtengerenji 2007; Piot et al. 2007), microenterprise has the potential to mitigate the effects of HIV prior to and after transmission.

Microenterprise and Women

Among the approximately one billion individuals living in poverty, women shoulder a disproportionate share of this burden: they earn 30–40% less than men for the same work, and most are employed in the informal sector, which places them at higher risk for income insecurity and poor working conditions (UNIFEM 2000). These factors may increase the vulnerability of women to HIV by extending their dependence on men and further limiting their ability to refuse sex, negotiate condom use, and get out of risky relationships (Rao 2002).

IMAGE Study

The few examples of microenterprise as an approach to HIV prevention have occurred in Southern Africa, India, and the USA. The Intervention for Microfinance and Gender Equity (IMAGE) study is the largest, most rigorous trial examining the effects of a microfinance and HIV education intervention for women in South Africa (Pronyk et al. 2008). The study was a randomized, prospective, community-level intervention in eight pairs of villages in rural South Africa with multiple structural-level outcomes. The intervention targeted women and was comprised of small group microfinance lending based on the Grameen Bank model and gender-focused training. Although the intervention was not associated with significant reductions in incident HIV, it was associated with significant reductions in physical and sexual partner violence among participants. The study also found significant improvements across a broad range of empowerment indicators.

Several smaller trials examining the effects of microenterprise as an HIV prevention tool have been conducted among female sex workers (FSWs), given the role of economic deprivation in some women's decision to enter sex work, and the occupational hazard of HIV for many FSWs. For example, a small randomized clinical trial among FSWs in Chennai, India, that trained FSWs to make canvas bags found that the intervention was associated with a significantly lower number of sex partners, significant increases in income, and a significantly lower number of paying clients per month at the 6-month follow-up (Sherman et al. 2010).

These two studies provide a snapshot of the types of interventions that have utilized a microenterprise approach to HIV prevention among women. Lessons learned from these and other microenterprise interventions should inform future work. First, the long-term survival of microenterprise activities by those that have limited skills should include an array of related business topics to enhance the success and sustainability of business endeavors. Many participants may not have any experience in engaging in finance programs; therefore, savings-led programs may provide a safer introduction to microfinance, since they foster

asset development and financial independence without the financial risks associated with debt. Microenterprise programs could benefit from the inclusion of training content that incorporates an explicit gender focus, raises awareness about gender roles and cultural beliefs, and provides an opportunity for women to discuss often stigmatized subjects such as sexuality, HIV/AIDS, and gender-based violence in a safe environment. Such content could potentially increase the success of a microenterprise program by simultaneously increasing empowerment among women. These topics are particularly important when vulnerable women participate in economic empowerment programs, given that such empowerment can often threaten their sexual partners. Including partners as a component in such interventions is important to minimize any deleterious repercussions on participants and to sustainably target the context that often generates HIV risk among these women. Lastly, the pilot in India demonstrated that women were interested in and successful at learning a new skill – in this instance, tailoring.

Microenterprise approaches have great potential in reducing HIV risk exposure among women. Because economic deprivation and dependency can push some women into high-risk means of livelihood, such as sex work or other behaviors that place them at higher risk of HIV exposure, microenterprise economic activities can empower women to make safe choices and increase their opportunities, which in turn can reduce their vulnerability to HIV.

Microenterprise and People Living with HIV and AIDS

People living with HIV and AIDS (PLWHA) are economically vulnerable for a myriad of reasons. For PLWHA, poverty poses a formidable obstacle to accessing antiretroviral treatment, due to both individual-level factors (such as prioritization for survival: food, childcare, or school fees) and structural-level factors (such as distance and transportation costs, long wait times, inadequate health infrastructure). For those that are able to initiate antiretroviral therapy (ART), financial losses resulting from HIV (debt, job loss, costs of medical care) present roadblocks to

socioeconomic recovery. In sum, PLWHA suffer increased levels of poverty, in part because of discrimination and in part because of the extra medical burden they face (Viravaidya et al. 2008).

Very little programmatic attention has been paid to the potential for microenterprise for HIV/AIDS stigma reduction efforts (Viravaidya et al. 2008). While a number of microfinance institutions operate in high HIV prevalence areas, most have been cautious to target HIV-positive/affected individuals. Concerns about utilizing microenterprise initiatives with PLWHA include greater rates of absenteeism, loan default, repayment delinquency higher drop-out rates, and premature death (Barnes 2005; Datta and Njuguna 2008).

CARE's Cote d'Ivoire Village Savings and Loan Pilot

In 2007, CARE, a humanitarian organization, launched pilot village savings and loan activities as a part of their HIV prevention and mitigation program in Cote d' Ivoire. Qualitative methods were used to explore the socioeconomic effects of the loan activities on HIV-/AIDS-related factors. Study participants reported using funds from income-generating associations (IGAs) to pay for ART, thereby increasing adherence and access to treatment. Improved health, in turn, then increased the likelihood that community members would loan them money, which then increased their capacity to carry out economic activities, and the use of revenue from IGAs to pay for medical expenses improved perceptions of PLWHA as they were viewed as contributors (Holmes et al. 2011).

A randomized controlled trial examined the impact of child savings accounts as an economic empowerment intervention among AIDS-orphaned youth in rural Uganda. The evaluation found that girls of families who possessed child savings accounts had more protective attitudes or attitudes that aligned with safer sex behaviors. The authors concluded that adolescent girls might be able to develop protective attitudes toward sexual risk taking through economic empowerment programs that address gendered social norms (Ssewamala et al. 2010).

These two studies exemplify ways in which PLWHA's economic vulnerability can be reduced

through involvement in microenterprise. First, income generation can directly translate into ART adherence, but in order to do so, microenterprise programs must be cognizant of the physical limitations that PLWHA may face, as these limitations affect the types of income-generating activities that are feasible for PLWHA. Before microenterprise programs are developed to target PLWHA, it would be pertinent to invest in research to understand which IGAs are most appropriate for PLWHA, based on their health status. Another important consideration is the type of activities that programs targeting PLWHA should offer. Because HIV-related expenses are typically recurring, it is important to choose activities that can yield profit all year long and are stable. Finally, microenterprise programs that include training sessions on risk behavior can shift attitudes and norms around behaviors that may place individuals at increased risk for HIV exposure, by increasing individual self-efficacy and risk perception.

Microenterprise is a tool that is well suited for PLWHA and those affected by HIV/AIDS. Savings-led community-managed microfinance, by providing PLWHA access to money, can mitigate the negative socioeconomic impacts of HIV/AIDS. Microenterprise can generally increase PLWHA financial security and well-being, and even when the benefits are small and are not able to restore business to pre-illness levels, microenterprise can provide a means to pay for food and medicine.

Conclusion

Microenterprise lends itself well to improving health outcomes, because when families have fallen into poverty or are economically vulnerable, health often emerges as a key reason. Specific to HIV/AIDS, reducing poverty is without doubt one of the major strategies for use against the HIV pandemic (Longuet et al. 2009).

While women and PLWHA are both important target populations with regard to HIV exposure, microenterprise can play a role in reducing risk among almost any population. This is due to the fact that microenterprise approaches are rooted in the promotion of access to resources that can

increase economic well-being, which provides individuals the means to have the opportunity to mitigate some of the negative factors that may place them at higher risk for adverse health outcomes.

In sum, microenterprise programs provide an innovative way to address the ongoing challenges of poverty that underlie a range of health issues. Opportunities are now emerging for microfinance institutions and organizations to increase their potential benefits by more directly addressing health-related concerns. There is mounting evidence that implies that combining economic and health interventions can create powerful synergies and broaden program effects. However, interventions and evaluations of such interventions are limited. There is a need to rigorously evaluate such interventions to determine what is the best combination of program components to maximize potential benefits in health and social outcomes. Finally, there is a need to plan for the extension and sustainability of such programs to create viable, self-financing microenterprise programs to encourage and ensure local ownership of such an approach.

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Microsporidiosis

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Definition

Microsporidia are a group of intracellular protists related to Fungi that can cause chronic diarrhea in patients with AIDS, but, depending on the

particular species involved, can also infect many other organ systems with keratoconjunctivitis being a common nongastrointestinal manifestation.

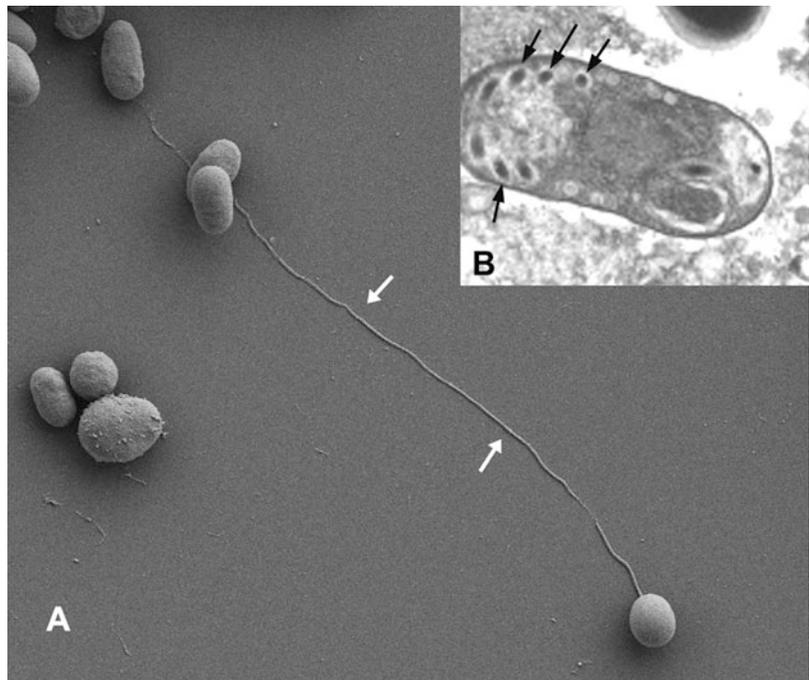
Introduction

The microsporidia are intracellular pathogens that infect both invertebrate and vertebrate hosts (Weiss and Becnel 2014). Microsporidia have a unique invasion organelle, the polar tube, that punctures host cells during invasion allowing transmission of this pathogen (Fig. 1). Given the wide host range of these organisms it is not surprising that they have been identified as human pathogens as they infect almost all animal phyla, including other protists. They were historically considered “primitive” protozoa; however, recent studies suggest that these organisms are related to the Fungi, either as a basal branch of the Fungi or as a sister group (Keeling 2009; Weiss and Becnel 2014), and are not primitive but have lost genes in their evolution as intracellular pathogens. The first description of these organisms was the identification of *Nosema bombycis* 150 years ago as the etiological agent of pébrine, an economically

important disease of silkworms, and the first description in mammalian tissues occurred in 1923. In their hosts, the majority of the microsporidia infect the digestive tract, but infections of almost all organ systems have been documented.

Microsporidia were first linked to infection in humans in 1959 with the description of a child with encephalitis, but until the HIV epidemic infections were only sporadically reported in humans (Franzen 1998). In 1985, intestinal microsporidiosis was reported to be associated with chronic diarrhea and wasting in patients with AIDS (Desportes et al. 1985) and the number of reported cases increased exponentially until the widespread use of combination antiretroviral therapy (cART) resulted in a decline in reported cases. The available literature supports that microsporidia display strength of association, coherence, and reproducibility with respect to being causative for a diarrheal syndrome in patients with AIDS (Coyle et al. 1996). Further proof of this association is provided by efficacy of albendazole, for *Encephalitozoon intestinalis*, and fumagillin, for *Enterocytozoon bieneusi*, in the treatment of diarrhea due to these pathogens. Besides gastrointestinal tract involvement,

Microsporidiosis,
Fig. 1 (a) Scanning electron micrograph (SEM) of a germinated *Anncaliia algerae* spore demonstrating the polar tube (arrow). (b). Transmission electron micrograph (TEM) of an *Encephalitozoon hellem* spore. Polar tube cross sections are indicated by arrows



infection with various species of microsporidia can also cause encephalitis, ocular infection, sinusitis, myositis, or disseminated infection (Weber and Bryan 1994).

In addition, to HIV/AIDS other causes of immune suppression, e.g., antibodies to TNF α , chemotherapy, immune modulating drugs and transplantation, have been demonstrated to be risk factors for microsporidiosis (Weiss and Becnel 2014). Microsporidiosis due to *E. bienersi* has been reported in patients with kidney, liver, or heart-lung transplants; Encephalitozoonidae infections in patients with kidney, pancreas, liver, or bone marrow transplantation; *T. acridophagus* in a patient with bone marrow transplantation; and *A. algerae* in a patient with lung transplantation. Microsporidiosis, however, is not limited to immune compromised hosts and infection can occur in immune competent individuals resulting in diarrhea (mostly due to *E. bienersi* and *E. intestinalis*) and other syndromes, such as keratoconjunctivitis (due to *Vittaforma corneae*, particularly in Southeast Asia).

Species and Life Cycle

The phylum contains over 1200 species distributed into at least 200 genera and the species shown in Table 1 have been demonstrated to be associated with disease in humans (Weiss and Becnel 2014; Weber and Bryan 1994).

Epidemiology

Studies conducted on patients with AIDS before 1998 and the widespread use of cART demonstrated that as many as 70% of patients with chronic diarrhea had microsporidia in their stool samples. There was no overall trend in these studies with regard to country of origin or other demographic characteristics. When combined, these studies identified 375 *E. bienersi* infections among 2400 patients with chronic diarrhea, for a prevalence of 15% in this population (Weiss and

Becnel 2014). In areas in which cART is not widely available the epidemiology of this disease has not changed and a similarly higher prevalence rate is present. Serosurveys in humans have demonstrated a high prevalence of antibodies to *E. cuniculi* and *E. hellem* ranging from 5% to 36% with the higher seroprevalence being seen in studies conducted in the tropics in patients with co-existent tropical infections, such as malaria or filariasis, or in HIV-infected populations. Positive serology has also been associated with travel to the tropics. In temperate countries, the average seroprevalence has been 5%. Although initially regarded as rare, these organisms are now understood to be common enteric pathogens that cause self-limited or asymptomatic infections in normal hosts. Cases of microsporidiosis have been identified from all continents except Antarctica.

Infection with microsporidia, in the majority of cases, occurs due to the oral ingestion of spores, and the site of initial infection is the gastrointestinal tract. Many of the species of microsporidia that infect humans are also found in animals, suggesting that human infection may be a zoonosis. For example, the Encephalitozoonidae are found in many mammals and birds, and the onset of human infection with these organisms has been associated with exposure to livestock, fowl, and pets. Other microsporidia are found in insects and surface water. *Nosema* and *Vittaforma* infections have been associated with traumatic inoculation of environmental spores into the cornea; exposure to hot springs was associated with ocular infections due to *V. corneae*. For both *Tubulinosema* and *Anncaliia* vector borne transmission has been suggested to occur. Spores of human pathogenic microsporidia have been found in municipal water supplies, tertiary sewage effluent, surface water, and ground water. Infectious spores are found in stool, urine, and respiratory secretions suggesting that person-to-person transmission occurs, as has been demonstrated in cohabiting homosexual men. Congenital transmission of *E. cuniculi* has been seen in many mammals including primates, but there are no reports in the literature of congenital infection in humans.

Diagnosis

Light microscopy is the standard technique for the identification of microsporidia using staining methods that produce differential contrast between the microsporidian spores and the debris

in clinical samples (Weber et al. 1992). Microsporidian spores are 1–5 µm in size, depending on the species, necessitating the use of a 60× or 100× objective for clear visualization. Chromotrope 2R (Fig. 2), calcofluor white (fluorescent brightener 28), or Uvitex 2B are

Microsporidiosis, Table 1 Microsporidia identified in human infections

Genus and species	Organ(s) involved	Animal hosts ^c
<i>Encephalitozoon</i>		
<i>E. cuculii</i> ^a	Liver, peritoneum, brain ^b , urethra, prostate, kidney, sinus, eye, bladder, gastrointestinal tract ^b , skin, disseminated disease	Mammals (rabbits, rodents, carnivores, primates)
<i>E. hellem</i> ^a	Eye ^b , sinus, lung, kidney, prostate, urethra, bladder, gastrointestinal tract, disseminated disease	Psittacine birds (parrots, lovebirds, parakeets), birds (ostrich, hummingbirds, finches)
<i>E. intestinalis</i> ^a	Gastrointestinal tract ^b , biliary tract and gall bladder, kidney, eye	Mammals (donkeys, dogs, pigs, cows, goats, primates)
<i>Enterocytozoon</i>		
<i>E. bienersi</i>	Gastrointestinal tract ^b , biliary tract and gall bladder, nose, lung	Mammals (pigs, primates, cows, dogs, cats), birds (chickens)
<i>Trachipleistophora</i>		
<i>T. hominis</i> ^a	Muscle, eye, sinus	Unknown
<i>T. anthropoptera</i> ^a	Brain, eye, disseminated infection,	Unknown
<i>Pleistophora</i>		
<i>P. ronneafiei</i>	Muscle	Unknown
<i>Pleistophora</i> sp.	Muscle ^b	Fish
<i>Anncaliia</i> ^d		
<i>A. vesicularum</i>	Muscle	Unknown
<i>A. algerae</i> ^a	Eye, muscle, skin	Mosquitoes
<i>A. connori</i>	Disseminated disease	Unknown
<i>Nosema</i>		
<i>N. ocularum</i>	Eye ^b	Unknown
<i>Vittaforma</i>		
<i>V. corneae</i> ^a	Eye, ^b bladder	Unknown
<i>Tubulinosema</i>		
<i>T. acridophagus</i> (and <i>Tubulinosema</i> sp.)	Muscle, disseminated disease (skin, liver, peritoneum, lung and retinal involvement)	Insects (<i>D. melanogaster</i> and grasshoppers)
<i>Endoreticulatus</i>		
<i>Endoreticulatus</i> sp.	Muscle ^b	Lepidopteran insects
<i>Microsporidium</i>		
<i>M. africanus</i>	Eye ^b	Unknown
<i>M. ceylonensis</i>	Eye ^b	Unknown

Adapted from Mandell, Douglas, and Bennett's *Principles and Practice of Infectious Diseases*. 7th Edition, Chapter 271: Louis M. Weiss. "Microsporidiosis." Churchill Livingstone (Elsevier), 2010

^aOrganism can be grown in tissue culture

^bCases reported in immune competent hosts

^cAnimals in which organism has been found other than humans

^dPreviously called *Brachiola* and *Nosema*



Microsporidiosis, Fig. 2 Chromotrope 2R stain of stool demonstrating spores of *Enterocytozoon bienewsi*

useful stains for stool or other body fluids. Generally, it is easier to identify microsporidian spores in body fluids other than in stool because of the absence of bacteria and debris, which can be confused with microsporidian spores. False positive samples, due to environmental microsporidia from insects or other sources, has not been a common problem in clinical samples. A number of molecular diagnostic tests have been developed for diagnosis of microsporidiosis using primers to small subunit rRNA genes which permit the identification of microsporidia at the species level without ultrastructural examination (Weiss and Vossbrinck 1998). These PCR techniques have been applied to biopsy specimens, urine, cultures, and stool specimens greatly facilitating diagnostic and epidemiologic studies. These tests are available in reference laboratories such as the Centers for Disease Control and Prevention. Monoclonal antibodies have been developed for *E. hellem*, *E. intestinalis*, and *E. bienewsi* but are not commercially available for diagnosis in human infections. Due to the presence of renal involvement with shedding of spores in the urine in those microsporidia that can cause disseminated infection, urine specimens should be obtained and examined whenever the diagnosis of microsporidiosis is considered. This has therapeutic implications because microsporidia that

disseminate (e.g., the Encephalitozoonidae.) are sensitive to albendazole, whereas those that do not disseminate (e.g., *E. bienewsi*) are resistant.

Endoscopy is useful for the diagnosis of microsporidiosis in cases of chronic diarrhea. Because microsporidian infections usually involve mucosa or epithelium, cytologic preparations are especially useful for diagnosis. In tissue samples, microsporidia can be seen with a modified tissue chromotrope 2R, tissue Gram stain (Brown-Hopp or Brown-Brenn), periodic acid-Schiff Giemsa, Steiner silver stain, or Luna stain. Microsporidia can also be seen on routine hematoxylin and eosin-stained sections. Spores can be demonstrated in fresh tissue by phase contrast microscopy as they are retractile and often birefringent. The definitive identification of the exact species of microsporidia causing an infection can be done using ultrastructural examination (e.g., electron microscopy) or molecular techniques (e.g., PCR). The isolation of microsporidia from clinical specimens is not a routine procedure and is available in only a few specialized research laboratories. Serological testing has been used in animals, but no tests are commercially available for human infections. In addition, serology has not been useful in patients with HIV infection in the diagnosis of microsporidiosis.

Clinical Manifestations

The various microsporidia infecting humans and described infections are summarized in Table 1. Although most reported cases of microsporidiosis involve diarrhea, the spectrum of diseases caused by these organisms has expanded to include keratoconjunctivitis, disseminated disease, hepatitis, myositis, sinusitis, kidney and urogenital infection, ascites, and cholangitis (Weiss and Becnel 2014; Weber and Bryan 1994; Weber et al. 1997).

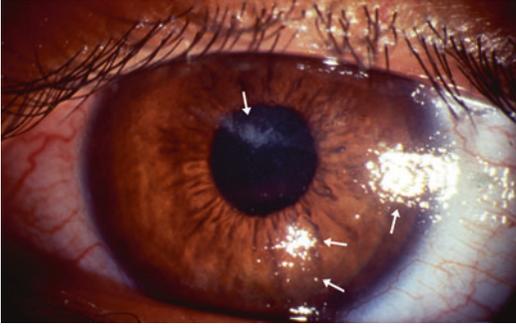
Gastrointestinal Tract Infection: At least 90% of cases of gastrointestinal microsporidiosis are due to *E. bienewsi* and the remaining cases are mostly due to *E. intestinalis*. In patients with AIDS infection it often manifests as chronic diarrhea, and stool examination may reveal multiple pathogens in these patients. In addition to

diarrhea, granulomatous hepatitis caused by Encephalitozoonidae has been reported in patients with AIDS and infection of the biliary system epithelium due to *E. bienersi* can lead to sclerosing cholangitis. Microsporidiosis due to *E. bienersi* is a cause of diarrhea in travelers, children in low income countries, and patients undergoing, kidney, lung, liver, or bone marrow transplantation. In immune competent patients, the diarrheal syndrome is self-limited, but in patients with immune suppression the diarrhea can be persistent and result in a wasting syndrome. Clinical manifestations include watery, nonbloody diarrhea, nausea, diffuse abdominal pain, and fever. In immune competent patients, the diarrhea syndrome is usually self-limited; however, in those with immune suppression the diarrhea can be persistent and result in a wasting syndrome. Infection with *E. bienersi* does not result in enteritis or ulceration; however, infection causes villous blunting and crypt hyperplasia. From three to ten bowel movements occur daily that consist of loose to watery stool without blood or fecal leukocytes with associated malabsorption exacerbated by eating. Within epithelial cells *E. bienersi* spores and proliferating forms are found on the apical surface, e.g., they are not present on the basal surface below the nucleus of these polarized cells or in the lamina propria. *E. bienersi* can also infect cholangioepithelium causing sclerosing cholangitis, cholangiopathy, or cholecystitis with associated abdominal pain, nausea, vomiting, fever, and elevation of alkaline phosphatase. Imaging studies can demonstrate dilated biliary ducts, irregularities of the bile duct wall, and gallbladder abnormalities such as thickening, distention, or the presence of sludge. In patients with AIDS, the prevalence of *E. bienersi* infection increases with declining CD4⁺ T cell counts and patients with CD4⁺ T cell counts less than 50 cells/ μ l are at high risk. In these patients, infection is associated with increased mortality rates. Systemic dissemination is not described for *E. bienersi* infection; however, there are reports of this organism in nasal mucosa (perhaps due to aspiration) with associated cough, dyspnea, wheezing, and interstitial infiltrates. Other members of the

Enterocytozoonidae, such as *Nucleoporea salmonis*, are found in lymphocytes and are capable of disseminated infection in their host species.

Three members of the family Encephalitozoonidae are associated with disease in humans – *E. cuniculi*, *E. hellem*, and *E. intestinalis* (originally called *Septata intestinalis*). All of these organisms can cause disseminated disease involving any organ system. They are associated with numerous clinical syndromes including gastroenteritis, keratitis, sinusitis, bronchiolitis, nephritis, cystitis, urethritis, prostatitis, hepatitis, fulminant hepatic failure, peritonitis, cerebritis, and skin nodules. *E. intestinalis* is the second most common microsporidian cause of diarrhea and in some geographic areas can be more common than *E. bienersi*. Unlike *E. bienersi*, spores and proliferative forms of *E. intestinalis* are found on both the apical and basal sides of infected intestinal epithelial cells as well as in cells in the lamina propria. Infection of the gastrointestinal tract can be locally destructive resulting in necrosis and perforation of areas of bowel that presents as an acute abdomen. A characteristic septated parasitophorous vacuole can be seen surrounding the developing spores. *E. cuniculi* has not been described as a cause of diarrhea but has been described as a cause of hepatitis, hepatic failure, and peritonitis. *E. hellem* has also not been reported in cases of diarrhea but can cause disseminated infection and hepatitis. *Tubulinosema acridophagus* infection with peritonitis and hepatitis has been reported in the setting of an allogeneic bone marrow transplant.

Ophthalmologic Infection: While *E. cuniculi*, *E. hellem*, or *E. intestinalis* can all cause keratoconjunctivitis with a punctate keratopathy (Fig. 3) and corneal ulcers, the most common cause is *E. hellem* infection, especially in patients with HIV infection. *Trachipleistophora anthropoptera* has also been reported to cause a similar keratoconjunctivitis. *T. hominis* and *A. algerae* have also been reported in cases of keratitis. Ocular infection can be the presenting manifestation of disseminated infection due to any of the Encephalitozoonidae. Clinical examination demonstrates coarse punctate epithelial keratopathy and conjunctival inflammation resulting in redness,



Microsporidiosis, Fig. 3 Keratoconjunctivitis due to *Encephalitozoon hellem*. Arrows point to punctate lesions in cornea

foreign body sensation, photophobia, excessive tearing, blurred vision, and changes in visual acuity. Slit-lamp examination usually demonstrates punctate epithelial opacities, granular epithelial cells with irregular fluorescein uptake, conjunctival injection, superficial corneal infiltrates, and a noninflamed anterior chamber. Corneal scraping or biopsy material demonstrates microsporidian spores in the corneal and conjunctival epithelium. Inflammatory cells are rarely present. Infection with Encephalitozoonidae responds to topical fumagillin (Table 2) and when there is associated disseminated infection systemic albendazole is indicated. While these ocular infections are more common in patients with immune suppression they can be seen in immune competent hosts. As is true for many pathogens, contact lens use is a risk factor for infection. There are now numerous reports, involving over 300 patients, from India and Singapore, of *Vittaforma corneae* infection presenting as a keratoconjunctivitis in immune competent hosts. This infection presents as a seasonal keratoconjunctivitis often associated with soil exposure due to playing sports. These *V. cornea* infections have been treated with various topical agents including 1% voriconazole, 0.02% polyhexamethylene biguanide, albendazole, ciprofloxacin, and fumagillin with variable success. Several species of microsporidia (*V. corneae*, *Microsporidium africanus*, *Microsporidium ceylonensis*, and *N. oculorum*) have also been reported to cause deep stromal infections especially in cases with

associated ocular trauma. Treatment of these deeper infections has been problematic but has included various topical agents until keratoplasty or corneal transplantation.

Central Nervous System Infections: Cerebral microsporidiosis has been reported to occur in both immune competent and immune compromised humans (Weber et al. 1997). In other mammals, granulomatous encephalitis is a common complication of *E. cuniculi* infection. In the setting of AIDS, cerebral microsporidiosis has presented as a mass lesion with associated seizures and focal neurological complications similar to the presentation of *Toxoplasma gondii* encephalitis. Histopathology has demonstrated spores in cerebral parenchyma, perivascular spaces, and macrophages. *Trachipleistophora anthropoptera* has also been reported to cause cerebral microsporidiosis and an associated disseminated infection. Histopathology demonstrated birefringent spores in the gray matter with associated tissue necrosis.

Other Infections: Infections of the skin due to *A. algerae* (patients with leukemia), *Encephalitozoon* sp. (nodular skin lesions), and *Tubulinosema acridophagus* (red macules and papules in a patient with an allogeneic bone marrow transplant) have been reported.

There are several case reports of myositis with inflammation due to *Pleistophora ronniaefiei*, *Pleistophora* sp., *Trachipleistophora hominis*, *Tubulinosema* sp., *Endoreticulatus* sp., *E. cuniculi*, *Anncaliia vesicularum*, and *A. algerae*. Clinically these patients have had myalgias, weakness, elevated serum CPK and aldolase levels, and abnormal electromyograms consistent with inflammatory myopathy. The *A. algerae* infection occurred in a patient with rheumatoid arthritis treated with steroids and monoclonal antibody to tumor necrosis factor- α (TNF- α). A case of endocarditis (large right atrial vegetation attached to a ventricle pacing lead) due to *E. cuniculi* has been reported. Infection of the mandible, most likely due *Encephalitozoon* sp., has also been described in a patient with advanced AIDS. Several of the patients with microsporidian myositis responded clinically to albendazole. The *A. vesicularum*

Microsporidiosis, Table 2 Treatment of Microsporidiosis

Organism	Drug	Dosage and duration ^a
All microsporidian infections	Combinations antiretroviral therapy (cART) with immune restoration (an increase of CD4 ⁺ count to >100 cells/ μ m) is associated with resolution of symptoms of enteric microsporidiosis. All patients should be offered cART as part of the initial management of microsporidian infection	
	Severe dehydration, malnutrition, and wasting should be managed by fluid support and nutritional supplement Antimotility agents can be used for diarrhea control if required	
<i>Enterocytozoon bieneusi</i>	No effective commercially available treatment. Fumagillin (oral) has been effective in a clinical trial. <i>Alternatives:</i>	20 mg tid (e.g., 60 mg/day)
	Albendazole ^a resulted in clinical improvement in up to 50% of patients in some studies but was not effective in other studies. Nitazoxanide, 1000 mg bid with food for 60 days, has been used, but is less effective in patients with low CD4 counts	
<i>Encephalitozoonidae</i> infection (e.g., systemic, sinusitis, encephalitis, hepatitis)		
<i>E. cuniculi</i>	Albendazole	400 mg bid ^b
<i>E. hellem</i>	Albendazole	400 mg bid
<i>E. intestinalis</i>	Albendazole	400 mg bid
<i>Encephalitozoonidae</i> keratoconjunctivitis	Fumagillin solution ^c (70 μ g/mL) Patients may also need albendazole ^a if systemic infection is present	2 drops every 2 h for 4 days then 2 drops 4 times a day ^d
<i>Trachipleistophora hominis</i>	Albendazole	400 mg bid
<i>Anncaliia</i> (formerly <i>Brachiola vesicularum</i>)	Albendazole	400 mg bid
	\pm Itraconazole	400 mg qd
Tubulinosema (<i>T. acridophagus</i> and <i>Tubulinosema</i> sp.)	There was no response to Albendazole (400 mg/d) in the published cases. Oral fumagillin 20 mg TID would be a reasonable choice	
<i>Endoreticulatus</i> sp.	Albendazole	400 mg bid

Adapted from Costa and Weiss (2000, pp. 1–16)

^aAlbendazole, 400 mg bid

^bThe duration of treatment for microsporidiosis has not been established. Relapse of infection has occurred on stopping treatment. Patients should be maintained on treatment for at least 4 weeks, and most patients should continue treatment until their CD4 count is higher than 200 cells/ μ L for at least 6 months following the initiation of cART

^cFumidil B (fumagillin bicyclohexylammonium; Mid-Continent Agrimarketing, Overland Park, KS, USA) is used at 3 mg/mL in saline (final concentration of fumagillin, 70 μ g/mL)

^dEye drops should be continued indefinitely; relapse is common on stopping treatment

infection that caused myositis in patient with AIDS responded clinically to a regimen of albendazole plus itraconazole.

Respiratory tract and sinus involvement has been reported in microsporidiosis. Encephalitozoonidae have caused rhinitis, sinusitis, bronchitis, and nasal polyposis often with associated

disseminated infection. In patients with *E. hellem* keratoconjunctivitis sputum examination demonstrated spores even without associated respiratory symptoms. *E. cuniculi*, *E. hellem*, and *E. intestinalis* have all been reported to cause bronchiolitis with or without pneumonia. Sinus biopsies in AIDS patients with chronic sinusitis

have demonstrated microsporidian spores in epithelium and supporting structures.

The Encephalitozoonidae cause granulomatous interstitial nephritis in mammals, including humans. Infection can be asymptomatic with shedding of spores. Infection is associated with tubular necrosis, with the lumen of the tubules containing amorphous granular material. Glomerular involvement is rarely seen. Interstitial nephritis due to microsporidiosis has been seen in patients with AIDS as well as in patients with renal transplantation. Infection can also result in necrotizing ureteritis or cystitis that can be seen on cystoscopy. Genital tract infection with *Encephalitozoon* sp. and with *V. corneae* has been associated with prostatitis with abscess formation. It is not known if these organisms can be sexually transmitted.

Treatment and Prevention

Treatment for microsporidiosis is reviewed in Table 2 (Weiss and Becnel 2014; Costa and Weiss 2000). In patients with impaired immune function restoration of immune function (e.g., cART in AIDS patients) has proven effective in the treatment of microsporidiosis by ameliorating disease, clearing the parasite and reversing intestinal damage. Therefore, restoration of immune function should always be part of the treatment plan for microsporidiosis. Fumagillin and albendazole are two therapeutic agents that are useful for the treatment of microsporidiosis. Albendazole is a β -tubulin-binding agent that is effective against all Encephalitozoonidae at a dose of 400 mg twice daily for 2–4 weeks. Unfortunately, the β -tubulin genes of both *Enterocytozoon bienewisi* and *Vittaforma corneae* have amino acid substitutions that provide resistance to albendazole and clinically it has shown little success against *E. bienewisi* (at most a 50% improvement in symptoms, but persistence of the parasite in stool). Albendazole is effective for treating microsporidiosis due to *Trachipleistophora* or *Anncaliia* with reports of improvement in myositis due to either pathogen. Despite efficacy, relapse of disease and recurrence of parasites have been

seen in cases of microsporidiosis in immune compromised hosts suggesting that, in the setting of persistent immune dysfunction, maintenance therapy may be needed.

Fumagillin, which inhibits methionine aminopeptidase type 2, is also effective in the treatment of microsporidiosis; however, this drug is not commercially available for the treatment of humans. Both fumagillin (Fumidil B) and a fumagillin derivative (TNP-470) have demonstrated efficacy in vitro and in vivo (in animals and insects) against many different microsporidia including *E. cuniculi*, *E. hellem*, *E. intestinalis*, *V. corneae*, *E. bienewisi*, *N. apis*, *N. kingi*, *Octosporea muscaedomesticae*, *P. anguillarum*, *Sphaerospora renicola*, *Loma salmonae*, and *Nucleospora salmonis*. A trial of this drug in AIDS patients with diarrhea due to *E. bienewisi* demonstrated that fumagillin at a dose of 60 mg/day for 2 weeks eliminated infection (Molina et al. 2002). This efficacy has been confirmed in subsequent case reports of both AIDS patients and transplant patients with *E. bienewisi* infection. Nitazoxanide was shown to have activity in an AIDS patient with diarrhea and *E. bienewisi* infection, but this has not been studied in a series of patients. Itraconazole and other azoles have demonstrated efficacy for microsporidia other than *E. bienewisi* and are recommended for some disseminated infections. Metronidazole, atovaquone, quinacrine, trimethoprim-sulfamethoxazole, azithromycin, paromomycin, and furazolidone have had no significant activity and are not recommended as therapeutic agents.

There are limited data on effective preventive strategies for microsporidiosis, and no prophylactic agents have been identified for these organisms. It is reasonable to screen close contacts of patients with index cases of microsporidiosis for the presence of these organisms. Microsporidian spores can survive and remain infective in the environment for prolonged periods. Although the epidemiology of the microsporidia that infect humans has not been fully elucidated, it is likely they are food- or water-borne pathogens, and the usual sanitary measures that prevent contamination of food

and water with animal urine and feces should decrease the chance for infection. Severely immune compromised patients may wish to consider using bottled or filtered water in some settings. The most effective prophylaxis for microsporidiosis is the restoration of immune function in immune compromised hosts and cART with immune restoration can produce remission during active microsporidiosis.

Conclusion

While only recently widely recognized as human pathogens, it is now clear that microsporidiosis is more common than previously appreciated and often zoonotic. New human pathogenic microsporidia are being recognized due to increased awareness of these organisms. In patients with HIV infection on antiretroviral therapy the incidence of this opportunistic infection has declined; however, it has not completely disappeared. These organisms can cause a wide range of clinical syndromes beyond the classic presentation of chronic diarrhea. Treatment can be problematic as there are no commercially available active drugs for the most commonly seen species *Enterocytozoon bieneusi*; however, immune reconstitution can result in cure of infection in the setting of AIDS and is an important therapeutic modality.

Cross-References

- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [Immunopathogenesis of HIV Coinfections](#)
- ▶ [Mucosal Immunity to HIV-1](#)

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Molecular Biology of HIV-2

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Definition

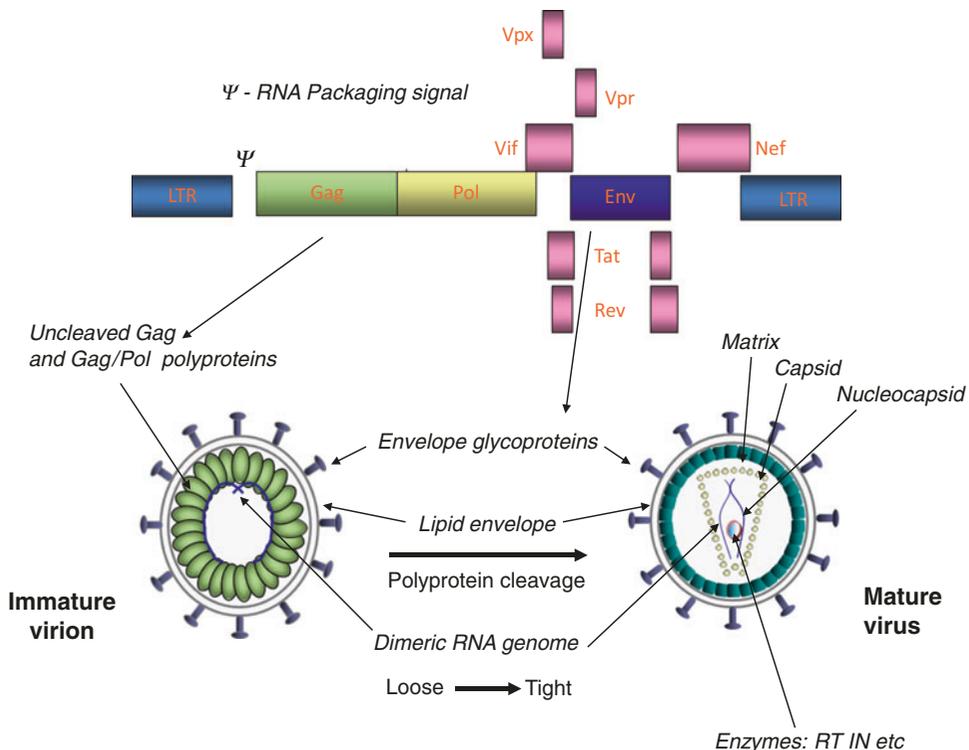
This entry describes the molecular biological aspects of HIV-2 and how they contrast with that of HIV-1.

Introduction

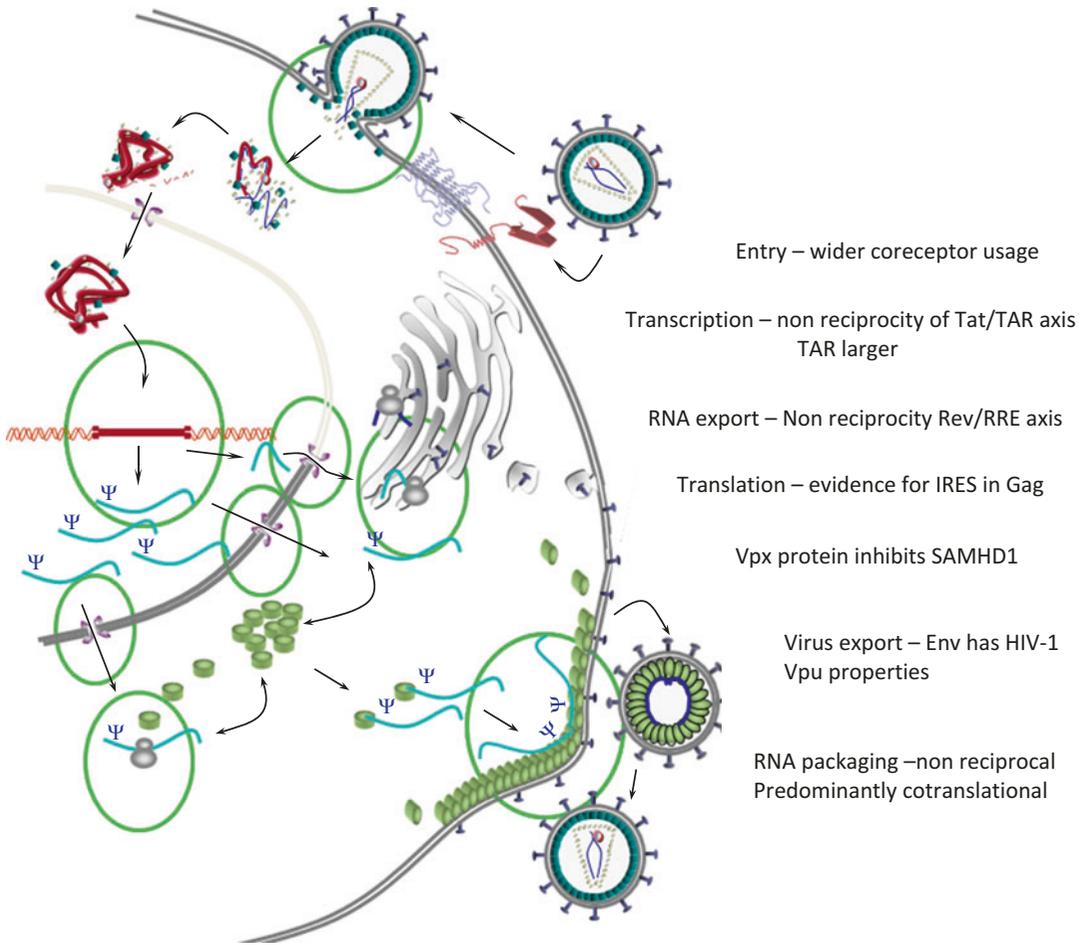
Since the first identification and cloning of the second human immunodeficiency virus HIV-2 (History of the AIDS epidemic, ► [Epidemiology of HIV-2 infection in Europe](#), ► [Recombinant forms of HIV-2](#), ► [The epidemiology of HIV-2 infection in West Africa](#), ► [The phylogeography of HIV-2 infection in West Africa](#)), it has been clear from an abundance of clinical studies that this is a much less pathogenic virus than HIV-1 (► [Dual HIV-1 and HIV-2 infection](#), ► [HIV-2 diagnosis and viral load measurements](#)) and that it has a number of genetic molecular and structural differences from HIV-1. In seeking to understand the more often (though not always) benign outcome of infection with HIV-2, many studies have focused on these differences in seeking an explanation for this and also for the potential insights which it might give to understanding the pathogenicity of HIV-1 and ways to combat those features which make the latter virus so much more

virulent. Both viruses are zoonotic and have had to adapt to the same new host, humans, within the last 100 years; the differences are thus perhaps greater than would be expected. The molecular biology of HIV-1 is covered elsewhere in the Encyclopedia; hence this entry does not aim to be a comprehensive description of HIV molecular biology. Rather it has been limited to areas in which important similarities or, more usually, differences between the two viruses have been identified.

The major open reading frames whose function has been investigated are shown in Fig. 1. While overall they are recognizably similar to those of HIV-1 (► [Virus Assembly](#)), there are obvious differences from the size of some ORFs and untranslated regions through to the contrasting absence or presence of specific accessory genes; HIV-1 uniquely has *vpu* and HIV-2 has *vpx*. Probably more important however are functional differences between homologous genes. These are summarized in Fig. 2 and discussed below.



Molecular Biology of HIV-2, Fig. 1 Major genes of HIV-2 and immature and mature virion structure



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Molecular Biology of HIV-2, Fig. 2 Major differences in lifecycle between HIV-1 and HIV-2

Perhaps the most striking contrasts are seen in the processes involved in the viral life cycle, and these are described in detail at the end of the article.

All exogenous retroviruses characteristically contain three open reading frames, from 5' to 3' *gag*, *pol*, and *env* which encode the structural genes of the core, the viral enzymes, and the envelope glycoprotein, respectively. They assemble as shown in an immature particle and then the protease gene cleaves the core proteins to form the mature infectious particle (Fig. 1) (Virus structure). Inside the virus HIV-2, like all retroviruses, carries two copies of its genetic material in the form of RNA linked as a dimer at their 5' ends.

HIV-2 Gag

HIV-2 *gag* is similar in organization and genetic structure to that of HIV-1, but the Gag polyprotein gene product is larger at 57 kDa compared to 55 kDa for HIV-1. Gag comprises four major domains with functional elements: from the N-terminus – matrix (MA-p17), capsid (CA-p26), nucleocapsid (NC-p7), and the C-terminal p6 (Briggs and Krausslich 2011). Two spacer proteins exist flanking NC: at the N-terminus p2 and at the C-terminus p1. The sequential proteolytic processing of Gag begins with an initial cleavage between p2 and NC followed by p1/p6 separation and MA/CA

cleavage. Finally, NC and p1 separate and p2 is cleaved from CA. This cleavage sequence is tightly controlled, and there appears to be a very strong interdependence between the correct rate and sequence of cleavage and the presence of a dimeric RNA genome. Mutations in the HIV-2 genomic RNA dimerization signal lead to aberrant rates of the first two cleavages with an accumulation of the p41 MA/CA fusion protein. In vitro revertants, particularly a mutation, 70TI, in MA, can correct this processing defect and at the same time restore RNA dimerization (L'Hernault et al. 2012), suggesting that viral assembly is a complex interplay between the RNA and the protein. This matrix reversion restores replication competence to the mutant virus despite the persistence of the dimerization mutation.

HIV-2 Gag has been expressed successfully in vitro and is capable of assembling into virus-like particles. VLP assembly occurs when Gag is expressed as a baculovirus recombinant. HIV-1 can multimerize in *Saccharomyces* spheroplasts, a yeast-based assembly system, but HIV-2 fails to. This seems to be dependent on the membrane-binding surface of MA in the helix 2 region. In other cell-free systems, multimerization of Gag appears to be identical to that of HIV-1 and is likewise dependent on the cellular HP68 protein. HIV-1 and HIV-2 Gags can, in certain combinations, form virus-like particles by co-assembly and generate functional virus particles. HIV-2 viral assembly (► [Cofactors of HIV Assembly](#)) shows some dependence on incorporation of the correct RNA (see below). HIV-2 assembly and virus export are dependent on the ESCRT (endosomal sorting complexes required for transport) pathway (► [Budding](#), ► [HIV-1 Assembly Cofactors](#)), and Gag binds TSG101 (tumor susceptibility gene 101) (Weiss and Gottlinger 2011). HIV-2 Gag which is expressed from the unspliced viral mRNA may have an autoregulatory role in controlling viral RNA splicing through an interaction with PRP4, a serine-threonine kinase which inhibits phosphorylation of splicing factor SF2 (Bennett et al. 2004).

The Vpx and Vpr proteins of HIV-2 are incorporated into viral particles through interaction with the P6 region of Gag.

HIV-2 Protease

The aspartyl protease (PRO) of HIV-2 at the N-terminal of the *pol* gene product, like that of HIV-1, is a homodimer which when cleaved from the GagPol polyprotein is responsible for the processing to the completion of the Gag and the GagPol precursors during viral maturation and budding. The proteins are extremely similar in the two viruses. Given that their target sites for cleavage are slightly different, they, unsurprisingly, show differences in their rate of cleavage of different target peptide sequences (► [Anti-Retroviral Therapy and Drug Resistance in HIV-2 Infection](#)) (Tomasselli et al. 1990). However, functionally, they are similar enough such that in a construct expressing the HIV-1 homodimer as a single chain, the substitution of one of the subunits for an HIV-2 sequence leads to a functional chimeric protease. The variations in proteolytic specificity of the two enzymes have clinical relevance in that in general the licensed HIV-1 protease inhibitors have a lower potency against HIV-2 and in a number of cases a structural basis for this has been shown (Camacho 2012).

HIV-2 Reverse Transcriptase

This enzyme (Reverse Transcription) is responsible for converting the RNA genome in the virus into a DNA which is integrated into the cell the virus infects. HIV-1 and HIV-2 reverse transcriptase (RT) molecules are heterodimers of a differently processed single protein which, after proteolytic cleavage, forms a p66/p51 heterodimeric molecule (Herschhorn and Hizi 2010). Both enzymes use the natural tRNA (Lys3) as a primer and in vitro the HIV-2 RT can prime successfully on the HIV-1 RNA template. Unlike protease there are significant structural differences between the two viral enzymes. Heterodimer formation between subunits of the two enzymes does not occur efficiently; in particular p66 (HIV-2) and p51 (HIV-1) assembly is very inefficient although the reciprocal combination assembles more easily. Both lentiviral RTs show a similar low fidelity of polymerization, and in assays of DNA-dependent DNA polymerase

activity, HIV-1 and HIV-2 show a six- to ninefold greater successful extension of a mis-paired nucleotide than the RT from other retroviruses such as murine leukemia virus. This error-prone process is clearly a major contribution to viral variation in both HIVs. Structural and functional differences have been revealed by detailed crystallographic analysis. It is known that HIV-2 RT is singularly resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs) and the binding site of NNRTIs, the so-called NNRTI pocket, is structurally quite different in the two enzymes (► [Anti-Retroviral Therapy and Drug Resistance in HIV-2 Infection](#)). Perhaps more interesting is the suggestion that structural differences in the two enzymes influence their *mode* of developing resistance to individual drugs (Boyer et al. 2006). For example, HIV-2 tends to develop resistance to AZT by accumulating mutations that reduce incorporation of AZTTP, whereas HIV-1 tends to develop resistance by mutations which enhance excision of AZTTP. HIV-1 RT is intrinsically more efficient at ATP-mediated excision of AZTMP than HIV-2 RT, and the mutagenic pathway to resistance enhances this capability.

HIV-2 Integrase

This enzyme cuts and pastes the newly reverse-transcribed viral DNA into the cellular chromosome (► [Integration](#), ► [LEDGF/p75, Cofactors of Integration](#), ► [Transportin-SR2 \(TNPO3\), Nuclear import](#)). HIV-2 integrase (IN) is quite significantly different from that of HIV-1. The two genes show only 60% homology (Roquebert et al. 2008). Perhaps, surprisingly, susceptibility to therapeutic inhibitors is very similar in the two viruses. Minor differences appear to be seen in mutations which lead to resistance to these drugs, although the clinical impact of this is not yet apparent.

HIV-2 Env

This is structurally similar to all lentiviral envelopes comprising a heterodimer of a

transmembrane protein gp36 (TM) and a surface glycoprotein gp125 (SU) arranged as trimers (► [The HIV-2 envelope: structure, diversity and evolution](#)). Similarly to HIV-1, a variable domain of SU, the V3 loop, is critical for binding to the primary cell receptor CD4 (► [Attachment/Binding](#)). The hydrophobic terminus of the TM protein is involved in the process of virus/cell fusion during entry. The kinetics of HIV-2 interaction with the CD4 and CXCR4 receptor and coreceptor are different from those of HIV-1 envelope with the rate of engagement with CXCR4 following CD4 binding, leading to virus/cell fusion occurring around twice as fast (Gallo et al. 2006).

There are two important differences in the HIV-2 envelope compared to HIV-1 (Choe et al. 1998). One is in its coreceptor usage. Whereas HIV-1 largely uses CCR5 (Selection of CCR5 using viruses; transmission) or CXCR4 as its coreceptor (► [CXCR4, Co-receptors](#)), HIV-2 is far more promiscuous and can use CCR1, CCR2, CCR3, CCR4, CCR5, CXCR2, and CXCR4. Other cell surface chemokine receptors including BONZO and BOB have also been shown to support HIV-2 entry. The efficiency with which the virus can use these different receptors is very variable, and although the option is there for the virus to use a range of surface molecules to trigger virus/cell fusion, in vivo it is not clear how much of its diversity of capability is actually utilized.

The second surprising feature of the HIV-2 envelope is that, in addition to its receptor-binding capabilities, it has the functionality of the protein Vpu in HIV-1 (Le Tortorec et al. 2011), leading to enhanced virus export from the infected cell. The efficiency of enhancement of HIV-2 particle release by HIV-2 Env is comparable to that of HIV-1 Vpu. The HIV-2 envelope can clearly counteract the antiviral effects of the cellular protein BST2/tetherin as effectively as HIV-1 Vpu, and it does this by sequestration of the cellular protein in a perinuclear compartment. This arrangement of a Vpu-like function of envelope is shared with the envelope glycoprotein of feline immunodeficiency virus.

HIV-2 Tat

Tat (► [Tat expression and function](#)) is a small protein produced from a bicistronic spliced RNA and is a powerful transactivator of the HIV promoter in the viral long terminal repeat (LTR) sequence (Marcello et al. 2001). It functions by binding the nascent viral RNA transcript at the 5' end, annealing to an RNA helix loop structure, the Tat response element (TAR). Tat protein of HIV-2 is almost 50% longer than that of Tat-1 at 130 amino acids versus 86. It has similar cysteine- and arginine-rich domains. The nonreciprocity of transactivation is described below.

HIV-2 Rev

Rev (► [Rev expression and function](#)) is another small regulatory protein, again produced as the spliced product of two short ORFs (Kjems and Askjaer 2000). It is essential for nuclear export of incompletely spliced HIV RNAs from the nucleus; the mode of activity is through its binding to a *cis*-acting RNA sequence in the envelope gene region, the Rev response element (RRE), a complex RNA structure. This nucleoprotein complex uses the cellular Crm1/RanGTPase pathway (► [CRM1](#)) to export its RNA cargo to the cytoplasm. As is the case for Tat, there is a lack of reciprocity in activity between HIV-1 and HIV-2. There has been surprisingly little comparative biology done of the Rev/RRE regions of HIV-1 and HIV-2. One explanation for the lack of reciprocity may be the requirement for Rev to multimerize on the RRE, forming what has been termed as a “molecular rheostat”; the HIV-1 RRE does not facilitate multimerization of HIV-2 Rev (Garrett and Cullen 1992). Despite considerable expansion of the understanding of the roles of Rev in HIV-1 beyond that of nuclear export of RNA to influences on translation and genome encapsidation, similar studies have not been performed in HIV-2.

HIV-2 Nef

Since its original description as “negative factor,” the most 3' open reading frame of HIV, Nef has been shown to have multiple activities. In HIV-2, as in HIV-1, Nef can down-modulate cell surface expression of CD4 in infected cells involving direct interaction of Nef with the cytoplasmic tail of CD4. The site of binding of Nef-2 is distinct from but overlaps with that of HIV-1 and the process involves the mu chain of adaptor complexes which are components of clathrin-coated pits. HIV-2 Nef has also been shown to down-modulate MHC-II, but unlike SIV both HIV-1 and HIV-2 Nef proteins fail to down-modulate CXCR4. Perhaps the most fascinating is the demonstration that the T-cell receptor (TCR) is down-modulated by HIV-2 Nef and by related simian immunodeficiency virus (SIV) proteins but that this function has been lost in HIV-1. This has been suggested to explain the high level of T-cell activation (► [Chronic immune activation](#)) found in HIV-1-infected individuals which correlates with its higher pathogenicity (Kirchhoff 2010). The correlation between TCR down-modulation, decreased T-cell activation, and pathogenicity has been questioned since it was shown that, despite T-cell receptor down-modulation and decreased T-cell activation, there are still HIV-2-infected patients with relatively rapidly progressive disease, implying that other factors can influence the disease phenotype (► [HIV-2 infection: the role of immune activation in pathogenesis](#)).

HIV-2 Vpr

All primate lentiviruses encode a 96 amino acid 14 kDa Vpr protein which is packaged into the viral particle at about 40–50 copies per virion. It appears to be physically less stable than Vpx and the D-X-A-X-X-L-L sequence motif in the HIV-2 Gag p6 protein which is used by Vpr for packaging is different from that in HIV-1. In general, the established properties of Vpr are the same in HIV-1 and HIV-2. It is essential for replication in

macrophages. It can cause G2 cell cycle arrest. Vpr enhances nuclear import of the pre-integration complex and is a transactivator of the long terminal repeat. In HIV-1 Vpr has been implicated in a significant amount of pathogenicity with suggested contributions to neurotoxicity, nephrotoxicity, and immune dysfunction. Given the differential in pathogenicity of the two viruses, it is of interest that in one report it is noted that HIV-2 Vpr is less able to trigger apoptosis in human cells compared to the HIV-1 protein (Kirchhoff 2010).

HIV-2 Vpx

Possession of a Vpx protein in HIV-2 (and SIV) distinguishes it from HIV-1 (Fujita et al. 2010). It was noted early that Vpx facilitated and, in some cases, permitted viral infection of primary peripheral blood mononuclear cells but was dispensable in *in vitro* cultured cell lines. The virus packages 2,000–3,000 copies of Vpx into the particle bound to the p6 region of Gag. Early studies suggested that Vpx was important for nuclear import of the pre-integration complex and completion of reverse transcription and the protein appears to have a novel nuclear localization signal. Early cellular binding partners of Vpx were identified as being proteasome associated, and it was also shown that Vpx could work *in trans*, allowing HIV-1 to infect primary human mononuclear cells, including dendritic cells and macrophages. The cellular target of Vpx turns out to be SAMHD1 (sterile alpha motif and histidine/aspartic acid domain controlling protein-1). This is a dGTP-stimulated triphosphohydrolase which cleaves dNTPs into the deoxynucleoside and inorganic phosphate. Vpx targets this protein which would otherwise deplete the availability of dNTPs for viral cDNA synthesis. The mechanism appears to be by binding SAMHD1 to a ternary complex of DDB1/Cullin 4 and DCAF-1 which binds to the N-terminus of Vpx. This complex then targets SAMHD1 for degradation by the cellular proteasome.

HIV-2 Transcription

There are similarities between transcriptional control of HIV-1 and HIV-2 in that both viruses are switched on by the activation of the T cell in which they reside and both have the powerful Tat/TAR transactivation mechanism (Garcia-Martinez et al. 1995; Marcello et al. 2001) (► [Tat expression and function](#), ► [Acetylation](#), ► [Histone deacetylase \(HDAC\): YY1](#)). The mechanism of transactivation is similar in both with Tat recruiting a Tat-associated kinase (TAK) and cyclin T1, leading to the phosphorylation of the C-terminus of RNA polymerase II which dramatically increases its processivity. Transactivation of HIV transcription has long been known to involve Tat binding to TAR, leading to full-length transcription of the RNA genome. In the absence of Tat, multiple short-truncated transcripts are formed. The HIV-2 promoter appears to be more prone to produce large numbers of short transcripts than the HIV-1 promoter, possibly relating to the structure of its duplicated TAR element. Interestingly, the overall number of these short transcripts does not change on Tat activation in HIV-2; instead there is an overall increase in the total number of transcripts, many of which now extend for more than 500 nucleotides from transcription initiation site.

Several groups have identified the fact that there was non-reciprocity of transactivation in that HIV-1 Tat was able to activate both HIV-1 and HIV-2, but HIV-2 Tat could only transactivate its own promoter. Part of the functional difference appears to reside in the arginine-rich basic domain which when the HIV-2 sequence is substituted into the HIV-1 Tat equivalent leads to reduced transactivation of the HIV-1 LTR. Structural predictions of the Tat-binding region of both viruses demonstrated that the newly transcribed RNA formed tight stem-loop structures, but that in HIV-1 this was a single stem loop, whereas in HIV-2 a second stem loop directly 3' and linked to the first was seen. *In vitro* affinity assays show that Tat-1 can bind both TARs with similar affinity but Tat-2 binds TAR-2 with a much greater

affinity than it does TAR-1. Exon-2 (which is inessential for transactivation by Tat-1) is very important for Tat-2 activity and this, in part, is due to its ability to increase the affinity of the protein for the TAR-2 RNA (Garcia-Martinez et al. 1995). Deletion studies suggested that the first stem loop of TAR-2 was sufficient for transactivation but that the addition of the second stem loop enhanced the effect. The HIV-2 TAR element appears to have two important dinucleotide bulges responsible for HIV-2 Tat binding, and surprisingly, mutation of these inhibits HIV-2 Tat transactivation but not that of HIV-1 Tat. There is also evidence that a cellular factor interacts with the 3' arm of the proximal stem loop structure of TAR-2.

Upstream of the TAR region in the transcription factor binding sites of the promoter, HIV-2 has only a single NF- κ B-binding site as opposed to two found in HIV-1, and this correlates with a greater response to T-cell activation signals of HIV-1. Additional *cis*-acting regions are found in the HIV-2 enhancer, including two purine-rich sites, PuB1 and PuB2, and an additional region proximal to PuB2 (pets) also contributes to promoter activation. Mutation of any of these four sites reduces the response of HIV-2 to T-cell stimulation. An *ets*-related transcription factor E1f-1 has been shown to bind specifically to the PuB1 and PuB2. A 43 kDa nuclear factor has been shown to bind to the pets sites. This has been identified as the oncoprotein DEK. Activation through the pets site appears to be dependent on protein phosphatase-2A (PP2A).

HIV-2 Translation

Transcription of the proviral DNA produces an approximately 9 kb full-length transcript which is spliced into singly and multiply spliced variants. All of these, like conventional cellular messenger RNAs, have a 5' methyl cap and a poly(A) tail (Bolinger and Boris-Lawrie 2009). There is some evidence in HIV-1 of skewing of the preference for a particular form of capping, but this has not been investigated in HIV-2. There has been a degree of controversy in recent years over the primary method of translational control of

HIV transcripts. The high density of structured RNA regions in the 5' untranslated sequence might be expected to impair scanning from the 5' cap, and indeed, structures such as the TAR stem loop have been shown to be quite strong inhibitors of ribosomal scanning *in vitro*. Based on this, some groups have sought evidence of an internal ribosome entry site (IRES) in HIV-1 and HIV-2, and indeed, evidence of IRES activity has been detected in conventional dicistronic reporter construct assays. In HIV-1, the balance of evidence is in flux as to whether scanning or IRES-mediated translational initiation dominates; there is clear evidence from studies on UTR truncations that HIV-1 Gag can be translated quite effectively by ribosomal scanning, suggesting that the 5' structure is not in itself prohibitive of this mode of protein production. Nevertheless, the detection of IRES activity suggests that the virus may utilize both forms of translation, perhaps to optimize viral protein production during different parts of the cell cycle. In HIV-2 there has not been the same investigation of the practicalities of scanning and the majority of studies have focused on the existence of an IRES. This is an unusual form of IRES in that the structure appears to extend at least 50 nucleotides downstream of the Gag initiation codon into the coding region. There appear to be different isoforms of Gag protein produced with different start codons from within the Gag open reading frame, and there is evidence that the HIV-2 I.E. can recruit three initiation complexes onto the HIV-2 genomic RNA to initiate the production of these three Gag proteins. What the role of these three variants of Gag is has yet to be established. The apparent dominance of IRES-mediated translation in HIV-2 versus HIV-1 may relate to the more complex and extended TAR stem loop in HIV-2 which may be more inhibitory to scanning than the equivalent structure in HIV-1. Analogous to polio virus, the HIV-1 protease has been shown to cleave the poly(A)-binding protein (PABP). The protease being only released from its polyprotein precursor during viral assembly and budding would suggest that this was a rather late time point for this activity to influence translation. This is an area of active ongoing research which hopefully will become clearer with time.

HIV-2 Genomic RNA Packaging

The unspliced viral RNA is not only used as a template to translate the viral Gag and GagPol proteins; it is also the RNA which is encapsidated as a dimer into the viral particle (► [Virus Assembly](#)). The virus recognizes this RNA through the latter having a region at the 5' end which folds into a complex “knot”-like structure whose shape is sufficiently unique to distinguish it from the other RNAs in the cell in which this is bound by the virus Gag polyprotein (Lever 2007).

HIV-2 offers some similarities but interesting contrasts with HIV-1. An early clue was the observation that the major packaging signal region was upstream of the major splice donor, unlike in HIV-1 where it is downstream. The specificity of the packaging signal being confined to the unspliced RNA is, therefore, not present in HIV-2 and implies alternative mechanisms for genome selection. Interestingly, the distance from the RNA cap site to the packaging signal structure is similar in both viruses (and in all SIVs) and would be compatible with the recognition involving another molecular rheostat mechanism. Differences in packaging mechanism between the two viruses were further reinforced by a nonreciprocal relationship being identified, whereby HIV-1 could package HIV-2 RNA but not the reverse. Interestingly, the defect in reciprocity of HIV-2 extended to that of SIVmac Gag which was also able to package HIV-2 RNA, again nonreciprocally.

Like HIV-1 and distinct from simple viruses, such as murine leukemia viruses, capture of the genomic RNA was shown to be from the translating pool of full-length viral rather than from a separate pool of RNA destined specifically for packaging rather than translation to produce Gag. HIV-1 can package RNA from outside of this pool but HIV-2 is less promiscuous.

The RNA structures involved in genome dimerization and encapsidation bears some similarities to HIV-1 but again are distinct. In HIV-2 a palindromic sequence at the base of a stem loop in the RNA constitutes the major authentic *in vivo* dimerization and encapsidation signal. On the tip of the same stem loop, a second palindromic

sequence occurs which can influence RNA dimerization *in vitro* but is clearly dispensable for efficient packaging and dimerization in wild-type virus. In HIV-2 a clear association between packaging of a dimeric genome and particle assembly has been shown with abnormal morphology and an increase in viruses containing double capsids seen in particles bearing a mutation in the dimer linkage site. Recently a tight interdependence (L'Hernault et al. 2012) of correct Gag/GagPol protein processing and the presence of a functional RNA dimer site have been identified suggesting perhaps that dimeric RNA can enhance initial stages of Gag assembly and that after polyprotein cleavage, the nucleocapsid protein then enhances the transformation of the loose RNA dimer to the tight RNA dimer characteristic of that found in the mature particle.

Conclusion

This short entry has highlighted differences between the molecular biology of HIV-1 and HIV-2 and demonstrated how closely related viruses can evolve quite different mechanisms to achieve similar ends, that of successful infection of their host. The contrasts explain the value of comparative studies in helping to understand the biology of both pathogens.

Cross-References

- [Acetylation](#)
- [Chronic Immune Activation in HIV](#)
- [CRM1](#)
- [CXCR4, Coreceptors](#)
- [Epidemiology of HIV-2 Infection in West Africa](#)
- [HIV-1 Assembly Cofactors](#)
- [HIV-1 Rev Expression and Functions](#)
- [HIV-2 Diagnosis and Viral Load Measurements](#)
- [HIV-2 Envelope: Structure, Diversity, and Evolution](#)
- [HIV-2 Infection: The Role of Immune Activation in Pathogenesis](#)
- [Integration](#)

- ▶ [Recombinant Forms of HIV-2](#)
- ▶ [Role of Histone Deacetylases 1 and Yin Yang 1 Protein in Proviral Latency](#)
- ▶ [Role of LEDGF/p75 in Cell Biology and Disease Pathogenesis](#)
- ▶ [Role of Transportin-SR2 \(Transportin-3, TRN-SR2, TNPO3\) in HIV Replication](#)
- ▶ [Tat Expression and Function](#)
- ▶ [Virus Assembly](#)

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Mother-to-Child Transmission of HIV-1: Role of Receptor Usage and Target Cells

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Definition

Mother-to-child transmission (MTCT) of HIV-1 occurs during pregnancy, delivery, and breastfeeding, although to different proportion. The latest reports still count approximately 330,000 new infections in babies although a significant decrease was observed since introduction of efficient preventive measures. Viral genotype and phenotype gave little evidence of a specific pattern associated with MTCT of HIV-1 and thus

are not useful absolute predictive markers of transmission. HIV-1 infection of children is initiated in most cases by an R5 virus, but viruses with other phenotypes can be isolated when the mother carries those. A sophisticated balance between inhibitory and facilitating factors as well as of competent target cells in the placenta, the breast milk, and at gastrointestinal level may redirect the transmission event.

Mother-to-Child Transmission of HIV-1: Epidemiological Notes

Mother-to-child transmission (MTCT) of HIV-1 can occur in utero, during delivery, and during breastfeeding. Indeed, transmission to the fetus or infant occurs through the placenta or by swallowing of large amounts of infected biological fluids as the amniotic fluid during gestation or blood and vaginal secretions during delivery and predominantly milk during breastfeeding. In the absence of any prevention strategy, the cumulative viral transmission accounts for 25–45% with 5–10% infections occurring in utero, 10–15% intrapartum, and 10–20% via breastfeeding. This latter one can increase with prolonged feeding practices.

In the last decade the introduction of antiretroviral therapy (ART), elective cesarean section, and exclusive bottle feeding as a prophylactic mean to prevent (► [Preventing HIV-1 MTCT](#)) MTCT of HIV-1 has contributed to an important reduction of newly infected babies in those areas of the world that can afford it. Although resource-limited countries have not yet reached full access to the whole array of these prophylactic measures, a significant trend in the reduction of new infections in babies has been achieved. According to the UNAIDS latest report, an estimated 330,000 children (► [Children, Epidemiology of HIV/AIDS](#)) became infected with HIV-1 in 2011 as compared to the more than 500,000 newly infected children occurring 10 years before. WHO (“History of the AIDS Epidemic”) and UNICEF have recently launched a global plan towards the elimination of new HIV infections among children by 2015 and keeping their

mothers alive, called “Eliminating Mother-to-Child Transmission.” The primary goal is to reduce by 90% the number of new infections in children and by 50% the number of HIV-related death in women during pregnancy, childbirth, and puerperium.

Maternal Viral Chemokine-Receptor Usage: Which Virus is Transmitted?

Maternal viral genotype and phenotype gave little evidence of a specific pattern associated with MTCT of HIV-1 and thus are not useful absolute predictive markers. Most maternal isolates are able to use CCR5 as co-receptor alone or in association with CXCR4 (“► [HIV and Sexual Violence](#)”) Co-receptors or other chemokine receptors, independently from transmission. A minority of pregnant women carries CXCR4-using viruses and even to a lower degree viruses able to use exclusively CXCR4, i.e., X4 viruses. Similarly, mothers harboring viruses with more flexible use of the CCR5 co-receptor (termed R5 broad viruses), such as those able to use chimeric CCR5-CXCR4 receptors, are not at higher risk of transmission than those carrying viruses able to infect via CCR5 exclusively, called R5 narrow (Cavarelli et al. 2008).

Unfortunately, due to the limited number of studies performed, it is difficult to define if chemokine receptors other than CCR5 and CXCR4, which are often used by HIV-1 of subtypes (HIV-1 subtype – overview and predominance) other than B, may be relevant for MTCT. Reports describing MTCT rates according to different viral subtypes were not always concordant. Pregnant women infected with subtype C virus appeared to transmit in utero more frequently than those with subtype A, D, or recombinant forms with an A or D envelope. In specific this was ascribed to a higher vaginal mucosal shedding of virus in those mothers with a subtype C virus infection.

If selection of specific viral phenotypes occurs during infection, there should be an association between viral phenotype and transmission. It is well established that HIV-1 infection of children is

initiated in most cases by an R5 virus. However, CXCR4-using viruses can be isolated from children, whose mothers carry a virus population with mixed R5 and X4 phenotype. Furthermore, a more in-depth analysis of the R5 virus variants showed that R5 broad viruses are not hampered during transmission and are indeed often isolated from newborns (Cavarelli et al. 2008). Of note is that isolation of R5 broad viruses with an enlarged chimeric receptor usage or of CXCR4-using viruses from neonates is predictive of an accelerated disease progression towards AIDS and death in the absence of a highly active antiretroviral therapy (HAART) (Cavarelli et al. 2010). This underlines that prevention of MTCT of HIV-1 should target both R5 and X4 viruses, as either viral phenotype can be detrimental to the baby and induce a fast disease progression.

Receptor Expression on HIV-1 Target Cells in MTCT

The availability of receptive cells able to replicate the virus may bias transmission towards a given virus phenotype. Indeed different lymphocyte subsets may become preferential target for R5 or X4 virus variants, accordingly. In vivo CCR5 is expressed mainly by the CD45RO⁺ subset of effector memory T cells, whereas CXCR4 is dense on the surface of naive ("Naive T cells") CD45RA⁺ T cells. Activated/memory T cells are those trafficking to peripheral lymphoid organs within the mucosal surfaces, where they form conjugates with dendritic cells (DCs) or macrophages, and thus represent the very early cellular targets for infection deputed to viral spreading. In vivo and in vitro studies confirmed that R5 virus isolates preferentially infect CCR5⁺-activated memory T cells, whereas X4 virus isolates infect CXCR4⁺ naive cells. Interestingly, the cord blood-derived CD4⁺ T cells are largely naive and do not express CCR5. In accordance with this observation, an in vitro study showed that CD4⁺ T cells derived from cord blood need to be activated to replicate HIV-1. These data support that in neonates, the naive CD4⁺ T cells are the predominant cell type, and thus, infection with

X4 viruses should be theoretically favored (Tuttle et al. 2004). However, a recent study showed an increased replicative capacity of R5 as compared to X4 viruses in a T-cell line derived from in vitro PHA-activated infant T cells as compared to that derived from adult cells (Mariani et al. 2012). This observation would suggest that infant cells are more susceptible to R5 strains than adult cells. Thus, a bias towards a specific viral phenotype goes beyond receptor expression on T cells. In addition, besides CD4⁺ T lymphocytes, HIV-1 can infect other cells of the immune systems, like macrophages ("► [Macrophages in HIV Immunopathogenesis](#)") and DCs. Cord blood-derived monocyte/macrophages have an increased susceptibility to HIV-1 infection in vitro with R5 but not X4 virus compared to adult cells.

Taken in consideration that the fetus and the infant independently from the transmission route ingest the virus, cells resident in the gut mucosa may play a crucial role in transmission. Indeed, CCR5 + CD4⁺ T cells with a memory phenotype are abundant at the level of the gut epithelium and in the gut lymphoid aggregates of the fetus and infant. Interestingly, in contrast to infant's CD4⁺ T cells derived from blood, these cells in the gut are highly susceptible to HIV-1 even without prior activation with cytokines, thus, supporting the possibility of a preferential transmission of R5 viruses through this route (Bunders et al. 2012).

DCs and Langerhans cells (LC), which are abundant at mucosal sites, have also been implicated in transmission, but little is known in infants. Indeed, Langerin expressed on LC and the C-type lectin DC-SIGN expressed on immature DCs have a high affinity for the HIV-1 gp120 surface protein and therefore can capture virus and transfer it to CD4⁺ T cells. In addition, immature DCs present in the periphery express high levels of CCR5. Analogously LCs have a preferential susceptibility to infection with CCR5-using viruses due to the lack of CXCR4 expression on their cell surface. Taken together these data suggest that there is a balance between different target cells, which at the end should favor transmission of R5 viruses.

In Utero Transmission: The Role of the Placenta

While in the simian immunodeficiency virus (SIV) (“► [SIVmac Infection of Macaques, Immunopathogenesis of](#)”) model it has been proposed that in utero infections occur when the infected amniotic fluid is ingested and the virus crosses the mucosal surfaces in the gastrointestinal tract of the fetus, for HIV-1 the data are not conclusive. Indeed, some early studies could detect HIV-1 proteins in the amniotic fluid of infected pregnant women, whereas other recent ones did not detect any virus in the amniotic fluid despite a concomitant high plasma viral load exceeding 10^5 copies/ml. Thus, it is likely that in utero transmission occurs primarily through the placenta although ingestion by the fetus and intake through the gastrointestinal route cannot be excluded.

In utero HIV-1 transmission seems to be relatively rare during the first trimester of pregnancy and usually occurs during the last trimester. This hints to the presence of effective natural control mechanisms particularly during the first months of gestation and suggests that the placenta itself possibly acts as a barrier for the virus. Contrasting data have been reported on the susceptibility of the trophoblasts to cell-free HIV-1 infection. These cells usually express CXCR4 and CCR5, but rarely the CD4 molecule and the mechanism of entry into trophoblasts have still to be elucidated. Furthermore, it is still under debate whether the varying expression levels of CCR5 on placental cells may augment the risk of MTCT of HIV-1 (Joubert et al. 2010). Instead, it was clearly shown that the interaction and fusion between trophoblastic cells and HIV-1-infected cells allow the virus to cross the trophoblastic barrier in an *in vitro* model.

Once trophoblastic cells carry HIV, molecules as DC-SIGN and ICAM-1 were implied to play a role in the viral passage to Hofbauer cells or T lymphocytes (Arias et al. 2003). Moreover, the identification of genetic variants of functional DC-SIGN, which were associated with an increased risk of in utero HIV-1 infection, further supports the implication of DC-SIGN in HIV-1

dissemination across the placenta (Boily-Larouche et al. 2012). Indeed, syncytiotrophoblasts and Hofbauer cells obtained from the placenta during the early and late stages of pregnancy were shown to carry HIV-1. Interestingly, these cells were not infected when the pregnant women received ART during gestation, clearly supporting the usefulness of such intervention strategy.

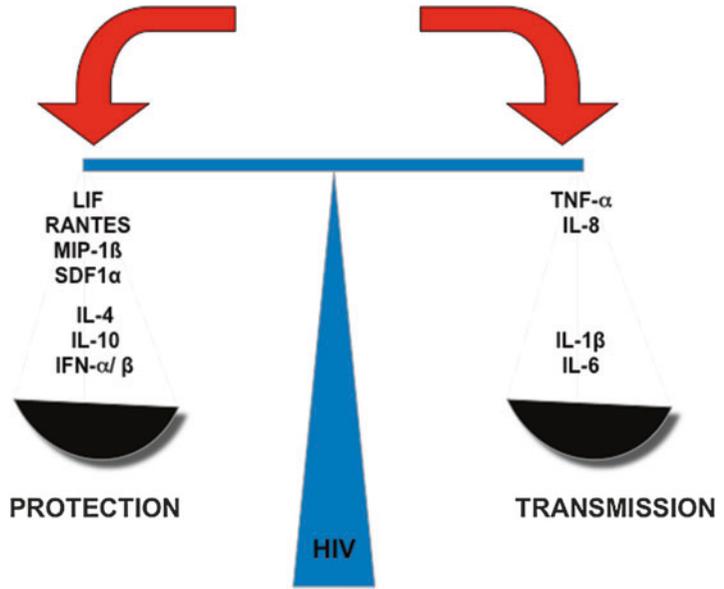
At the maternofetal interphase, the placenta is in close contact with the decidua, i.e., the maternal uterine mucosa, which contains plenty of target cells for HIV-1. In specific, decidual CD14⁺ cells are a preferential target for R5 viruses, whereas T lymphocytes for X4 viruses (Marlin et al. 2009). The same study showed that HIV-1 with R5 phenotype has a replication advantage in decidual cells over that with X4 phenotype (Marlin et al. 2009). Despite this, transmission through the placenta is a rare event, and the placenta may protect the fetus from infection through several mechanisms. The trophoblastic cells do not replicate HIV due to a restriction at postentry level. Furthermore a balance of cytokines and chemokines at placental level may direct the infection of trophoblastic cells. Recently Marlin et al. showed that soluble factors secreted by decidual cells inhibit HIV-1 infection (Marlin et al. 2011). The balance of pro- and anti-inflammatory factors (“IFN Signalling and Induction of Restriction Factors”) at the maternofetal interface, which is involved in stimulating leukocyte recruitment and angiogenesis and in regulating placental trophoblast invasion during pregnancy, may possibly play also an important role in controlling HIV-1 transmission (Fig. 1). Interestingly, a similar balance of inflammatory factors occurs also in other mucosae such as the gut mucosa, with the ultimate goal to regulate tolerance to the microflora and to prevent pathogen invasion.

Breast Milk: A Facilitator and Inhibitor of Transmission

During breastfeeding the neonate first and then the child ingest colostrum and milk at increasing volumes (Van de Perre et al. 2012). The breast milk of HIV-1-infected mothers contains large amounts of

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Fig. 1 Role of the cytokine and pro-/anti-inflammatory factor balance of the placental environment in the control of HIV-1 infection. Pro-inflammatory cytokines, such as TNF- α , IL-6, IL-8, and IL-1 β , have been shown to enhance HIV-1 infection, whereas type 1 interferons (IFN- α and IFN- β), the leukemia inhibitory factor (LIF), and chemokines (Rantes, MIP-1 β) that bind to the HIV-1 entry co-receptors can inhibit HIV-1 infection



free virus and of HIV-infected lymphocytes and macrophages, which may initiate transmission during breastfeeding (Rousseau et al. 2004). It was clearly demonstrated that duration of exposure to virus via breastfeeding correlates with increased risk of transmission. Besides cell-free virus it has to be considered that also cell-associated virus transmission likely occurs. Whether cell-associated HIV may play a more relevant role in transmission via breastfeeding than does cell-free virus has to be clarified yet.

Breast milk CD4+ T cells have particular characteristics that distinguish them from circulating blood lymphocytes, as, for example, high frequency of cell activation and expression of memory and mucosal homing markers. These specific features may very well favor the establishment of active replication of HIV in these cells. Furthermore maternal milk contains HIV-1-infected macrophages. Interestingly, R5 HIV-infected macrophages but not X4 HIV-infected lymphocytes are able to transmigrate across the fetal oral epithelia to reach the submucosa, where they can easily be involved in cell-to-cell transfer of the virus (Tugizov et al. 2012).

Breast milk may have, however, also a protective role and reduce the risk of infection. Indeed, several antiviral factors have been identified, as,

for example, mucin 1 (MUC1), the blood group antigen LewisX, and bile salt-stimulated lipase (BSSL). All these act through a common mechanism, as they inhibit binding of cell-free HIV-1 to DC-SIGN and prevent transfer of infection to CD4+ T cells (Pollakis et al. 2011). The DC-SIGN binding properties of breast milk obtained from different lactating mothers, however, are not constant suggesting mother-dependent protection levels for HIV-1 transmission during breastfeeding (Stax et al. 2011).

A recent study reported a significant inhibition of cell-free HIV-1 with breast milk from HIV-positive and HIV-negative Tanzanian women (Lyimo et al. 2012). The effect was independent of the viral tropism and did not correlate with the presence or levels of individual cytokines, chemokines, or growth factors in the milk, including factors that serve as blocking ligands for the HIV-1 co-receptors, CCR5 and CXCR4. However, a correlation was found between HIV-inhibitory activity and the sialylated form of LewisX antigen (sialyl-LewisX) in the milk of HIV-positive women. Surprisingly, breast milk from the same subjects on the contrary enhanced infection via cell-associated HIV in vitro. Enhancement did not correlate with any of the abovementioned factors. These data indicate that

distinct factors in breast milk can modulate cell-free and cell-associated HIV-1 infection: the balance between these factors may bias the transmission event.

Enhancement of cell-cell interaction and HIV-1 infection by breast milk may result from several factors, which remain largely to be defined. Upregulation of cell surface molecules needed for efficient attachment and interaction of HIV-infected milk cells to target cells in the intestinal epithelium or submucosal tissue may play a major role. Breast milk contains high levels of soluble ICAM-1, which are involved in the formation of the virological synapse between HIV-1-infected cells and target cells; however, no correlation was found between the level of ICAM-1 in breast milk or of antibodies against ICAM-1 and cell-associated HIV-1 infection (Valea et al. 2011). Alternatively, activation of HIV-1 expression from latently infected cells may be promoted by the presence of stimulatory factors in breast milk. In vitro stimulated HIV-infected milk cells secrete higher levels of virus than the comparable population of cells from peripheral blood. Interestingly, activated milk CD4+ T cells from women receiving HAART produce HIV-1 in vitro (Valea et al. 2011), suggesting that these cells may constitute a reservoir that is relatively refractive to treatment. Moreover, localized and systemic inflammatory responses in the mother, which increase the levels of pro-inflammatory cytokines in the milk, may contribute to enhance HIV-1 release from infected cells and favor transmission.

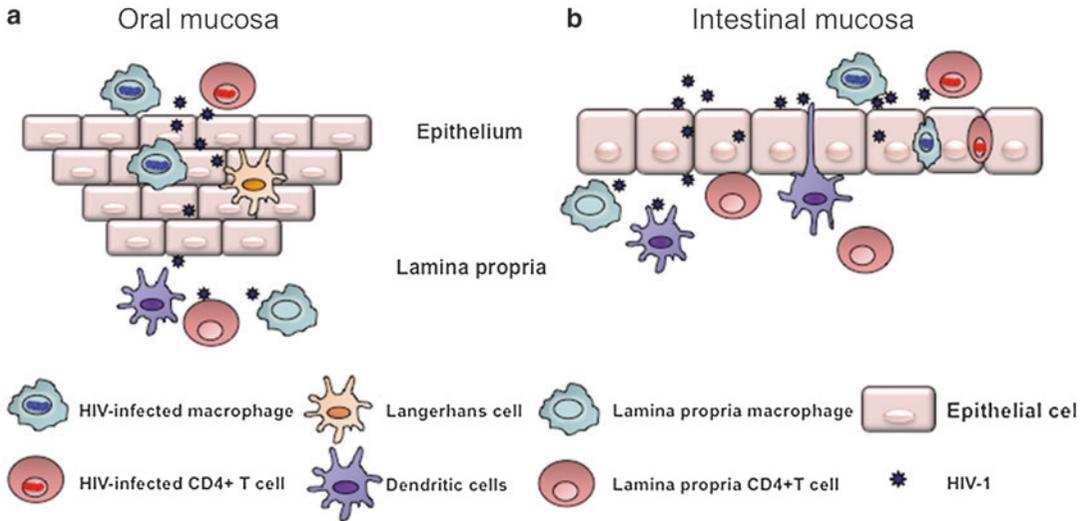
The Gastrointestinal Tract: A Primary Site of Infection

The interaction of HIV-1 with the mucosal epithelia of the oropharyngeal and gastrointestinal tract is the first step in the establishment of systemic HIV-1 infection during MTCT after ingestion of HIV-1 from maternal blood and cervical mucus during delivery or from breast milk. Tissue morphology and integrity, as well as distribution of relevant cell types within the mucosa can greatly

influence viral transmission, and the ability of HIV-1 to initiate systemic infection may vary considerably among the different mucosal types. The intestinal tract with its large extension of the epithelial surface provides an important site for viral uptake and consequent transfer to underlying and confining target cells. After crossing the mucosal barrier, the main target cells for HIV-1 replication are the CD4+ T (“▶ [Central Memory CD4 T cells](#)”) lymphocytes, which are rapidly depleted both in the periphery and in the mucosal tissues. However, many cell-type variants of both lymphocytic and monocytic lineage can be found in the mucosal tissues, which are potential targets for the incoming virus.

HIV spread across fetal/neonatal oropharyngeal epithelia is an important route of MTCT. While MTCT through the neonatal oral epithelium is considered to be common, in adults oral transmission occurs apparently less frequently. Virions can traverse both adult and fetal oral epithelial cells by transcytosis (Tugizov et al. 2011); however, infectivity of the virions is greatly diminished during the passage through the adult cells, but not through the fetal cells. This appears to be due to the high-level expression of anti-HIV innate proteins, the β -defensins, and the secretory leukocyte protein inhibitor (SLPI) by the adult oral epithelial cells. Cell-free HIV-1, which crossed the fetal oral epithelia, is able to further transfer infection to CD4+ T cells, DCs, and macrophages. Antibodies against galactosylceramide (GalCer) and heparan sulfate proteoglycan (HSPG) but not against CCR5 and CXCR4 reduce infection, suggesting that chemokine receptors do not play a primary role in transmission through the fetal/infant oral mucosa (Tugizov et al. 2011). Moreover macrophages but not CD4+ T cells can transmigrate across the oral mucosa (Fig. 2a).

The tonsil's mucosa contains M cells lying above regions where DCs are juxtaposed with CD4+ lymphocytes, whereas LCs cluster in the subepithelial papillae of the buccal mucosa. Accordingly in the primate MTCT model, infection was shown to occur through the surface mucosa of tonsils, where specialized M cells and DCs may transport HIV to the interior of the



Mother-to-Child Transmission of HIV-1: Role of Receptor Usage and Target Cells, Fig. 2 Oral and intestinal mucosal epithelia are key portals of entry for HIV MTCT. (a) Cell-free HIV transmigration across the stratified oral mucosal epithelium leads to passage of virions into the intraepithelial and subepithelial virus-susceptible cells. HIV-infected lymphocytes do not transmigrate across oral epithelia; however, HIV-infected macrophages penetrate into fetal epithelia and reach the lamina propria. (b) Cell-free HIV also transmigrates across

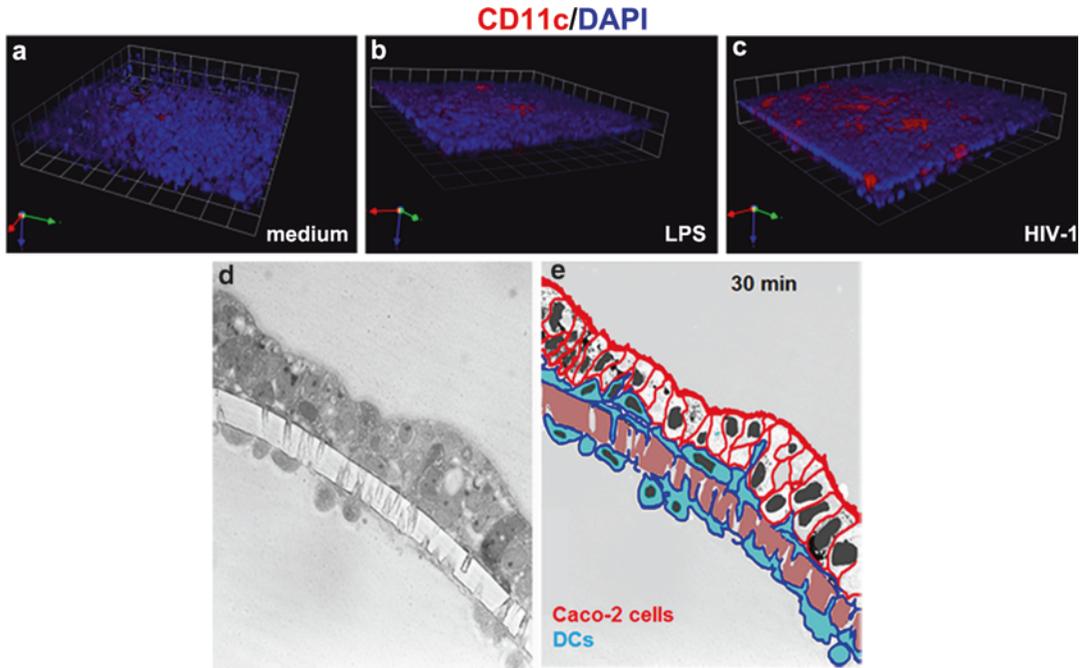
monostratified intestinal epithelia. Moreover subepithelial DCs extend dendrites across the epithelium to shuttle luminal virions. Both HIV-infected lymphocytes and macrophages transmigrate across fetal intestinal epithelia. Transcellular spread of cell-free and HIV-infected cells across oral and intestinal mucosal epithelia may lead to infection of intraepithelial and submucosal HIV-susceptible cells, including T lymphocytes, Langerhans/DCs, and macrophages

tissue. Tonsil lymphoid cells have an increased susceptibility to infection with HIV-1 compared to PBMC, which may in part be ascribed to the increased expression of early activation markers and the viral co-receptor CXCR4, relative to PBMC.

Transmission of HIV-1 through the gastrointestinal epithelial monolayer has been object of intensive studies. Different pathways have been suggested (Fig. 2b): cell-free and cell-associated viruses of R5 or X4 phenotype bind the GalCer receptor and cross the enterocytes via transcytosis. However, primary jejunal epithelial cells incubated with HIV-1 carry over only R5 viruses to receptive target cells, whereas M cells in Peyer's patches of the digestive epithelium, which deliver samples of foreign material directly to the close intraepithelial lymphoid cells, transport selectively X4 viral variants through a chemokine-receptor-mediated mechanism. In

addition, DCs in jejunum explant cultures are the predominant target cell of R5 HIV-1 early after infection and leave thereafter the tissue to transmit in trans the virus to lymphocytes (Shen et al. 2010). Bunders et al. recently showed that the fetus and the newborn gut mucosa and submucosa contain abundant levels of CD4 + CCR5+ T cells, which lay within the epithelial lining (Bunders et al. 2012). Being these cells in direct contact with the lumen may facilitate their infection with R5 viruses. In summary, some of the described mechanisms support a preferential transmission of CCR5-using viruses others instead provide evidence of the transmission of X4 viruses as well.

DCs or LCs of the intestinal and vaginal mucosa were repeatedly described to be the first target for HIV-1 and the vehicle for the virus to reach replication competent cells. More recently cell-free R5 but not X4 HIV-1 were described to attract colonic lamina propria-resident DCs to extend their cellular



Mother-to-Child Transmission of HIV-1: Role of Receptor Usage and Target Cells, Fig. 3 Migration of DCs across a monolayer of epithelial cells. Caco-2 cells were grown on Transwell filter to form a confluent monolayer, and then DCs were let to adhere to the bottom of the filter. Cell-free HIV-1 (a, d, e), LPS (b, positive control), or medium (c, negative control) were incubated on the apical side of the Caco-2 monolayer for 1.5 h. Filters were processed for confocal microscopy. (a–c) Three-dimensional rendering of representative fields obtained with Volocity 5.0 of the Caco-2/DCs coculture stained

with DAPI nuclear dye (all cells; blue) and mouse anti-human DC-SIGN-PE (DCs; red) are shown. DCs migrated between the epithelial monolayer in response to HIV-1 and LPS stimulation, but not in response to medium. (d, e) Semi-thin sections for transmission electron microscopy (d) and the corresponding explicative color mask (e, Caco-2 cells red, DCs blue, filter gray) show the morphology and the spatial organization of cells. DCs are disposed along the lower face of the filter, inside the membrane pores, and intercalated in between Caco-2 cells

processes through the intact epithelium (Fig. 3). Migrated DCs sample luminal virions and are able to transfer infection to CD4+ T cells (Cavarelli et al. 2013). The migratory process was dependent upon the interaction of the HIV envelope protein with CCR5, and therefore, providing CCR5-using viruses with an additional mechanism to gain access to the intestinal mucosa. It will be important to understand if these DCs will reside in the mucosa to be involved in antigen presentation and/or be able to reach the lymph nodes to spread further the infection. Unraveling this mechanism may help to identify the relevant cells involved during the early phases of infection and develop effective prevention strategies.

Conclusion

As of today the viral phenotype was not identified as a predictive marker of MTCT of HIV-1. The transmitted virus, when isolated from the newborn, is, however, predominantly of R5 phenotype. While the placenta is a player in the transmission during gestation, the gastrointestinal mucosa instead, with its large extension of the epithelial cell monolayer, is certainly involved in transmission during labor, delivery, and breastfeeding via ingestion of infected biological fluids. Different cellular pathways seem to be determinant, each of them showing a preferential transmission of one or the other viral phenotype.

Transmission through the mucosal surfaces is a portal of entry to reach the underlying cells, which are target for infection with HIV-1 and deputed to spread infection to other cells and tissues. Preventing the infection of the first target cell is the ultimate goal of all preventive interventions. Both vaccine and non-vaccine biomedical interventions could provide unique as well as complementary opportunities towards elimination of MTCT of HIV-1.

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MTCT HIV-1 Transmission Update: Transmission Routes and Mechanisms

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Definition

HIV can be transmitted from a mother to her child (mother-to-child transmission or MTCT) during pregnancy (in utero), labor/delivery (intrapartum), or via breastfeeding (postpartum). Mother-to-child transmission or vertical transmission of HIV occurs in 25–40% of pregnancies without intervention. With medical interventions including antiretroviral prophylaxis to both the mother and the infant, cesarean section and, breastfeeding avoidance, MTCT has been decreased to less than 2% in resource-rich settings, yet MTCT is still above 10% in resource-poor settings due to limited access to effective interventions.

Introduction

Vertical transmission, or mother-to-child transmission (MTCT) of HIV-1, may occur during pregnancy (in utero), during labor/delivery (intrapartum), or throughout the breastfeeding period (postpartum). Since the first report of vertical transmission of HIV in 1982, dramatic improvements in prevention of MTCT have been made. Despite these successes, an estimated 330,000 of the approximately 1.5 million children at risk of contracting the virus are infected annually (UNAIDS 2012).

Use of preexposure and postexposure prophylactic antiretroviral (ARV) treatment to interrupt MTCT has been implemented with great success in both resource-rich and resource-limited

settings. In resource-rich settings, universal antenatal testing, ARV use, caesarian section, and avoidance of breastfeeding have resulted in MTCT rates under 2%. Unfortunately, in low-to-middle income countries, poor access to antenatal testing, ARV treatment, and delivery services has limited the effectiveness of interventions (Luzuriaga 2007). In 2011, UNAIDS estimated that only 48% of HIV-positive pregnant women received the most effective ARV regimens to prevent MTCT and only 42% of infants received prophylaxis (UNAIDS 2012). Currently, implementation science is focused on reducing these significant gaps in intervention through a combination of best service delivery practices and health systems approaches. Provision of ARV agents is necessary but not sufficient to eliminate MTCT; health systems must be able to deliver services from the antenatal period through the end of breastfeeding in order to dramatically reduce MTCT (Chi and Bolton-Moore et al. 2013; Mofenson 2013). The ultimate goal of these efforts is to reduce MTCT globally to <5%.

Routes of Transmission

HIV-1 transmission from women to their infants may occur through the placenta, maternal genital secretions, or breast milk (Tobin and Aldrovandi 2013). Although the fetus and neonate are exposed to HIV at multiple time points, infection is rather inefficient. Without any intervention, 5–10% of pregnancies result in utero transmission, 10–20% of deliveries result in intrapartum infection, and 5–15% of breastfeeding infants become HIV infected (1995). Understanding how these immunologically vulnerable infants escape infection at any of these three stages could provide important insights into the mechanisms of natural protection.

Transmission and Infection

In all three routes of MTCT, infection of the infant occurs with HIV traversing an anatomical barrier and productively infecting the infant's CD4+ T cells. Viral amplification and dissemination to other tissues then occurs. It is still uncertain

whether HIV transmission occurs as a result of cell-free virions or infected cells. Both have been detected in blood, amniotic fluid, cervicovaginal secretions, and breast milk. Compared with levels in blood, mucosal fluids generally contain significantly lower amounts of virus (Shepard and Schock et al. 2000). MTCT risk is associated with both the levels of cell-free and cell-associated virus. As these two variables are highly correlated, it is difficult to disentangle which is more influential; however, studies have shown a stronger association between cell-free virus and HIV transmission (Lehman and Farquhar 2007; Fiscus and Aldrovandi 2012; Tobin and Aldrovandi 2013).

Within an HIV-infected individual, there are millions of HIV variants referred to as a quasi-species. However, during all forms of HIV transmission, usually only a single variant establishes productive infection in the new host. This is referred to as a “genetic bottleneck” (Shaw and Hunter 2012). The biologic basis of this genetic bottleneck is unknown but does not appear to be stochastic since transmitted forms appear to share certain viral characteristics. HIV entry necessitates viral interaction with cellular receptors, specifically the CD4 molecule, and a chemokine coreceptor, usually C-C chemokine receptor type 5 (CCR5) or C-X-C chemokine receptor type 4 (CXCR4). The overwhelming majority of transmitted variants use the chemokine receptor CCR5.

Whether the genetic bottleneck occurs within the transmitting compartment (e.g., maternal genital secretions or within breast milk) or later within the newly infected host is uncertain. Studies comparing viral variants in breast milk and plasma have found that despite a quantitative restriction of viral variants in breast milk, the variants in milk are genetically indistinguishable to those in maternal plasma. Thus, the transmission bottleneck does not occur in this transmitting compartment (Heath and Conway et al. 2010; Tobin and Aldrovandi 2013). Once the infant is infected, the variant that establishes infection replicates and dominates initially; however, over time, the HIV-1 population in the infant becomes more diverse through viral mutation as the virus replicates.

HIV can be divided into several subtypes or clades; particular subtypes are found in different locations globally and may impact the likelihood of MTCT. A few controversial and smaller studies have found that HIV clade C, the predominant clade in sub-Saharan Africa, is more likely to be transmitted and may be preferentially transmitted in utero compared to other subtypes (Lehman and Farquhar 2007; Selvaraj and Paintsil 2013).

Maternal Characteristics and Transmission Risk Factors

Regardless of the route of infection, maternal health and HIV-related characteristics influence the risk of transmission. The most predictive risk factors of MTCT are the levels of HIV in the mother and the maternal CD4+ T-cell count. HIV is generally measured in the blood as HIV RNA, often referred to as “viral load.” The level of maternal viremia is associated with disease progression and all forms of transmission. The higher the level of HIV RNA, the greater the risk; however, there are well-described cases where women with “undetectable” viral loads have transmitted HIV to their infants. HIV viremia, and thus risk of transmission, is highest at the time of primary HIV-1 infection and during advanced disease when the immune system cannot control viral replication. Women are at increased risk of HIV acquisition during pregnancy and subsequently at increased risk of transmitting the virus to both their infants and their sexual partners during their primary viremia (Gray and Li et al. 2005; Keating and Hamela et al. 2012; Mugo and Heffron et al. 2012).

The second important risk factor is maternal CD4+ T-cell count, which declines with advancing disease. CD4+ T cells are the major targets of HIV infection, and by both direct and indirect mechanisms, HIV infection results in progressive reduction of cells expressing CD4. As CD4+ T cells decrease, the likelihood of transmission increases. Healthy adults typically have CD4+ T-cell counts of 500–1,200 cells/ μ L, and when the CD4+ T-cell count is less than or equal to 200 cells/ μ L, a person is classified as having

AIDS. The majority of MTCT occurs in women who have a CD4+ T-cell count of less than 350 cells/ μ L.

Inflammation is a well-established risk factor for transmission of HIV. Local inflammation and systemic inflammation are associated with increased risk of MTCT. Inflammatory cytokines can activate CD4+ T cells, increasing the quantity of readily available targets for HIV infection; additionally, the inflammatory response may allow for easier passage of HIV across the mucosa. Women with conditions associated with inflammation, such as sexually transmitted infections (STIs), bacterial vaginosis, chorioamnionitis, mastitis, placental malaria, and other opportunistic infections (i.e., tuberculosis), are at increased risk of MTCT (Lehman and Farquhar 2007; Selvaraj and Paintsil 2013).

Both adaptive and innate maternal immune responses are associated with protection against MTCT (Lehman and Farquhar 2007). Levels of maternal HIV-neutralizing antibodies and CD8+ cytotoxic T-cell responses are associated with protection, while inflammatory cytokines and chemokines are associated with increased transmission. However, most of these studies are limited by small sample sizes. Data on the protective effects of certain genetic markers are more robust. Children who possess a 32-base pair deletion in the CCR5 receptor are much less likely to become infected, as the preferred coreceptor is non-functional. Children who share human leukocyte antigen (HLA) alleles with their mothers are at increased risk of HIV acquisition presumably due to a virus that has preselected fitness for the infant.

Mechanisms of Transmission

MTCT Transmission: In Utero

With in utero transmission, maternal HIV must traverse the placenta, productively infect an infant's T cells, and then begin viral replication to establish infection in the infant. Although HIV infection can occur throughout gestation, early in utero infection is associated with an increased risk of spontaneous abortion. Most in utero transmission occurs late in the third trimester. As only

5–10% of all pregnancies of HIV-infected women result in in utero infection, it is likely that a breakdown of the maternal-fetal barrier must occur in order for HIV to cross the placenta. Conditions where the maternal-fetal barrier is breached (i.e., prolonged rupture of membranes) or inflamed (i.e., chorioamnionitis) during pregnancy are associated with a marked increased risk in HIV infection/transmission (Bond et al. 2007). Furthermore, placental micro-transfusions (small exchanges of blood) increase the risk of transmission through the direct mixing of HIV-infected maternal blood with that of the infant (Selvaraj and Paintsil 2013). As mentioned above, HIV can only productively infect activated CD4+ T cells; yet most infant T cells are quiescent. This quiescent or tolerogenic state may help the infant escape infection.

MTCT Transmission: Intrapartum

During childbirth, the infant's mucosal membranes are exposed to maternal genital secretions and blood, which contain HIV. Over the course of labor and delivery, several factors have been identified that increase the risk of MTCT. High levels of HIV-1 in maternal blood or genital secretions, low maternal CD4+ T-cell counts, and inflammatory conditions (e.g., STIs) increase the risk of transmission to the infant. During parturition, prolonged rupture of membranes prior to delivery dramatically increases the risk of transmission, potentially due to ascending spread of HIV from the lower genital tract. Additionally, breaches in the infant's skin through the use of scalp electrodes or forceps during labor/delivery are associated with increased risk of transmission. Interestingly, the first twin is more likely than the second twin to be HIV infected during delivery presumably due to the first twin's longer exposure to HIV in the birth canal. Cesarean section reduces MTCT during labor and delivery if performed prior to the initiation of labor or, in other words, prior to the breakdown of the maternal-fetal barrier at the onset of labor.

MTCT Transmission: Postpartum

If the infant escapes HIV in utero and intrapartum infection, the child is still at risk of infection from

breast milk. Breastfeeding transmission of HIV may occur at any point during lactation. The amount of virus in breast milk, type of breastfeeding (mixed feeding or exclusive breastfeeding), duration of breastfeeding, as well as presence of breast and oral inflammation are important determinants of breast milk transmission.

The amount of HIV in breast milk is controlled by the mammary epithelial barrier, which is highly effective at reducing the passage of HIV from the blood to the breast milk. In the absence of inflammation, levels of HIV in milk are 100-fold less than those in the maternal circulation. Breast milk also contains antimicrobial and anti-inflammatory agents that decrease the incidence of infection (Tobin and Aldrovandi 2013). It appears that transmission is most likely when the mammary epithelium is permeable. Cellular tight junctions regulate the breast epithelial permeability; early in lactation these tight junctions are still forming and are thus more permeable. Additionally, the epithelium is more “leaky” in women who do not exclusively breastfeed and during the time of weaning. This increase in permeability is associated with increased levels of HIV in breast milk.

Risk of MTCT is modified based on woman’s breastfeeding pattern. Several large studies have demonstrated that exclusive breastfeeding [provision of only breast milk for the first 6 months postpartum] reduces the risk of transmission by almost half compared to mixed feeding [provision of breast milk and other foods or liquids]. This finding seems counterintuitive as an exclusively breastfed infant will be exposed to more breast milk and therefore more HIV compared to a child who is mixed fed. Why exclusive breastfeeding has such a profound effect on transmission risk is uncertain. Two non-mutually exclusive mechanisms have been proposed: (1) mixed feeding disturbs the gastrointestinal epithelium of the infant making establishment of infection easier, and (2) nonexclusive breastfeeding harms mammary epithelium causing increased permeability. With the first mechanism, new foods and potentially pathogens are introduced during mixed feeding that can lead to intestinal inflammation that is exacerbated by removal of the

anti-inflammatory agents found in breast milk. With the second mechanism, the mammary epithelial tight junctions may be disrupted due to less frequent emptying of the breast and subsequently levels of HIV in the breast milk may be increased.

Not only how a woman breastfeeds but how she stops breastfeeding appears to impact the risk of transmission to the neonate. As breastfeeding decreases over the course of weaning, in a mechanism similar to nonexclusive breastfeeding, potentially the mammary epithelium becomes more permeable. Abrupt weaning, or stopping breastfeeding quickly, is associated with increases in breast milk viral load. If comfort feeding or re-lactation takes place, the breast milk has a much higher level of HIV-1 RNA compared to preweaning breast milk (Kuhn and Kim et al. 2013). Additionally, timing of breastfeeding cessation is important since the infant is at risk of HIV infection as long as s/he breastfeeds. However, early weaning (prior to 6 months) has been associated with increased infant morbidity and mortality due to gastroenteritis (Read 2012). More research is needed to inform recommendations for women on best practices and timing for safe weaning in HIV-infected women.

As with in utero and intrapartum transmission, inflammation has been associated with higher risk of HIV transmission. Mastitis, or inflammation of the breast, and breast problems (i.e., cracked/bleeding nipples or abscess) can increase the risk of transmission, likely through a transient increase in HIV-1 RNA levels due to increased mammary epithelial permeability. Subclinical mastitis, defined by an elevated sodium-potassium ratio, has also been correlated with an increased risk of transmission (Lehman and Farquhar 2007; Tobin and Aldrovandi 2013). Unfortunately, subclinical mastitis is not easily detectable and makes clinical intervention and prevention difficult. Not only does inflammation in the mother increase risk of transmission, inflammation in the child may increase the risk. Introduction of new foods during the period of mixed feeding may cause inflammation in the mouth of the child, facilitating easier passage of HIV across the mucosal barrier. Breaches in infant oral mucosa during disease-related inflammation such as in the case of oral

Candida infection likely increase the risk of transmission (Wood and Chahroudi et al. 2013). Prevention of mastitis and other inflammatory illnesses in the mother and child can help reduce the risk of MTCT.

In resource-rich settings, avoidance of breastfeeding is recommended; however, this is not an option in many lower-resource settings. Making breastfeeding a safer option through anti-retroviral therapy, exclusive breastfeeding, and good breast health with prevention of mastitis can reduce the likelihood of transmission.

Conclusion

Over the past decades, millions of children have been exposed to HIV in utero, intrapartum, and postpartum. However, infection is not absolute; the majority of HIV-exposed infants will not become infected. Risk factors for HIV transmission include levels of maternal virus, maternal health, and HIV disease stage. Several maternal and infant immune and innate responses prevent transmission from occurring.

Models of antiretroviral care and treatment have dramatically decreased the risk of transmission globally; in parts of the developed world, transmission risk is less than 2% in the presence of ARV therapy. Interruption of MTCT with ARV has paved the way for preexposure and post-exposure prophylaxis in other contexts (i.e., sexual transmission). Prevalence of HIV has decreased globally; however, 50% of those living with HIV are women. In sub-Saharan Africa, women make up more than 59% of those living with HIV (Anon n.d.). Therefore, infants will continue to be at risk of contracting HIV through mother-to-child transmission.

Through provision of ARV and other prevention services, MTCT can be significantly reduced, as observed in resource-rich settings. Going forward the availability of and adherence to effective antiretroviral therapy in combination with safer breastfeeding practices could dramatically improve HIV-free child survival in resource-limited settings. Understanding the mechanisms of vertical transmission can provide important

insights into natural immunity and protection. These insights can be translated to a deeper understanding of HIV and improving prevention methods in the future.

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Mucosal Immunity to HIV-1

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Definition

Mucosal immunity refers to innate and adaptive immune responses that defend mucosal surfaces, which are the surfaces of the body that line passageways exposed to the external environment, such as the respiratory, gastrointestinal, and reproductive tracts. These defenses protect the body against potentially pathogenic microbes, including HIV-1. This review focuses on immune defenses in the female reproductive tract, the male reproductive tract, and the gastrointestinal tract, which are the major portals of entry for HIV-1.

Female Reproductive Tract

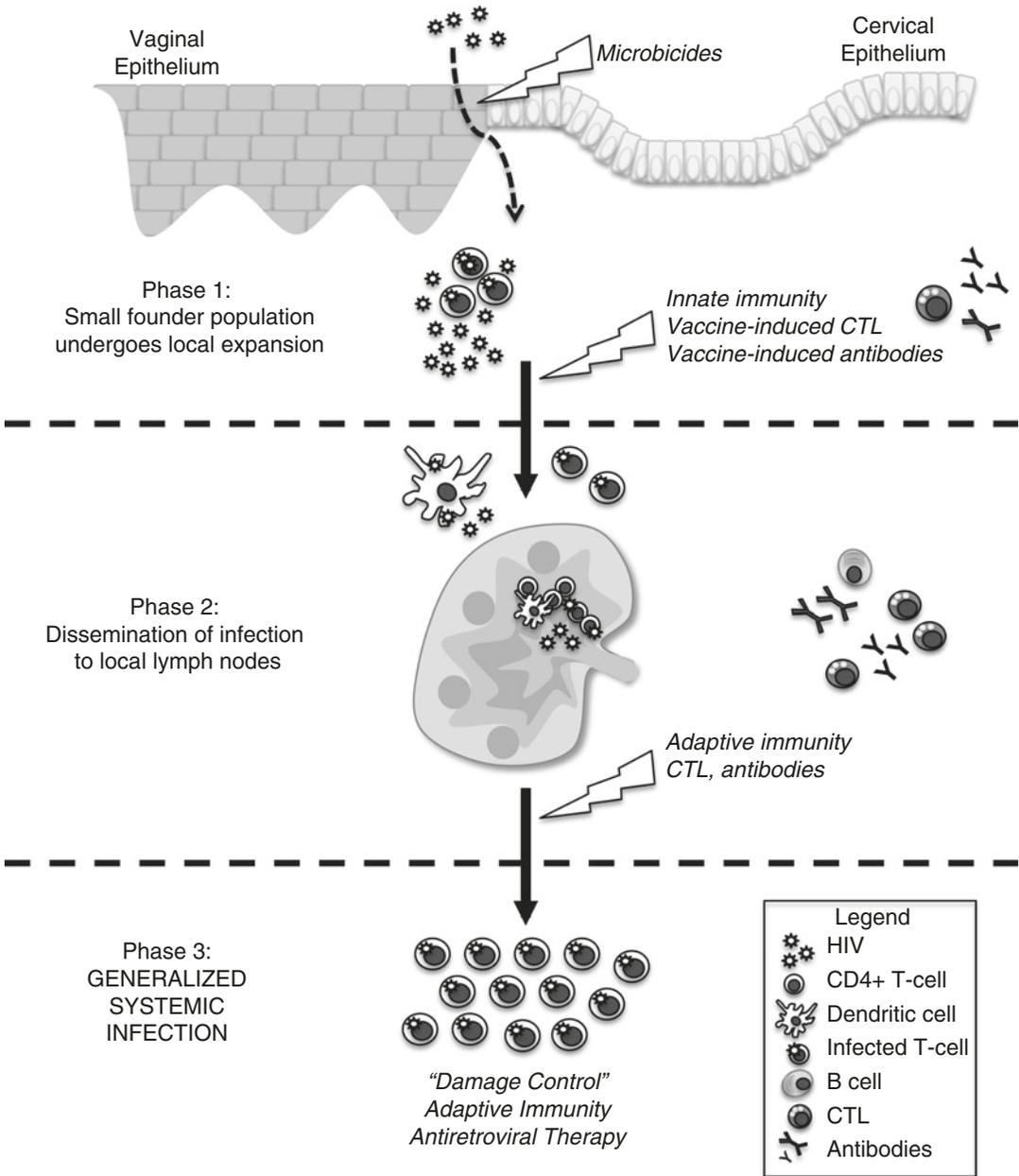
FRT and HIV-1 transmission (► [HIV-1 Sexual Transmission, Overview](#)). Women (► [Women, Epidemiology of HIV/AIDS](#)) account for approximately half of the 34 million individuals with HIV-1; nevertheless, transmission via the female reproductive tract (FRT) (► [Identifying Opportunities to Block HIV-1 Transmission in the Female Genital Tract](#)) is relatively inefficient, with an estimated relative probability of 1 transmission per 200–2,000 exposure events (Hladik and

McElrath 2008). Factors affecting transmission efficiency to the FRT are numerous and include HIV-1 viral load in the male partner's semen; the presence and thickness of vaginal mucus, which varies with the menstrual cycle; other changes in the mucosa occurring throughout the menstrual cycle; the presence of inflammation or lesions due to other sexually transmitted infections; and the composition of local microbial flora (Hladik and McElrath 2008; Xu et al. 2013).

The actual sites within the FRT where initial HIV-1 transmission and viral amplification occur remain controversial. The lower female reproductive tract, consisting of the vagina and ectocervix, is protected by a stratified squamous epithelium. In contrast, the upper tract, including the endocervix and uterine endometrium, is lined by a single layer of columnar epithelium that is easier to traverse than the multilayered epithelium of the lower tract (Fig. 1, top panel). The cervical transformation zone, or squamocolumnar junction, where the stratified squamous epithelium of the ectocervix and the columnar epithelium of the endocervix meet, is rich in CD4+ T cells, macrophages, and dendritic cells and therefore may be highly susceptible to HIV-1 transmission (Hladik and McElrath 2008).

Despite the increased thickness of lower reproductive tract epithelium relative to that of the upper tract, infection can and does occur via the lower tract: experimentally hysterectomized macaques can be infected after intravaginal inoculation; HIV-1 infection has been documented in women who lacked a uterus at birth; and a randomized clinical trial showed no reduction in HIV-1 acquisition in women using a diaphragm as compared to the control group (Hladik and McElrath 2008; Xu et al. 2013).

Cervical ectopy, a condition defined as extension of the simple columnar epithelium of the endocervix to cover a portion of the ectocervix, is associated with increased risk of acquiring HIV-1 and other STI including human papillomavirus and *C. trachomatis* (Wira and Fahey 2008; Rodriguez-Garcia et al. 2013). Cervical ectopy is most prevalent in adolescent girls and can also occur during pregnancy and oral contraceptive use.



M

Mucosal Immunity to HIV-1, Fig. 1 Idealized female genital mucosa showing potential points of intervention to prevent systemic infection. In the top panel, stratified squamous epithelium of the vagina is shown on the left, with the squamocolumnar junction or transition zone shown in the center and the simple columnar epithelium of the endocervix on the right. "Phase 1" in this figure refers to the earliest stage of mucosal infection, when virions have penetrated the epithelium and a small founder population is undergoing local expansion. During this time, microbicides, innate immune responses, and/or vaccine-induced adaptive responses can theoretically limit virus

propagation and expansion. If the virus is not eliminated at this stage, the infection is disseminated to local and regional lymph nodes, where it further expands and infects susceptible target cells. Antibody-secreting cells and CTL present in lymph nodes may restrict further replication. However, if immune responses in mucosal tissues and lymph nodes are insufficient, the infection rapidly becomes systemic. From that point forward, adaptive responses can provide "damage control" by limiting viral loads, but complete eradication of infection is unlikely (Figure redrawn and modified from Shacklett and Shattock (2004; Haase (2011))

The endocervical canal is lined with mucus (► [HIV-1 Transmission; Influence of Body Secretions](#)) (known as the “mucus plug”), which serves as an additional physical barrier to the entry of pathogens. The consistency of cervical mucus changes during the menstrual cycle, thinning during ovulation in order to enable fertilization. The extent to which fluids and/or cells containing HIV-1 can traverse the cervical mucus barrier, leading to direct infection of susceptible cells in the upper FRT, is unclear and may also vary with the menstrual cycle (Wira and Fahey 2008).

Initial target cells (► [HIV-1 Transmission, Cell-Types Associated with](#)). Mechanisms of transmission across the intact vaginal epithelium are difficult to study, but have been modeled in vivo using the simian immunodeficiency virus (SIV) model and ex vivo using explant cultures. These studies suggest that both cell-free and cell-associated virions can successfully establish infection through the female reproductive tract. The precise events that allow HIV-1 to traverse the genital epithelium remain controversial but may include transcytosis across intact epithelial cells; transport through gaps between epithelial cells, or through breaches in the epithelial layer; binding and uptake by Langerhans cells in the genital epithelium, followed by transfer of virion particles to T cells; and direct infection of intraepithelial CD4+ T cells (Hladik and McElrath 2008; Xu et al. 2013; Fig. 1). Viral penetration is believed to be rapid and has been detected in the rhesus macaque model by 30–60 min following experimental intravaginal exposure to SIV. Studies in this model system have identified resting vaginal CD4+ T cells expressing CCR5 (HIV-1 Transmission; Association with Host Genotypes) but negative for activation markers HLA-DR and Ki67, as early targets for infection (Haase 2011). The susceptibility of vaginal CD4+ T cells to infection is also illustrated by the rapid depletion of these cells following intravenous exposure to SIV (Xu et al. 2013).

Early viral dissemination. Extensive studies to determine the nucleic acid sequences of initial transmitted/founder viruses have revealed that the earliest transmitted strains utilize CD4 and CCR5 (HIV-1 transmission dynamics and HIV-1

transmission selection) as their primary receptor and coreceptor, respectively. Furthermore, infection is usually initiated by a single viral genotype, which then gives rise to a small cluster of infected cells near the portal of entry (Haase 2011). A local inflammatory response ensues, leading to attraction of additional susceptible target cells. Infected cells then spread via afferent lymphatics to draining lymph nodes, where further amplification and dissemination can occur (Haase 2011) (Fig. 1, middle panel).

In the rhesus macaque model of SIV exposure, an inflammatory cascade begins in the cervicovaginal mucosa by day 1 postinfection. At this point, there is increased expression of the chemokine MIP-3 α (CCL20), which drives an influx of CD123+ plasmacytoid dendritic cells (pDC) into the mucosa. The pDC then release type I interferons, which are important mediators of antiviral innate immunity. However, pDC also release beta chemokines MIP-1 α (CCL3) and MIP-1 β (CCL4); these chemokines attract additional CCR5+ CD4+ T cells that serve as targets for viral infection and amplification (Haase 2011; ► [HIV-1 Transmission; Cell-Types Associated with](#)). This model for early viral dissemination has been termed a “broadcasting” model because it begins with a single focus of infection, from which an inflammatory cascade leads to rapid amplification of the viral “signal.” The infection spreads from the initial site of infection to local lymph nodes and subsequently disseminates throughout the body (Fig. 1, middle and lower panels).

The role of immune activation (► [Immune Activation and HIV Transmission](#)). Since soluble molecules that promote inflammation contribute to rapid viral dissemination, it has been proposed that anti-inflammatory agents might be used to limit or prevent viral “broadcasting.” The hypothesis that immune quiescence can inhibit viral dissemination from mucosal foci of infection was tested by Li and Haase, who demonstrated that topical application of the anti-inflammatory compound glycerol monolaurate (GML) to cervicovaginal mucosa of rhesus macaques could prevent local cytokine/chemokine production and protect the macaques from mucosal SIV

infection (Haase 2011). This finding suggested that anti-inflammatory compounds could be developed for use as microbicides to prevent HIV-1 acquisition in high-risk humans.

Other factors contributing to transmission risk. Other sexually transmitted infections (STI), including *C. trachomatis*, chancroid (*H. ducreyi*), and herpes simplex viruses, can increase susceptibility to HIV-1 transmission by increasing local inflammation, recruiting infectable target cells, and through other mechanisms. Bacterial vaginosis may also facilitate HIV-1 transmission (Shacklett and Greenblatt 2011).

Semen and seminal plasma contain immunosuppressive agents that alter the immune microenvironment of the female reproductive tract in order to facilitate conception and implantation. These include high concentrations of TGF- β and prostaglandin E2, which can both inhibit innate immune responses and promote the differentiation of regulatory T cells (Sabatte et al. 2011). In vitro, seminal plasma also induces cervical epithelial cells to produce several proinflammatory cytokines and chemokines that could lead to infiltration by neutrophils and macrophages. Finally, amyloid fibrils derived from the semen protein prostatic acidic phosphatase have been shown to enhance HIV-1 infectivity in vitro. These fibrils, known as semen-derived enhancer of virus infection (SEVI), have been shown to promote non-specific attachment of virus particles to target cells under laboratory conditions (Sabatte et al. 2011). However, the effects of SEVI fibrils on HIV-1 transmission in vivo remain to be demonstrated.

Innate responses in the FRT (HIV & SIV, Innate Immune Responses to). The reproductive mucosa can deploy a wide range of innate immune defenses against viral infection (Iwasaki 2010). In addition to the previously cited physical barriers, these include soluble factors such as secretory leukocyte protease inhibitor (SLPI), lactoferrin, alpha and beta defensins, the antimicrobial peptide cathelicidin (LL-37), and various cytokines and chemokines. Several recent studies have implicated the serine protease inhibitor trappin-2/elafin as a potential biomarker of protection against HIV-1 acquisition (Rodriguez-Garcia et al. 2013).

Adaptive responses in the FRT (► HIV & SIV, CD4 T Cell Responses to; ► HIV & SIV, CD8 T Cell Responses to). Studies evaluating the kinetics of acute phase mucosal CD8+ T-cell (► HIV & SIV, CD8 T Cell Responses to) responses have reported that these responses emerge “too little and too late” near the site of infection to prevent viral dissemination to draining lymph nodes (Haase 2011). HIV-1-specific CD8+ (► HIV & SIV, CD8 T Cell Responses to) and CD4+ T-cell (► HIV & SIV, CD4 T Cell Responses to) responses remain detectable in the reproductive tract throughout the chronic phase of infection (Shacklett and Greenblatt 2011).

Detailed characterization of HIV-1-specific T-cell responses throughout the female reproductive tract is lacking, mainly due to the difficulties inherent in obtaining sufficient material for study. Nevertheless, HIV-1-specific CD4+ and CD8+ T cells have been detected in both the upper and lower RT of women with chronic HIV-1 infection, as well as in cervical tissue of highly exposed, persistently seronegative (HEPS) women (Rodriguez-Garcia et al. 2013).

Several studies have reported the detection of HIV-1-specific immunoglobulin A (IgA) in cervicovaginal secretions from HEPS women. This IgA has been reported to neutralize primary HIV-1 strains in vitro and to block viral transcytosis across an intact monolayer of cultured epithelial cells. However, this topic remains controversial, as other studies of HEPS cohorts have failed to detect such antibodies in secretions (Shacklett and Greenblatt 2011).

Hormonal influences on HIV-1 susceptibility. Vaginal epithelial thickness varies through the menstrual cycle: thickness of the vaginal epithelium peaks at ovulation in response to the surge in estradiol (Wira and Fahey 2008). In postmenopausal women, loss of estradiol leads to permanent thinning of the vaginal epithelium. Changes in the vaginal epithelial barrier may contribute to an increased risk of HIV-1 transmission among older women; however, this has not been proven.

Immune responses in the upper and lower FRT are affected differently by hormonal fluctuations during the menstrual cycle. In the lower tract,

cytotoxic T-cell (CTL) activity remains relatively constant throughout the cycle (Wira and Fahey 2008). However, levels of IgG and IgA in cervical secretions decline by 10–100-fold at mid-cycle, immediately after ovulation, and increase again near the end of the cycle. Soluble mediators of innate immunity, including lactoferrin, SLPI, and HBD2, also decline at mid-cycle and rebound late in the cycle just before menses. Taken together, these observations suggest a potential window of vulnerability in the lower reproductive tract at mid-cycle and during the secretory (luteal) phase due to suppressed immunoglobulin production and innate responses (Wira and Fahey 2008).

In the upper reproductive tract, CTL responses vary during the menstrual cycle. Under hormonal influence, lymphoid aggregates form in the uterine endometrium, consisting of a B-cell core surrounded by CD8⁺ T cells and macrophages. CTL activity within these aggregates is greatest during the proliferative phase, but the aggregates attain their maximal size, with reduced CTL activity, during the secretory phase. This finding suggests particular vulnerability of the upper tract to infection during the secretory phase due to suppressed cell-mediated immunity (Wira and Fahey 2008). These lymphoid aggregates are absent from postmenopausal women, whose uterine CTL activity is more robust than that of premenopausal women.

Hormonal contraception and HIV-1 acquisition. In rhesus macaques, progestin application increases susceptibility to vaginal SIV transmission, likely by promoting thinning of the vaginal epithelium. However, the effects of progestins on human vaginal epithelial thinning are reportedly less pronounced (Rodriguez-Garcia et al. 2013; Xu et al. 2013). Several studies have assessed whether use of progestin-based contraceptives affects HIV-1 susceptibility in women. Literature on this topic remains controversial, and interpretation of available data has been complicated by differences in study design. Some studies have raised concern about an association between use of depot medroxyprogesterone acetate (DMPA) and increased risk of HIV-1 transmission; however, this finding has not been universal (Polis and Curtis 2013). Further studies will be required to

firmly establish the relationship between hormonal contraception and the risk of acquiring HIV-1 and other STI.

Male Reproductive Tract

Sites of HIV-1 entry. In men, HIV-1 transmission via the genital tract can occur via the foreskin, where numerous T cells, macrophages, and dendritic cells are located. Early studies to investigate the specific sites of entry via the male reproductive tract focused on the inner foreskin, which was believed to have a thinner layer of keratin than the outer foreskin, rendering it more susceptible to infection. However, subsequent studies found no difference in thickness of the keratin layer between inner and outer foreskin (Dinh et al. 2010).

Two recent studies tested the infectability of penile explants acquired from individuals undergoing gender reassignment surgery. In the first, explants of foreskin, glans, meatus, and urethra were found susceptible to infection with HIV-1 BaL, an R5-tropic strain (Anderson et al. 2011). In the second, urethral macrophages were susceptible to infection with cell-associated HIV-1 (Ganor et al. 2013). Thus, these studies confirm the infectability of sites other than the foreskin within the male RT. However, the male reproductive tract remains understudied in HIV-1 transmission and pathogenesis, and further studies are needed to better elucidate the sites of initial infection, virus production, and immune responsiveness to infection (Anderson et al. 2011).

Immune defenses. Human foreskin and penile urethral tissue express mucin genes, as well as soluble mediators of innate immunity including SLPI, lactoferrin, lysozyme, and interferon-beta. The urethral mucosa contains numerous cytotoxic CD8⁺ T cells. HIV-1-specific CD8⁺ T cells have been detected in semen of men with chronic HIV-1 infection, but neither the presence nor the frequency of these cells correlated with reduced levels of HIV-1 RNA in semen (Sheth et al. 2005). The urethral mucosa also contains plasma cells secreting IgA, IgM, and to a lesser extent, IgG (Anderson et al. 2011). Compared with studies of

the female reproductive tract, relatively few studies have explored immune responses of the male reproductive tract, and there remain major gaps in our understanding of this important site of infection (Anderson et al. 2011).

Circumcision (► [Medical Male Circumcision, Infant Circumcision, Prevention of HIV](#)). Early epidemiological evidence from sub-Saharan Africa suggested that male circumcision might reduce the risk of HIV-1 acquisition. Subsequently, three randomized trials were conducted in sub-Saharan Africa (South Africa, Kenya, and Uganda, respectively), to test whether circumcision of adult heterosexual males reduced their risk for acquisition of HIV-1. In all three trials, circumcision reduced the participants' risk of HIV-1 acquisition by 50–60% (reviewed in (Anderson et al. 2011)).

The protective effect of foreskin removal is thought to be due in part to the presence of HIV-1 target cells in the foreskin epithelium and in part to trapping of virus and infected cells by the foreskin, facilitating acquisition through the foreskin and the other sites that it covers. Circumcision also reduces the prevalence of other STI that can enhance HIV-1 acquisition due to increased levels of genital inflammation. However, studies to evaluate whether male circumcision reduces transmission of HIV-1 to female sexual partners have not demonstrated a similar protective effect (Weiss et al. 2009), and observational studies to determine the effects of circumcision on transmission among men who have sex with men (MSM) have yielded mixed results.

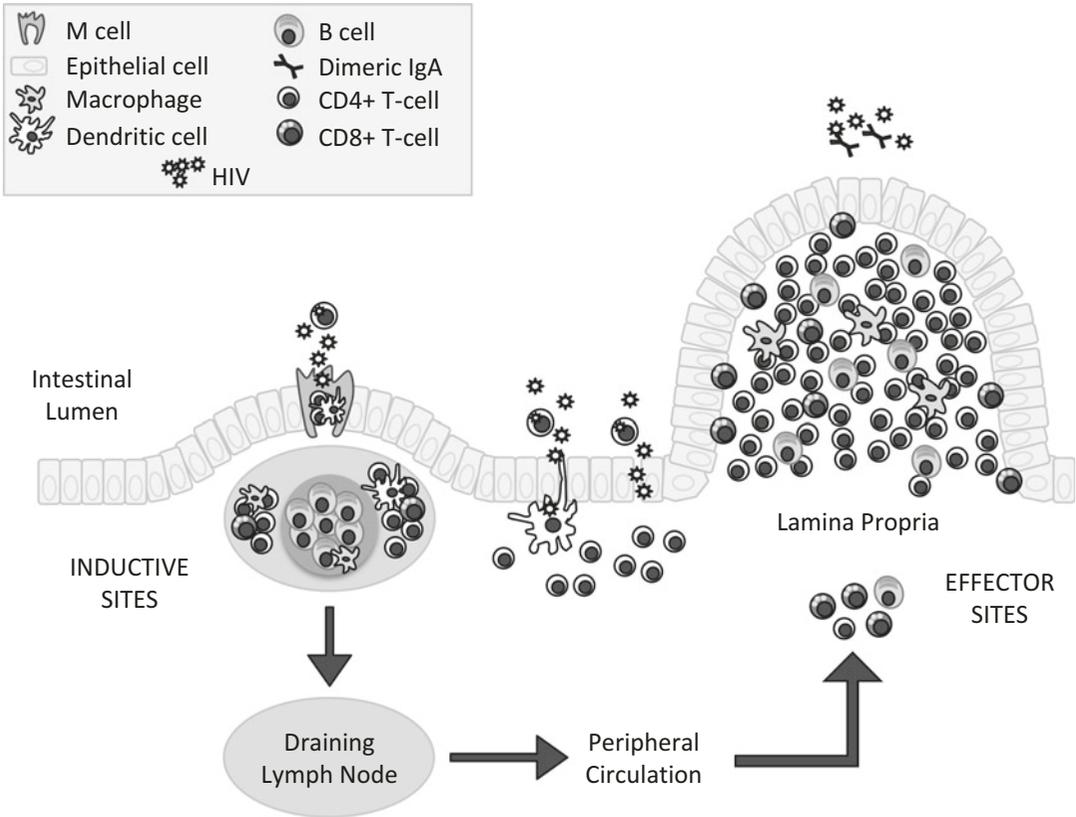
The Gastrointestinal Tract

Background. The gastrointestinal tract has been characterized as the largest lymphoid organ in the body, housing a majority of the body's lymphocytes as well as a large array of commensal organisms. Because of its role as an immunological barrier, in contact with dietary antigens and microbial flora, the GI tract maintains a tolerogenic environment; nevertheless, it must also be able to mobilize the defenses necessary for rapid response to mucosal pathogens. AIDS-associated

diarrhea and wasting syndromes were among the most common clinical features documented in the early years of the HIV/AIDS epidemic. Subsequent research revealed that the GI tract is a major site of viral replication, CD4+ T-cell depletion, and epithelial disruption irrespective of the route of initial HIV-1 infection. Furthermore, recent studies have provided evidence linking gut epithelial barrier dysfunction and microbial translocation to the generalized immune activation that has long been associated with HIV-1 disease.

Rectal transmission (► [Men who have sex with men \(MSM\)](#)). HIV-1 transmission frequently occurs during anal intercourse; however, as for other sites of HIV-1 transmission, the precise mechanisms leading to viral penetration of the GI mucosa remain unclear (Shacklett and Anton 2010; Fig. 2). During receptive anal intercourse, microabrasions and breaches can form in the simple columnar epithelium lining the rectal mucosa, allowing virus to access susceptible T cells in the underlying lamina propria. Intraepithelial dendritic cells may also bind virion particles and transfer them to nearby CD4+ T cells. Alternatively, virions may be taken up by intestinal epithelial cells and/or M cells via transcytosis and subsequently transmitted to CD4+ T cells. Several of these possible mechanisms are illustrated in Fig. 2. A recent study used SPECT/CT imaging of radiolabeled surrogates of cell-free and cell-associated HIV-1 to monitor movement of these surrogates in the distal GI tract during simulated intercourse. The radiolabeled molecules reached their peak concentrations at a distance of 10–20 cm from the anal verge, corresponding to the rectosigmoid junction (Louissaint et al. 2012).

Gut CD4+ T-cell depletion. Regardless of the initial pathway for viral penetration, the predominant target cell for productive HIV-1 infection in the GI tract is the CCR5+ CD4+ T cell. In the mid-1990s, several groups reported that CD4+ T cells in the gastrointestinal lamina propria underwent profound depletion during acute HIV/SIV infection. This depletion occurs rapidly, beginning 4–7 days postinfection, irrespective of the route of initial infection, as demonstrated by studies in rhesus macaques. The mechanisms driving CD4+ T-cell depletion include direct



Mucosal Immunity to HIV-1, Fig. 2 Idealized gastrointestinal mucosa. Inductive sites are organized structures, including Peyer's patches and lymphoid aggregates, where antigen presentation occurs. Effector sites are the epithelium and lamina propria. Intraepithelial lymphocytes are primarily CD8+ cytotoxic T cells, while the lamina propria is rich in activated memory CD4+ T cells expressing CCR5 that serve as targets for HIV. CD8+ T cells and antibody-secreting plasma cells (shown in legend as "B cells") are also found in the lamina propria. Lymphocytes primed in mucosal inductive sites move through efferent lymphatics

to the draining lymph nodes, eventually rejoining peripheral circulation at the thoracic duct. After circulating through peripheral blood, they selectively return to mucosal effector sites based on expression of specific chemokine receptors and integrins, as described in the text. HIV may infect the gastrointestinal mucosa by several mechanisms, including transcytosis across intact epithelial cells or M cells, by adhering to dendritic cells, or by passing through breaches in the epithelial layer (Figure redrawn and modified from Shacklett and Anton (2010))

viral infection and apoptosis of uninfected "bystander" cells (Shacklett and Anton 2010).

► **Th17 cells.** T helper cells secreting IL-17, known as Th17 cells, are a subset of CD4+ T cells that are important for mucosal defense against bacteria and fungi and also secrete cytokines that are critical for the maintenance of gut epithelial integrity (Cecchinato and Franchini 2010). However, Th17 cells can also contribute to inflammatory responses and have been implicated in the pathogenesis of human inflammatory bowel diseases (IBD). In humans, differentiation of Th17

cells requires expression of the transcription factor ROR γ t and exposure to polarizing cytokines including IL-23.

Th17 cells in the GI tract are rapidly depleted during acute HIV/SIV infection. This rapid loss may be due to preferential infection of Th17 cells; however, this point is controversial. Comparison of pathogenic and nonpathogenic models of SIV infection suggests that loss of these cells contributes to immune activation (Cecchinato and Franchini 2010). Nonpathogenic SIV infection of sooty mangabeys or African green monkeys is

characterized by retention of mucosal Th17 cells and absence of microbial translocation and immune activation. In contrast, pathogenic SIV infection of rhesus macaques leads to loss of intestinal Th17 cells, decreased intestinal barrier function, and reduced containment of bacterial infections such as *S. typhimurium* (Cecchinato and Franchini 2010). Partial restoration of intestinal Th17 cells has been reported in individuals on HAART (Cecchinato and Franchini 2010).

T_{reg} cells (► [Role of Regulatory T Cells during HIV Infection](#)). In contrast to the proinflammatory nature of Th17 cells, another subset of CD4⁺ T cells, termed regulatory T cells or T_{reg}, exerts anti-inflammatory functions and induces tolerance against “self” antigens. Like Th17 cells, T_{reg} are abundant in mucosal tissues. Their development is induced by expression of transcription factor Foxp3, retinoic acid, and cytokines including TGF- β . In the mouse, the pathway leading to T_{reg} development is considered a reciprocal pathway that can be polarized to induce either T_{reg} or Th17 development, depending upon the cytokine environment. In the context of HIV-1, T_{reg} may play a beneficial role by limiting harmful inflammation; however, evidence suggests that they also inappropriately suppress HIV-1-specific adaptive responses, thereby exacerbating the disease process. Accordingly, the balance between Th17 and T_{reg} may influence HIV-1 disease progression by modulating the equilibrium between inflammation and adaptive immunity in mucosal tissues.

Epithelial damage and microbial translocation (► [Microbial Translocation](#)). Systemic immune activation in HIV-1 infection has been recognized since the early days of the epidemic, but its etiology was unclear. This persistent activation is characterized by elevated plasma levels of proinflammatory mediators, high T-cell turnover, and elevated markers of T- and B-cell activation. Immune activation often persists despite successful reduction of viremia by antiretroviral therapy (ART) and is strongly predictive of morbidity. Indeed, individuals on ART remain at risk for many conditions, including cardiovascular disease, malignancy, and type II diabetes, which are associated with inflammation and immune activation.

Several studies have provided evidence that microbial translocation is a major contributor to the persistent immune activation observed in HIV-1 infection (Sandler and Douek 2012). First, numerous groups have reported elevated markers of microbial translocation in plasma of HIV-1-infected individuals as compared to healthy controls; these include bacterial lipopolysaccharide (LPS), soluble CD14 (sCD14), and bacterial 16S ribosomal DNA (rDNA). Second, in the SIV model (► [Non-Human Primate Models of HIV Transmission](#)), increased deposition of microbial products has been detected in tissues of rhesus macaques (which exhibit systemic immune activation and progress to disease following SIV infection), as compared to sooty mangabeys (which do not). Third, by several measures, gut epithelial barrier integrity is impaired in HIV-1 infection; these include loss of tight junction proteins, increased enterocyte apoptosis, increased plasma levels of intestinal fatty acid-binding protein (I-FABP), and increased villous atrophy. Taken together, these findings led to the hypothesis that impaired intestinal barrier function in HIV-1 infection contributes to increased translocation of commensal microbial products from the intestinal lumen into the circulation (Sandler and Douek 2012). These findings may lead to therapeutic interventions that can be used to complement HAART in HIV-1-infected individuals.

Cytotoxic T cells (CTL) in the GI tract (► [HIV & SIV, CD8 T Cell Responses](#) to). Several reports have addressed the nature of HIV-1-specific T-cell responses in the gastrointestinal tract during chronic infection, with most studies focusing on immune responses in sigmoid colon and/or rectal mucosa (Shacklett and Anton 2010). Generally, the breadth and specificity of CD8⁺ T-cell responses were demonstrated to be similar in gut mucosa and peripheral blood. In some studies, polyfunctional Gag-specific rectal CD8⁺ T-cell responses were positively associated with CD4⁺ T-cell count and inversely related to plasma viral load, suggesting a relationship between strong mucosal responses and immune control. In one such study, polyfunctional Gag-specific CD8⁺ T cells were significantly more abundant in rectal mucosa of “elite controllers,” rare

individuals able to contain HIV-1 viremia in the absence of antiretroviral therapy, than in “non-controllers,” individuals with viral load greater than 10,000 copies/mL of plasma. Similarly, gut CD4⁺ T-cell responses in elite controllers were unusually robust and polyfunctional. Not surprisingly, elite controllers showed relative preservation of gastrointestinal CD4⁺ T cells in contrast to typical progressors. MHC class I alleles HLA-B57 and B27 have been associated with HIV-1 control, and responses restricted by these alleles are also abundant in rectal mucosa of controllers. In individuals on HAART, gut HIV-1-specific CD4⁺ and CD8⁺ T-cell responses are generally weak to undetectable and tend to be monofunctional rather than polyfunctional (Shacklett and Anton 2010).

Gut B cells in HIV-1 infection (► [HIV & SIV, B Cell Responses](#) to). The effects of HIV-1 infection on antibody-producing cells in the gastrointestinal tract have not been extensively studied. As noted above, conflicting reports exist concerning the detection of HIV-1-specific IgA in mucosal secretions of HEPS individuals. However, in chronically infected individuals, HIV-1-specific IgA is typically absent or present at very low levels in mucosal secretions as well as in plasma, and this finding has been associated with reduced proportions of IgA-secreting plasma cells in the gastrointestinal lamina propria (Shacklett and Anton 2010). Several mechanisms have been proposed to account for this defect. These include impaired isotype switching in mucosal inductive sites, possibly related to HIV-1 Nef expression; impaired interactions with ► [T follicular helper cells](#); impaired IgA secretion by gut epithelial cells; or impaired homing of IgA-producing plasmablasts to the gastrointestinal lamina propria (Chaoul et al. 2012).

Mucosal B-cell impairment likely begins during the acute phase of HIV-1 infection. Acute infection induces polyclonal B-cell activation in both blood and gut, stimulating the production of antibodies specific for non-HIV-1 antigens as well as autoantigens. Acute HIV-1 infection also leads to a loss of germinal center architecture in Peyer’s patches, with an abundance of apoptotic T and B cells. Further studies are needed to address

the extent of mucosal B-cell impairment in HIV-1 disease and the mechanisms driving this impairment.

Gut immune reconstitution on HAART (► [Antiretroviral Medications, Adult Care and Treatment](#)). The GI tract is a major target for HIV-1 and SIV replication and CD4⁺ T-cell depletion. Although the extent of depletion can vary, studies in rhesus macaques have shown depletion of approximately 90% of gastrointestinal CD4⁺ T cells within the first weeks following infection [reviewed in (Shacklett and Anton 2010)]. Despite combination antiretroviral drug regimens that effectively reduce viral load to undetectable levels, reconstitution of CD4⁺ T cells in individuals on HAART is often incomplete. This may be due to a variety of factors, including patient compliance, drug pharmacokinetics and pharmacodynamics, persistent immune activation and disruption of lymphoid tissue architecture, and disruption of mucosal T-cell homing pathways.

A large number of cross-sectional and longitudinal studies have been performed to determine the extent and kinetics of mucosal CD4⁺ T-cell reconstitution in subjects on HAART (Costiniuk and Angel 2012). Early studies focused mainly on individuals with chronic and advanced infection, while more recent studies have tested whether more substantial T-cell recovery can be accomplished when HAART is initiated during acute or early infection. Outcomes have varied, with the extent of reconstitution varying from extremely low to virtually complete. For a detailed review of this topic, see Costiniuk and Angel (2012).

A consequence of increased immune activation in HIV-1 infection is the deposition of collagen fibrils in lymphoid nodes and the gastrointestinal tract (► [Collagen Deposition and Fibrosis](#)). Collagen deposition disrupts normal lymphoid tissue architecture and inhibits the cell-cell interactions that would normally drive the reconstitution process in patients on HAART. As compared to peripheral lymph nodes, CD4⁺ T-cell depletion in gut of individuals with chronic HIV-1 infection was found to correlate with the magnitude of collagen deposition. HAART did not completely restore gut CD4⁺ T cells, but treatment during early infection led to better restoration of CD4⁺

T cells with a central memory phenotype (Costiniuk and Angel 2012).

Mucosal homing may play an important role in gut CD4⁺ T-cell reconstitution during HAART. T cells primed in mucosal inductive sites, such as Peyer's patches and mesenteric lymph nodes, are programmed to express adhesion molecules and chemokine receptors that direct their eventual return to mucosal effector sites including the gut lamina propria. These molecules include integrin $\alpha\beta 7$, whose ligand is mucosal addressin cell adhesion molecule-1 (MAdCAM-1), and CCR9, a chemokine receptor whose ligand is thymus-expressed chemokine (TECK) or Ck β -15/CCL25. MAdCAM-1 is expressed on high endothelial venules in mucosal tissues, and CCL-25 is constitutively expressed in human small intestine but not in the colon. If expression of any of these molecules is perturbed, mucosal T-cell homing may be reduced (Costiniuk and Angel 2012).

Oral Mucosa and HIV-1 Transmission

Adults. Evidence from a variety of published studies suggests that the risk of HIV-1 transmission via oral-genital contact between adults is significantly lower than that associated with genital-genital or genital-anal sexual contact. Several factors likely contribute to the low risk of oral transmission. These include the structure of the buccal mucosa, which consists of a multilayered squamous epithelium containing relatively few CD4⁺ T cells, as well as the presence of numerous innate immune factors present in saliva that can inactivate the virus. HIV-1-specific antibodies are also detected in saliva. For a detailed review of this topic, see Campo et al. (2006).

Perinatal transmission (► [Mother to Child Transmission of HIV-1: Role of Receptor Usage and Target Cells](#)). Unfortunately, significant breast-feeding increases the risk of HIV-1 transmission from mother to child. Antiretroviral therapy administered to the mother around the time of delivery significantly decreases the likelihood of mother-to-child transmission, but does not provide protection from transmission via breast-feeding. The site of transmission in the infant is

believed to be the upper intestine, as infants have a neutral gastric pH. For a review of this topic, see John-Stewart et al. (2004).

HIV-1 Prevention and Mucosal Immunity

Vaccines to induce mucosal immunity (► [HIV-1 Transmission Blocking Vaccines; How Feasible Are They?](#)). Studies in the SIVmac model, cited above, suggest that induction of a high frequency of HIV-1-specific CD8⁺ T cells in mucosal tissues, translating to a high ratio of effector to target cells in vivo, could result in protection from mucosal exposure. Other studies have demonstrated that passive transfer of neutralizing antibodies, either via vaginal administration in a microbicide preparation or systemically via the bloodstream, can protect from intravaginal SIV challenge. However, with one exception, the human HIV-1 vaccine trials performed to date have failed to provide encouraging protection. Notably, in the Phase IIb STEP/Phambili trial (HVTN 502), a replication-defective adenovirus type 5 vector expressing HIV-1 Gag, Pol, and Nef genes failed to protect from infection or reduce viral loads in vaccinees that seroconverted. However, the recent RV144 trial, utilizing a prime-boost strategy involving a "prime" with canarypox vector expressing HIV-1 proteins followed by a boost with HIV-1 Env protein, provided 31% protection in a population of low-risk volunteers. The mechanisms of protection in this study are not fully understood, however, and mucosal immune responses were not measured. This trial nevertheless provided much-needed encouragement to the HIV prevention field (Bass 2013).

To date, two vaccine approaches have provided significant protection from pathogenic challenge in rhesus macaques: a live-attenuated virus derived from SIVmac with a deletion in Nef and a vaccine vector derived from rhesus cytomegalovirus (CMV) engineered to express SIV proteins (Picker et al. 2012). Both systems appear to induce widespread cell-mediated immune responses in tissues, including mucosal sites. Impressively, the CMV-based vaccine was shown to result in clearance of virus from 50% to 70% of challenged animals (Picker et al. 2012).

Microbicides (► **HIV-1 Transmission Blocking Microbicides**). To date, clinical microbicide trials have also met with mixed success. The CAPRISA 004 study, a Phase IIb trial performed in South Africa, found that vaginal application of a gel containing 1% tenofovir (an inhibitor of HIV-1 reverse transcriptase) was associated with a 39% reduction in HIV-1 acquisition risk overall and a 54% reduction in risk among highly adherent users when applied within 12 h before and 12 h after sex. In contrast, the VOICE study, which tested daily vaginal application of 1% tenofovir gel, was prematurely halted due to futility. Poor adherence in the VOICE study has been cited as a likely explanation for the outcome (Bass 2013). Many additional trials are underway, and new microbicide compounds, formulations, and delivery systems are under development for use in both women and men (Bass 2013).

Conclusions

As an infectious agent spread primarily through sexual contact, HIV-1 is a mucosal pathogen, and the study of HIV disease has provided clinicians and researchers with renewed awareness of the central importance of mucosal immunity in host defense. Although promising advances have been made toward an HIV “cure,” this goal remains elusive for the vast majority of HIV-1-infected individuals worldwide. Given this, intensive efforts are currently directed toward preventing transmission and providing treatment to those already infected. The next phase of “research for prevention” must continue to build knowledge in the field of mucosal immunology and apply the insights gained toward the development of more effective vaccines, microbicides, and antiretroviral drugs.

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Mucosal Pathogenesis in SIV Infection

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Definition

Mucosal pathogenesis is central to HIV infection and encompasses all the events triggered by HIV infection at the mucosal sites. The major virus targets are memory CD4⁺ T cells, which are

mainly presented at the mucosal sites. Thus, mucosal sites are the main sites of virus replication and CD4⁺ T cell depletion, with the majority of memory CD4⁺ T cells being whipped out from the mucosal sites in just 3 weeks from infection. This major immunological insult is further fueled by increased mucosal inflammation which results in breaches of the mucosal barrier, the translocation of intestinal microbes and microbial products from the intestinal lumen to the gut submucosa and then into the general circulation leading to chronic mucosal and systemic inflammation and immune activation that further fuel viral replication, mucosal damage, microbe release and ultimately result in HIV disease progression. As such, virus-host interactions should be targeted mostly at the mucosal sites in order to counteract the deleterious consequences of HIV infection.

Overview

Simian immunodeficiency virus (SIV) effects on the gastrointestinal tract are at the core of AIDS pathogenesis (Veazey et al. 2001; Dandekar 2007). The virus infects and kills activated memory and effector CD4⁺ T cells expressing the viral co-receptor CCR5, which are the major CD4⁺ T cell subset in the lamina propria of the gut and at the mucosal sites in general (Veazey et al. 2001). CD4⁺ T cell depletion in the gastrointestinal tract is therefore a hallmark of SIV infection. Similar to HIV-1 infection, in SIV-infected non-human primates, CD4⁺ T cell depletion occurs early, is substantial, and, if persisting through the chronic stage, is predictive for the clinical outcome of SIV infection. Mucosal CD4⁺ T cell depletion is also associated with chronic local immune activation and inflammation that trigger mucosal lesions and enteropathy. These processes damage the mucosal barrier and lead to leakage of the gut microflora into the general circulation, termed “microbial translocation.” This microbial translocation is a major cause of systemic immune activation and inflammation, which are key drivers of non-AIDS comorbidities, accelerated aging, and SIV disease progression. As such, gut dysfunction is a cornerstone feature of

HIV/SIV infection in driving disease progression (Brenchley et al. 2006).

Use of nonhuman primate models was critical for establishing this new paradigm in which the impact of HIV infection on the mucosa is a core determinant of HIV infection outcome (Dandekar 2007). The reports on the massive rapid depletion of the CD4⁺ T cells at the mucosal sites in nonhuman primates preceded the first observations in humans by a decade. Detailed comparative studies facilitated by invasive sampling at key time points of infection in multiple animal models with different outcomes of SIV infection (Fig. 1) permitted this major paradigm shift in AIDS pathogenesis. Furthermore, current studies in nonhuman primate models are instrumental for developing new therapeutic strategies aimed at limiting mucosal pathology, controlling microbial translocation and immune activation, and improving the overall prognosis of HIV infection.

Mucosal CD4⁺ T Cell Depletion During SIV Infection: Characteristics and Significance

Initial studies of the pathogenic SIV infection in macaques showed that massive depletion of mucosal CD4⁺ T cells occurred immediately after the peak of viral replication, with the majority of the mucosal CD4⁺ T cells being killed at mucosal sites in less than 3 weeks from the time of infection (Fig. 1a). These observations generated tremendous enthusiasm in the field and focused the research on understanding the events occurring during the first 3 weeks of infection.

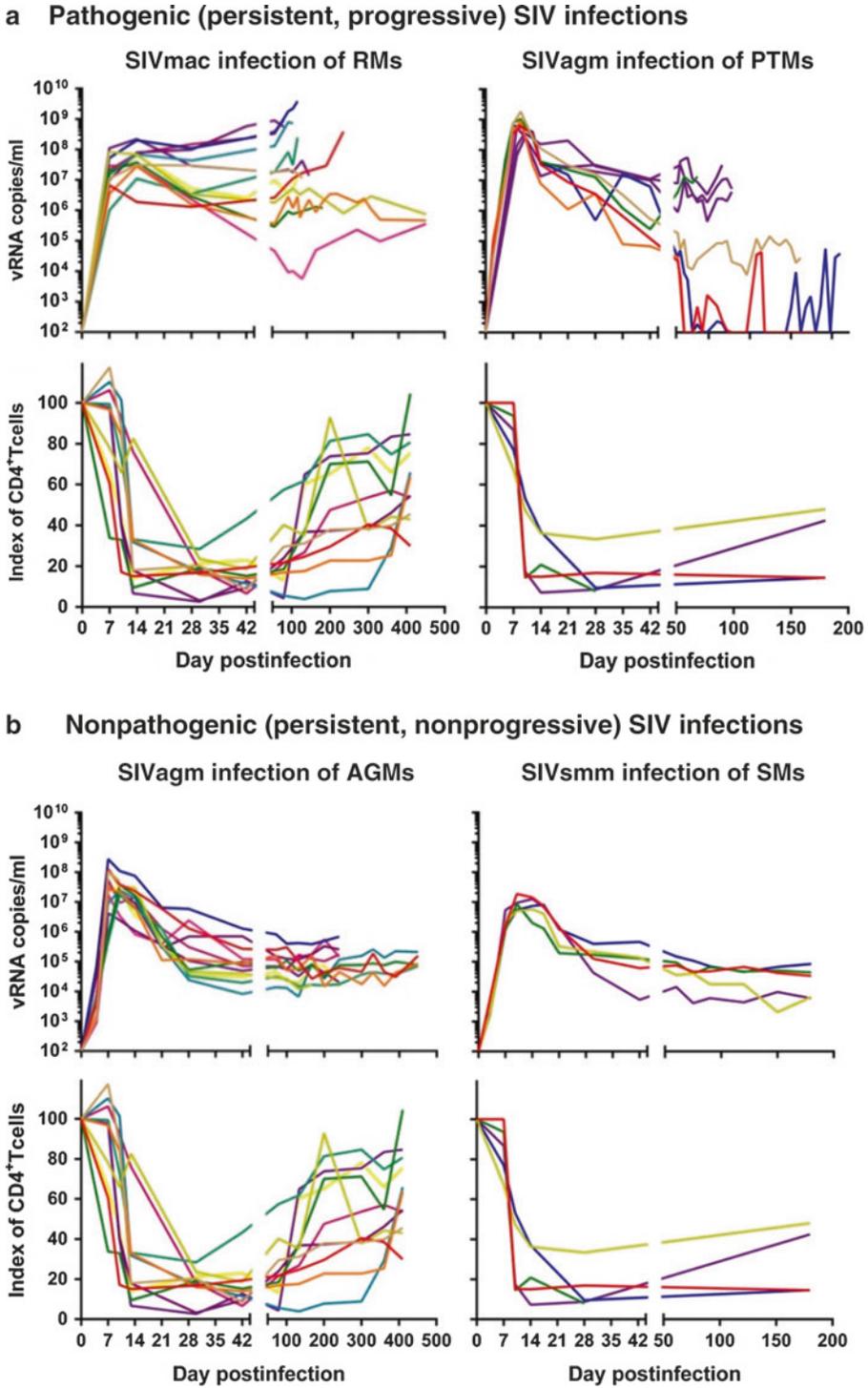
A more detailed understanding of mucosal HIV pathogenesis (Pandrea and Apetrei 2010; Grossman et al. 2006) was achieved with the inclusion in the overall picture of SIV pathogenesis of other nonhuman primate models with different outcomes of SIV infection. Comparative studies in various nonhuman primate hosts infected with an array of SIV strains, such as progressive SIV infections of rhesus macaques and pig-tailed macaques (Fig. 1a), persistent nonprogressive infections of the African nonhuman primates that are natural hosts of SIV (such as

African green monkeys and sooty mangabeys) (Fig. 1b), and spontaneously controlled SIV infections (such as the SIV_{agm} infection of rhesus macaques) (Fig. 1c), showed that massive CD4⁺ T cell depletion occurs in every pathogenic scenario, and therefore, acute mucosal CD4⁺ T cell depletion is not predictive for the virulence of SIV infection (Fig. 1). Instead, mucosal CD4 depletion that persists in chronic infection is predictive of disease progression and development of simian AIDS.

The initial mucosal CD4⁺ T cell depletions associated with SIV infection are (i) rapid (occurring within the first 3 weeks of infection); (ii) profound (impacting over 95% of the total population of mucosal CD4⁺ T cells and virtually the totality of SIV target cells, i.e., memory cells expressing the CCR5 co-receptor); (iii) exceeding the proportion of SIV-infected cells (thus suggesting that the mechanism of depletion is not only through direct effect of the virus, i.e., pyroptosis, but probably through additional mechanisms, such as bystander apoptosis); and (iv) persisting throughout the initial stages of chronic infection, irrespective of the virological and clinical outcome. As such, while the circulating CD4⁺ T cells are rapidly restored to near preinfection levels with the transition to chronic infection, the restoration of the CD4⁺ T cells at the mucosal sites is virtually nonexistent during the first months of chronic infection (Pandrea and Apetrei 2010; Okoye and Picker 2013).

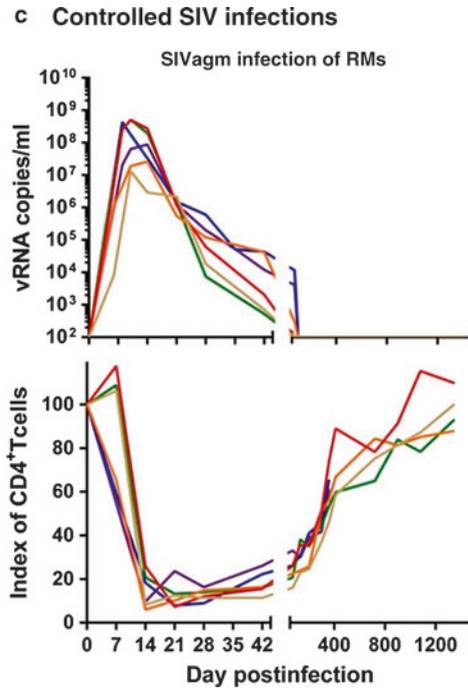
This disproportionate impact of the virus on the CD4⁺ T cells from the lamina propria of the gastrointestinal tract compared to those in circulation or in the lymph nodes supports the concept that the gastrointestinal tract is the primary site of virus replication and CD4⁺ T cell infection. Indeed, high levels of viral replication were documented in the gastrointestinal tract of acutely SIV-infected RMs, suggesting that a significant proportion of the mucosal CD4⁺ T cells are directly depleted by the virus.

Comparative studies between different animal models of pathogenic, nonpathogenic, and controlled infection also permitted identification of additional characteristics of CD4⁺ T cell depletion



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Mucosal Pathogenesis in SIV Infection, Fig. 1 (continued)



Mucosal Pathogenesis in SIV Infection, Fig. 1 Comparative dynamics of viral loads and mucosal CD4⁺ T cell depletion in (a) pathogenic, (b) nonpathogenic, and (c) controlled infections. Plasma viral loads (measured in viral RNA copies/ml) are depicted in parallel to the CD4⁺ T cell counts in the duodenal biopsies (expressed as an index of CD4⁺ T cells, i.e., changes from the preinfection levels). (a) Pathogenic models are represented here by the highly pathogenic SIVmac infection of rhesus macaques (RMs) and the SIVagm infection of pig-tailed macaques (PTMs), which has variable pathogenicity upon direct cross species

transmission but uniformly high pathogenicity upon serial passage (serially passed infections being depicted in violet). (b) Nonpathogenic models are represented by natural infections in African nonhuman primates, illustrated here by SIVagm infection in African green monkeys (AGMs) and SIVsmm infection of sooty mangabeys (SMs). (c) Controlled infections are represented here by macaque infection with cross species-transmitted SIVagm (that naturally infects AGMs). The X axis illustrates the duration of infection, while the Y axis illustrates the levels of viral loads or the levels of CD4⁺ T cell depletion at the mucosal sites

that are actually predictive for the outcome of SIV infection: (i) Depletion is correlated with the levels of acute viral replication, being minimal in animals in which the peak of viral load is below 10^6 viral RNA copies/ml of plasma. (ii) Restoration of mucosal CD4⁺ T cells is incomplete and tardive in progressive disease models. During the early stages of chronic pathogenic SIV infections, there is a relatively modest recovery of up to 10–25% of the preinfection levels, similar to HIV-1-infected patients (Fig. 1a). (iii) The degree of chronic restoration and preservation directly correlates with the levels of viral set point and the outcome of infection in pathogenic hosts (Fig. 1a, PTMs), with the best predictor of a better

clinical outcome being the restoration of memory CD4⁺ T cells (Grossman et al. 2006). In rapid progressor macaques, in which there is virtually no viral control, there is no recovery of CD4⁺ T cells at the mucosal sites (Fig. 1a).

Differently from pathogenic infections, in natural hosts there is a partial restoration of mucosal CD4⁺ T cells to up to 50–70% of the baseline levels during the chronic SIV infection (Fig. 1b) (Sodora et al. 2009). This restoration occurs independently of viral loads, in the context of the complete control of chronic inflammation and immune activation which is pathognomonic to nonpathogenic infections. This partial recovery of mucosal CD4⁺ T cells is not immediate, being

observed only after a few months of chronic infection (Fig. 1b).

Finally, spontaneously controlled SIV infections (that are equivalent to human elite controllers) associate a robust restoration of mucosal CD4⁺ T cells, with the caveat that complete restoration can only be observed years after viral replication, and residual immune activation and inflammation are completely controlled, a period which is necessary to enable healing of the mucosal lesions inflicted during the replicative stage of SIV infection (Fig. 1c).

In conclusion, mucosal depletion of CD4⁺ T cells occurs rapidly, is massive, and is a common feature of pathogenic, nonpathogenic, and controlled SIV infection. Therefore, acute mucosal CD4⁺ T cell depletion is likely not predictive for the outcome of SIV infection. Conversely, CD4⁺ T cell restoration during chronic infection is highly indicative of favorable clinical outcome. Restoration is slow and incomplete and depends on both the levels of viral replication and the amount of immune activation and inflammation. A partial CD4⁺ T cell restoration is possible even if viremia is not completely controlled, allowing survival and lack of disease progression in non-human primates that control immune activation and inflammation to preinfection levels. However, CD4⁺ T cell recovery may be complete only in the animals that control both viral replication and immune activation, but restoration of CD4⁺ T cells at the mucosal sites is a very long process.

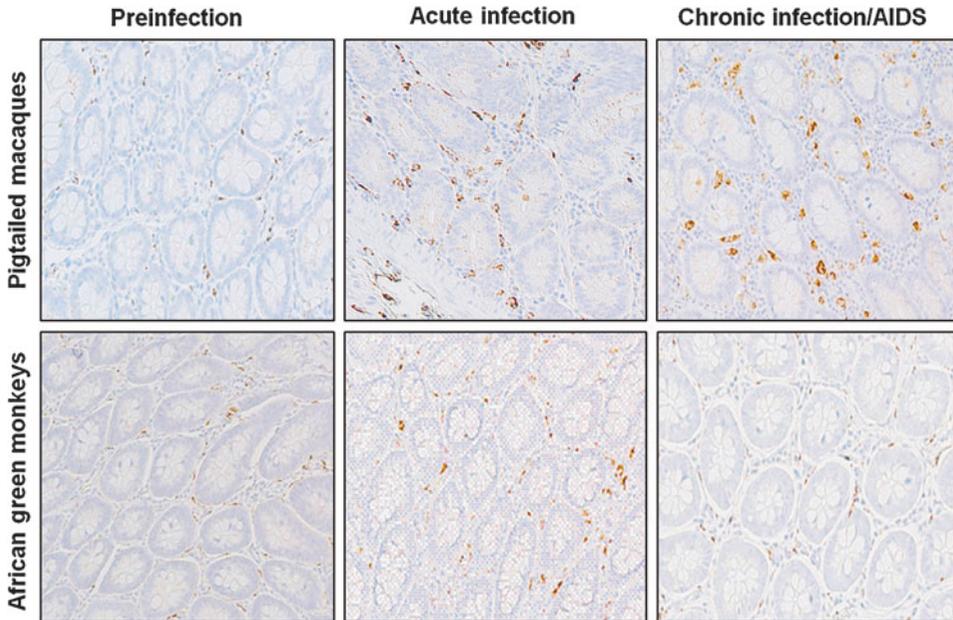
Immune Alterations in the Mucosal Tract During SIV Infection of Nonhuman Primates

The very active interactions between the virus and its target cells at mucosal sites trigger key pathogenic features of chronic SIV/HIV infection leading to disease progression.

Mucosal CD4⁺ T cell depletion involves all T cell subpopulations, yet there are subsets that appear to be particularly impacted by SIV infection (Okoye and Picker 2013). First, CD4⁺ T cells that express the CCR5 chemokine receptor are preferentially depleted. This is not surprising, as

CCR5 is the major co-receptor for both HIV and SIV. With the majority of the CD4⁺ T cells at the mucosal sites expressing CCR5 and CCR5-expressing CD4⁺ T cells having a memory phenotype, this explains the major impact of HIV/SIV on the mucosal memory CD4⁺ T cells. Memory cells being the preferential targets of HIV infection, depletion is more prominent at the effector sites, such as the lamina propria, while CD4⁺ T cells from the inductive sites (i.e., the Peyer patches) are less impacted by HIV infection (Veazey et al. 2001).

With regard to CD4⁺ T cell functionality, CD4 cells capable of producing IL-17 (Th17 cells), which are the cell subsets responsible for maintaining mucosal integrity, also appear to be preferential targets for infection (Hartigan-O'Connor et al. 2011; Paiardini et al. 2008). Therefore, most of the pathological features associated with CD4⁺ T cell depletion in the gut are related to the preferential loss of Th17 cells, which have a critical contribution to the immune dysfunction observed during pathogenic SIV infection: (i) Loss of Th17 cells results in a reduced production of IL-17 and IL-22, two cytokines that induce granulocyte colony-stimulating factors, which promote the recruitment of myeloid cells and neutrophils at the mucosal effector sites. (ii) Th17 cells are involved in the maintenance of mucosal integrity through induction of claudins, defensins, and mucins, which are components of the mucosal junctions and have antimicrobial activities, and as such, loss of Th17 has a direct impact on mucosal integrity. (iii) IL-22 produced by Th17 cells is involved in the division of epithelial cells. (iv) Low levels of Th17 cells are associated with increased expression by antigen-presenting cells of indoleamine-2,3-dioxygenase (IDO), an enzyme that is involved in the metabolization of tryptophan, and IDO metabolites directly inhibit Th17 cell differentiation (Brenchley and Douek 2008a). Increased levels of IDO are associated with decreased frequencies of CD103 antigen-presenting cells, which can induce Th17 cells. Altogether these features, which are specifically associated with pathogenic SIV infection and absent during the SIV infection of natural NHP hosts (in which Th17 cells are preserved), point to



Mucosal Pathogenesis in SIV Infection, Fig. 2 Changes in the mucosal macrophages in SIV_{sub}-infected African green monkeys versus pig-tailed macaques. Immunohistochemical staining of the CD68 cells from the mucosal sites demonstrate that macrophages accumulate in the lamina propria during the

acute stage of both progressive and nonprogressive SIV infections of macaques and African green monkeys, respectively. However, during the late stages of infection, the innate cells remain increased at mucosal sites only in the progressive infections of the macaques

a vicious circle that prevents maintenance of the Th17 population leading to the occurrence and enhancement of mucosal damage during the pathogenic SIV infection.

The impact of HIV/SIV infection on the innate immune cell populations at the mucosal sites is currently a subject of intense research. Progressive SIV infections are characterized by a reduction of both plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs) in both the peripheral blood and spleen and their homing to the gut. At the mucosal sites, both pDCs and mDCs were shown to release large amounts of proinflammatory cytokines, which are not produced by these cells in a normal mucosal environment. Due to their excessive immune activation, mDCs experience high rates of mortality. The same high rate of apoptosis and an altered functional profile are observed for the NKp44⁺ natural killer (NK) cells residing in the gut, an important innate effector population. As such, while the recruitment of innate immune cells to the gut

mucosa could facilitate virus control, the overproduction of cytokines and the high rate of mortality of these cells (which also triggers release of more inflammatory cytokines by surrounding cells) further enhance mucosal immune activation and inflammation. Interestingly, the recruitment of mDCs and macrophages to the mucosal sites also occurs during the nonprogressive SIV infections of the natural hosts or controller rhesus macaques (Fig. 2). This process is, however, only transient, is not associated with excessive production of inflammatory cytokines, and does not result in the death of these cells, strongly suggesting that the fate of the immune cell subsets and their functions in the gut is driven by the local environment. As such, the current view is that, being programmed to fight against the infections, the innate cells migrate to the gut in progressive, as well as in nonprogressive and controlled SIV infections. Yet, the innate cells become hyperactivated only in the pathogenic infections, due to their mucosal environment, which is altered by

both the virus and the translocated microbial products, and thus further fuel the inflammation, deepen the damage of the mucosal barrier, and contribute to the negative outcome of HIV/SIV infection.

Structural Alterations of the Gastrointestinal Tract Associated with SIV Infection

The SIV-associated immunological alterations at the mucosal sites result in structural and functional pathologies of the gastrointestinal tract. Virus replication, inflammation, and immune activation together with bystander apoptosis of the epithelial cells throughout the GI tract result in enterocyte loss and alterations of mucosal integrity (Brenchley and Douek 2008b).

There are multiple mechanisms of the enterocyte loss observed during HIV/SIV infections:

- (i) The virus itself can decrease glucose uptake by enterocytes through a Tat-mediated microtubule disruption;
- (ii) increased apoptosis of enterocytes through bystander effects, similar to other colitis (i.e., celiac disease).
- (iii) Increased production of proinflammatory cytokines, such as tumor necrosis factor (TNF) at the mucosal sites, is associated with increased apoptosis of the epithelial cells and perturbations of the tight epithelial barrier.

TNF-mediated enterocyte destruction was also reported to occur in subjects with inflammatory bowel disease. The increased inflammation and immune activation characteristic to chronic SIV infection are associated with enterocyte loss.

Enterocyte loss and subsequent intestinal alterations are associated with (i) low levels of serum citrulline (most of which is being produced by enterocytes); (ii) decreased ratio of the villous height/crypt depth (demonstrating atrophy); (iii) increased stem cell proliferation in the crypt (associated with malabsorption); (iv) increased

levels of intestinal fatty acid-binding protein (I-FABP), indicating enterocyte damage; and (v) abnormal enterocyte differentiation due to an impaired sodium glucose cotransport and to increased concentrations of intraepithelial calcium (Hartigan-O'Connor et al. 2011; Sandler and Douek 2012).

The massive destruction of the epithelial cells and decreased expression of the epithelial tight junction repair genes further result in focal breaches of the gastrointestinal epithelium. Alterations of the epithelial tight junctions are evidenced by discontinuous staining of the tight junction proteins in chronic SIV-infected rhesus macaques. These alterations in the tight junctions occur as early as 14 days post-infection, are the primary mechanism of the increased permeability of the epithelial barrier, and are clinically expressed as colitis, diarrhea, and malabsorption.

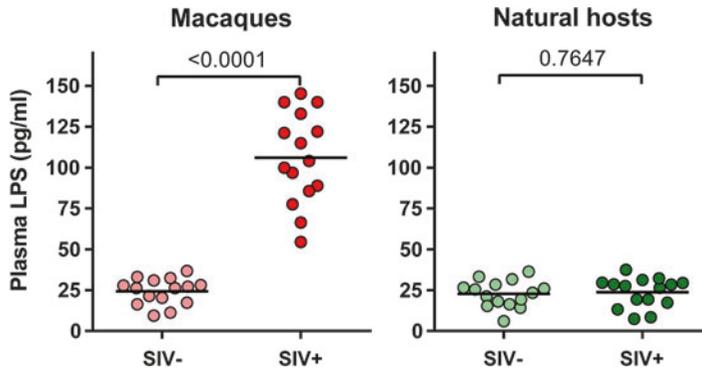
Villous atrophy and crypt hyperplasia were also reported in SIV-infected macaques and are associated with decreased levels of plasmacytes producing immunoglobulin A (IgA) leading to bacterial overgrowth.

These pathologies are specifically associated with pathogenic SIV infections in macaques and absent during SIV infections in African non-human primate hosts.

Microbial Translocation and Its Key Role in the Pathogenesis of AIDS

One of the most important consequences of the gut damage caused by loss of enterocytes and disruption of the tight junctions between the cells is the loss of barrier function of the gut and translocation of microorganisms from the intestinal lumen to the general circulation. This phenomenon is not specific to SIV/HIV infection, occurring in multiple clinical conditions in which mucosal epithelium is altered and gut permeability is increased (Brenchley and Douek 2012).

Microbial translocation is a key determinant of systemic chronic immune activation and inflammation, which are the most important drivers of SIV disease progression. The intestinal flora is large (approximately 10^{14} bacteria) and diverse,



Mucosal Pathogenesis in SIV Infection, Fig. 3 Comparative levels of microbial translocation in pathogenic and nonpathogenic SIV infections. (a) In rhesus macaques, the levels of microbial translocation significantly increase upon SIV infection as a result of the mucosal damage and are associated with persistent high

levels of systemic immune activation and inflammation. (b) In African green monkeys, the levels of microbial translocation are virtually unchanged upon SIV infection as a result of the maintenance of a healthy mucosal barrier. In these species, chronic immune activation and inflammation are controlled

and translocated microbial products include peptidoglycan, lipoteichoic acid, lipopolysaccharide (LPS), flagellin, ribosomal DNA (rDNA), and unmethylated CpG-containing DNA. These translocated microorganisms/products are bioactive, and they can induce potent proinflammatory responses through activation of a plethora of receptors [i.e., nucleotide-binding oligomerization domain 1 (NOD1) and NOD2, toll-like receptor 2 (TLR2), TLR4, TLR5, TLR6, and TLR9], which are expressed by multiple cell types, including innate immune cells (Sandler and Douek 2012). Therefore, microbial translocation can induce chronic activation of both adaptive and innate arms of the immune system and initiate a signaling cascade, leading to increased production of proinflammatory cytokines IL-1 β , IL-6, TNF, and interferons (Sandler and Douek 2012).

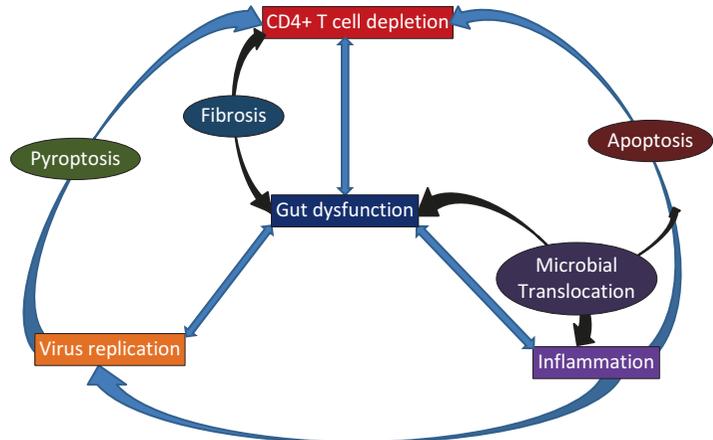
Microbial translocation is specifically associated with pathogenic SIV infection being nearly absent in African nonhuman primates that are natural hosts of SIV (Pandrea and Apetrei 2010) (Fig. 3), and studies in nonhuman primates have established a direct link between microbial translocation and chronic immune activation (Fig. 4). Chronically SIV-infected AGMs that do not progress to AIDS maintain a healthy mucosal barrier and lack evidence of microbial translocation, chronic immune activation, and systemic inflammation. However, intravenous administration of

LPS, either in single dose or in prolonged administration over a 3-week duration, resulted in increased levels of immune activation, inflammation, and coagulation markers. Similarly, alcohol or dextran sulfate administration to AGMs increased gut permeability, induced microbial translocation, and resulted in increased levels of immune activation and viral replication. Conversely, direct blockade of microbial translocation with sevelamer, a chelator of LPS in the gut, resulted in a significant reduction of systemic immune activation, inflammation, and coagulation markers. Altogether, these studies bring direct proof for the role of microbial translocation as a key determinant of immune activation and associated pathologies, such as non-AIDS comorbidities, in SIV infection (Fig. 4) (Deeks et al. 2013; Pandrea et al. 2015).

Due to the key role of microbial translocation in the pathogenesis of HIV/SIV infection, studies have also focused on characterization of the impact of infection on the gut microbiome. These studies failed to identify any significant alteration in the gastrointestinal bacteriome during the progressive SIV infection of macaques. These results are in disagreement with studies in humans reporting that dysbiosis, i.e., an altered balance in the composition of commensal microflora in the gut, is frequently observed in HIV-infected patients and is linked to immune

Mucosal Pathogenesis in SIV Infection,

Fig. 4 The vicious circle of gut dysfunction and its role in the pathogenesis of AIDS



activation. The significance of these differences between HIV-infected humans and SIV-infected macaques is not yet completely understood. Note, however, that the animal model allows for well-controlled sampling, at key time points of infection collected longitudinally, and in a context of a similar lifestyle and diet, with less impact from behavioral factors. These factors may significantly influence the way HIV and SIV infections alter the intestinal bacteriome and its role in driving the outcome of infection.

Gut viruses may also play a role in SIV-associated mucosal pathogenesis. A significant increase in the size of the fecal virome was reported to occur in the progressive SIV infection macaques, while no such change was detected in the non-progressive SIV infection of AGMs. Furthermore, potentially pathogenic viruses, such as adenoviruses, are specifically co-localized with the areas of structural damage of the gastrointestinal tract in progressively SIV-infected macaques.

Therapeutic Approaches Aimed at Limiting the Impact of Gut Dysfunction on the Outcome of HIV Infection

Administration of antiretroviral therapy (ART) dramatically improved the outcome of HIV infection and is one of the most effective medical achievements of modern medicine. In nonhuman primates, administration of ART has also been documented to improve the outcome of SIV

infection. However, the overall impact of ART on the chronic mucosal damage in HIV-infected humans is relatively limited, and resolving residual immune activation and inflammation in patients on ART is a major priority of current research. It is generally acknowledged that other therapies, in addition to ART, are needed to control mucosal damage and its consequences on the pathogenesis of AIDS.

There are multiple therapeutic options to limit the impact of gut pathology on the outcome of SIV/HIV infection (Pandrea and Landay 2012). Most of these approaches target microbial translocation. In addition to treatment with sevelamer, administration of prebiotics and probiotics to SIV-infected macaques increased frequency and functionality of antigen-presenting cells in the gut, enhanced reconstitution and functionality of CD4⁺ T cells, and reduced fibrosis of lymphoid follicles in the colon, suggesting that supplementing the intestinal flora may contribute to intestinal healing. Treatment of SIV-infected rhesus macaques with IL-21 resulted in preservation of intestinal Th17 cells and reduced microbial translocation. Administration of a combination of the antibiotic rifaximin and the anti-inflammatory agent sulfasalazine to acutely infected macaques resulted in a transient reduction in the plasma levels of sCD14, proinflammatory cytokines, and T cell activation and slightly ameliorated the CD4⁺ T cell decline. Further, monoclonal antibodies directed against the proinflammatory cytokines induced by NF- κ B activation may attenuate

immune activation. As an example, administration of an anti-TNF monoclonal antibody ameliorated some of the consequences of inflammation in SIV-infected macaques. Yet, as shown by recent studies of interferon and anti-interferon administration, blocking or boosting the cytokine responses is difficult and may lead to deleterious results. While none of the therapeutic interventions aimed at targeting the causes of chronic immune activation in chronically SIV-infected macaques completely reversed the pathological features triggered by SIV infection, the composite results of these studies show that targeting mucosal pathology has a real potential to improve the outcome of HIV infection and warrants additional studies.

Conclusion

Mucosal immune dysfunction is a key feature of the pathogenesis of SIV/HIV infection, being at the core of the vicious circle triggered by viral replication and followed by depletion of memory CD4⁺ T cells, mucosal inflammation, endothelial breaches, microbial translocation to general circulation and consequent chronic systemic inflammation and immune activation. In the current paradigm of HIV pathogenesis, each element of this vicious circle boosts the others, ultimately resulting in HIV disease progression. Strategies aimed at controlling progression of HIV infection should include interventions aimed at maintaining mucosal integrity and disrupting the vicious circle of AIDS pathogenesis.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [Microbial Translocation](#)
- ▶ [Mucosal Immunity to HIV-1](#)
- ▶ [Nonpathogenic SIV Infection of Sooty Mangabeys](#)
- ▶ [SIV Infection of African Green Monkeys](#)
- ▶ [SIVmac Infection of Macaques, Immunopathogenesis of](#)
- ▶ [Th17 Cells](#)

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Multicentric Castleman Disease

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Definition

Multicentric Castleman disease is a systemic disorder of lymph nodes characterized by inflammatory symptoms including fever, night sweats, cachexia, malaise, and lymphadenopathy together with laboratory abnormalities including anemia and hypoalbuminemia. Together these symptoms may be life threatening. The diagnosis is based on specific pathological findings in affected lymph nodes. In individuals infected with HIV, multicentric Castleman disease is almost exclusively caused by infection with a cancer-causing herpesvirus, Kaposi sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus 8). This may be considered a separate entity from the non-KSHV-related or idiopathic form of multicentric Castleman disease and from unicentric forms of Castleman disease. While these disorders have pathological and clinical similarities, the KSHV-related form is distinguished by its pathological characteristics and unique viral etiology.

Introduction

One of the earliest signs of the AIDS epidemic in the early 1980s was the recognition of an

increased occurrence of certain unusual tumors in the particular risk groups who also developed opportunistic infections. Tumors of lymphoid tissue were prominent among these, as was an unusual skin tumor called Kaposi sarcoma (KS). Along with common lymphoid cancers (lymphomas), by the 1990s, it was recognized that a hitherto uncommon lymphoid tumor called multicentric Castleman disease (MCD) was occurring at increased rates in people with HIV/AIDS. Following the discovery that a novel human herpesvirus, Kaposi sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus 8), was the causative agent of KS (Chang et al. 1994), it was soon appreciated that both a rare lymphoma and primary effusion lymphoma (PEL) and almost all cases of multicentric Castleman disease in people with HIV/AIDS were also caused by KSHV (Soulier et al. 1995).

Rather than being a true clonal malignancy or cancer, MCD is best considered a hyperproliferative state affecting mainly the lymph nodes and spleen. Its clinical features are those of uncontrolled systemic inflammation and lymphoid proliferation, especially fever, night sweats, cachexia, malaise, and lymphadenopathy. These occur together with laboratory abnormalities including anemia and hypoalbuminemia that are themselves at least in part consequences of systemic inflammation. In some cases, true clonal lymphomas may arise within lymph nodes affected by KSHV-MCD or in association with it (Oksenhendler et al. 1996). Until recently, outcomes for patients affected by this disorder were dismal. The time to survival from diagnosis of KSHV-MCD was usually only 1–2 years, as patients succumbed to the severe inflammatory symptoms or to secondary lymphomas. However, with the transformation of care of the underlying HIV/AIDS and an appreciation of the viral etiology and pathogenic mechanisms of KSHV-MCD, new therapeutic modalities are being developed that appear to be both improving short-term outcomes and reducing the long-term sequelae of KSHV-MCD, including secondary lymphomas.

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Clinical and Pathological Features of Multicentric Castleman Disease and Related Diseases: Historical Perspective and Current Understanding

Benjamin Castleman's name has been applied to a group of related but distinct disorders since his seminal description in 1954 of what is now known as unicentric Castleman disease, hyaline vascular variant (Waterston and Bower 2004). This initial description was of patients with a single (unicentric) slowly enlarging mass in the anterior chest (mediastinum), which on pathological examination demonstrated an unusual pattern of atrophy (technically involution) of the central portion of the lymph node follicles. This portion, called the germinal center, was instead replaced by protein material ("hyalinized") and an increase in local blood vessels. The cause of this disorder was, and remains, unknown. Patients rarely had systemic symptoms and responded to local approaches, specifically surgical resection or irradiation of the affected node.

Soon after this initial description, a group of patients with similar unicentric lymph node masses but distinct histopathological features was recognized. In these cases, the germinal center was less prominently abnormal. Rather, the affected nodes were characterized by sheets of mature lymphocytes (plasma cells) replacing the normal architecture of the node follicle and interfollicular spaces. Vascularity was again increased, and in some cases the germinal center was involuted or reduced in cell number. In some cases, these findings occurred in solitary sites away from the mediastinum. These patients were in most cases asymptomatic, but a proportion did exhibit systemic inflammatory symptoms (including fever, cachexia, sweats, and malaise) or hematologic cytopenias (anemia or thrombocytopenia). This disorder was named the plasma cell variant of Castleman disease.

Following this, it was further recognized that in a proportion of patients with Castleman disease – predominantly but not exclusively those with systemic inflammatory symptoms or cytopenias – more than one lymph node or group of nodes was affected by the same pathological process. Most,

but not all, multicentric cases had the histopathological features of the plasma cell variant. Thus the description of multicentric Castleman disease introduced an essentially clinical distinction, the presence of unicentric versus multicentric disease, to what had formerly been a histopathological classification and in doing so combined the two different pathological types (hyaline and plasma cell) under this new umbrella.

The etiology of these intriguing disorders remained elusive. In some cases, the nodal changes appeared to be secondary to an additional pathological process. Commonly this included lymphomas or certain slow-growing infections (including mycobacterial infection) occurring elsewhere, presumably giving rise to Castleman disease as a consequence of lymphoid growth factors being present in abnormal excess. In the 1970s and 1980s, a group of small molecules, now called cytokines, were discovered and found to act on lymphocytes to modulate their growth and inflammatory responses. It soon became evident that one such cytokine, interleukin 6 (IL-6), was responsible for many of the pathological and clinical findings of multicentric Castleman disease, as well as contributing to the clinical features of those with symptomatic unicentric disease (Yoshizaki et al. 1989). Notwithstanding the discovery of IL-6, the underlying cause of the IL-6 excess remains unexplained in most cases. Nonetheless, the development of inhibitors of IL-6 (antibodies to the molecule itself, or to prevent the molecule acting at its specific receptor) has led to the use of these agents as a therapeutic modality in patients with symptomatic idiopathic Castleman disease.

A further critical step in unraveling the etiology of many cases of MCD came with the discovery of KSHV. Because of its unusual epidemiology, including its tendency to occur in only some of the behavioral risk groups affected by HIV/AIDS, KS had long been suspected to be caused by an infectious agent. However, the agent remained a mystery until Chang and Moore identified novel herpes viral sequences in KS tissue (Chang et al. 1994). This virus was subsequently found in the affected lymph nodes in most cases of MCD in patients with HIV/AIDS and also in a minority of cases of MCD occurring

in persons without HIV. Further evidence for the role of KSHV in the etiology of these cases of MCD emerged with the recognition that symptomatic flares in these patients were associated with high levels of KSHV in the peripheral blood and pathological evidence of KSHV infection in the involved lymph nodes. Furthermore, these viral levels improved with treatment or disease remission (Oksenhendler et al. 2000).

Clinically, these KSHV-associated cases of MCD resembled their non-KSHV-associated counterpart. The disease course is characterized by intermittent flares of inflammatory symptoms as described above, together with widespread and often marked lymphadenopathy and prominent splenomegaly. Nonspecific gastrointestinal and respiratory symptoms are common, as are neurological symptoms including cognitive dysfunction and personality changes. Flares are often severe and can be fatal. Common laboratory abnormalities include anemia, thrombocytopenia, low serum albumin levels, low serum sodium, and elevated blood inflammatory markers such as C-reactive protein (CRP). The clinical course waxes and wanes, but until recently has generally been fatal within 2 years of diagnosis, with patients succumbing to the severe inflammatory syndrome and concurrent infections or progressing to lymphoma. The differential diagnosis of fever and adenopathy in the HIV-infected individuals is broad. As a result, KSHV-MCD may be difficult to diagnose and is often missed. Clinicians should be alert to the possibility of KSHV-MCD in patients with unexplained inflammatory symptoms and laboratory abnormalities resembling those described, and consider biopsy with adequate material (a substantial core or ideally excision of affected nodes) to establish the diagnosis.

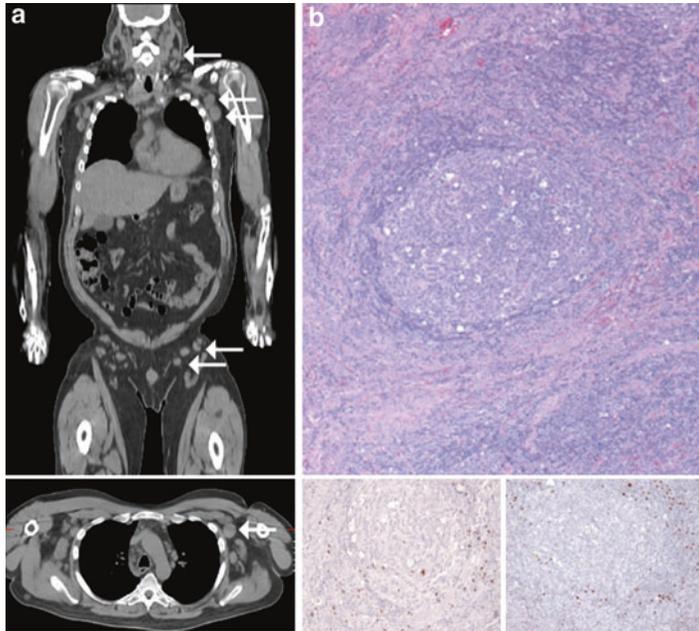
The pathology of KSHV-MCD bears substantial resemblance to idiopathic MCD. In affected nodes, the mantle zone and interfollicular zone are largely replaced by a plasma cell infiltrate forming characteristic concentric sheets (so-called onion skinning, named for the resemblance to the layered skins of the onion). These are polyclonal, but are usually restricted in secreting the lambda light chain and immunoglobulin M (IgM). There

are also increased numbers of blood vessels between follicles. However, unlike idiopathic MCD, a minority of cells in the affected node can be shown to be infected by KSHV using immunohistochemical stains for viral proteins. These occur predominantly toward the periphery of the follicle (Fig. 1). Furthermore, a proportion of these KSHV-infected cells are seen to express certain KSHV lytic proteins, suggesting the virus in its lytic (replicative) phase.

It can therefore be seen that the historical progression of the recognition of these disorders has led to the current nomenclature. This unfortunately commonly gives rise to confusion, combining as it does local and systemic diseases whose etiology, pathological and clinical characteristics, optimal therapy, and prognosis are each distinct. A summary of these different types is given in Table 1. It could be argued that, with the recognition of KSHV as the cause of a distinct group of cases of MCD, KSHV-associated MCD is best considered a separate disorder from the idiopathic variants (be it unicentric or multicentric), albeit sharing certain histopathological features. As the overwhelming percentage of cases of MCD in patients with HIV/AIDS are KSHV associated, the remainder of the chapter focuses on KSHV-MCD.

Pathogenesis of Multicentric Castleman Disease in HIV-Infected Patients: Role of Kaposi Sarcoma-Associated Herpesvirus and Immune Deficiency

The discovery of KSHV and its role as the cause of KS and MCD was pivotal in developing an understanding of these disorders and of the crucial role that HIV infection plays in promoting the pathogenesis of KSHV-MCD. KSHV is a herpesvirus, most closely related to Epstein-Barr virus (the cause of infectious mononucleosis). All herpesviruses including KSHV have in common a complex life cycle with two distinct phases. The first of these phases is *latency*, in which a minimal set of viral genes is expressed, many of which are directed at the twin goals of promoting survival of the host cell (and so the virus itself) by modulating



Multicentric Castleman Disease, Fig. 1 Radiological and pathological findings in KSHV-associated MCD. Radiographic findings in a patient with KSHV-associated multicentric Castleman disease and HIV (panel **a** upper and lower rows) demonstrate key features of the disease through structural and functional imaging. In the upper row, whole torso coronal images obtained by computed tomography show widespread lymphadenopathy (*white arrows*) involving the cervical, axillary, and inguinal regions together with mild hepatomegaly. This patient was previously splenectomized so the spleen is not seen, but it is commonly markedly enlarged. In the lower row, sagittal images at the level of the axilla show the axillary nodal abnormalities in detail. Pathological sections (panel **b**) stained with hematoxylin and eosin show characteristic

changes including the plasma cell infiltrate in distinct sheets and an increase in vascularity. The lower panels show KSHV-infected cells by immunohistochemistry: lower left demonstrates all KSHV-infected cells (using an antibody to a viral latent protein, LANA), and lower right shows those KSHV-infected cells in lytic phase and producing vIL-6 (using an antibody to vIL-6). Original magnification of all pathology sections was 20 \times (The authors gratefully acknowledge Dr. Stefania Pittaluga, Laboratory of Pathology, National Cancer Institute, for assistance with histopathological images, and Dr. Corina Millo, Positron Emission Tomography Department, Clinical Center, National Institutes of Health, for assistance with radiographic images)

cellular survival pathways and of impeding the normal host immune response to the virally infected cell. The second, or *lytic*, phase is characterized by viral replication and expression of the full complement of proteins encoded by the virus's own genome. The host cell's machinery is commandeered in the service of viral replication, being turned over to produce progeny virus particles that in turn infect other cells or are transmitted to other hosts. Importantly, all current antiviral therapies affect only replicating virus. No strategy has been effective in disrupting latent herpesvirus infection, and infection once established is lifelong.

Like other herpesviruses, KSHV modulates host pathways to facilitate its survival and replication. It does this in part by producing its own variations of certain critical host proteins, using genes acquired from the host by "molecular piracy" during the course of the virus's evolution. Notable among these is a viral version (homologue) of the aforementioned human cytokine IL-6, called viral interleukin 6 (vIL-6). In addition, KSHV directly affects the production of human IL-6 by several other mechanisms. The relationship of KSHV and its human host in normal circumstances is a delicate balance reflecting millennia of coevolution. Prior to the HIV epidemic, most individuals

Multicentric Castleman Disease, Table 1 Clinical and pathological characteristics of various forms of Castleman disease

	Unicentric		Multicentric	
	Hyaline vascular variant	Plasma cell variant ^a	Idiopathic	KSHV associated
Pathological characteristics	Involved node shows germinal center involution: lymphocytes are depleted and replaced (hyalinized). Between follicles, fibrosis and increased blood vessels are seen. Plasma cells are not a feature	Germinal centers usually uninvolved or paucicellular, while the adjacent mantle zone is replaced by plasma cell infiltrate in concentric sheets. These extend between follicles with increased blood vessels	Affected nodes almost always resemble those of unicentric plasma cell variant, but are multiple. In rare cases multiple nodes showing hyaline vascular change are seen instead	Mantle zone and interfollicular zone replaced by plasma cell infiltrate in concentric sheets. Increased vessels between follicles. A minority of cells are KSHV infected, some in “lytic” phase
Location	Single lymph node or local chain, commonly mediastinal, may be very enlarged	Single lymph node or local chain, commonly abdominal	Multiple nodes throughout body. Spleen may be enlarged	Multiple nodes throughout body, particularly above diaphragm. Spleen commonly enlarged
Clinical setting	Highly variable in age presentation, but commonly early adulthood. Occurs in both genders	Highly variable in age presentation, but commonly in early adulthood. Occurs in both genders	Highly variable in age presentation. Occurs in both genders	Most common in HIV infection, including well-controlled disease
Etiology	Unknown, may be reactive	Unknown, may be reactive. A proportion of cases appear to be reactive to intercurrent lymphoma	Unknown, may be reactive. A proportion of cases appear to be reactive to intercurrent lymphoma. Most manifestations relate to increase in interleukin 6 levels	KSHV/HHV8 infection, usually in the setting of immune dysregulation (HIV infection) Manifestations relate to increase in viral and human interleukin 6 and other cytokines
Clinical symptoms	Symptoms uncommon	Fevers, cachexia, night sweats, malaise occur in some cases	Fevers, cachexia, night sweats, malaise occur in almost all cases	Fevers, cachexia, night sweats, malaise. Nonspecific respiratory and gastrointestinal symptoms may also be seen
Laboratory features	May not have any abnormalities	Hematologic cytopenias (most commonly anemia, thrombocytopenia) and low albumin levels in a minority of cases	Hematologic cytopenias (most commonly anemia, thrombocytopenia) and low albumin levels with elevated C-reactive protein	Hematologic cytopenias (most commonly anemia) and low albumin levels with elevated C-reactive protein
Treatment	Local therapy, most commonly surgical resection; radiation has been used in cases not amenable to surgery	Less well defined than for hyaline vascular variant, but usually similar: Local therapy, most commonly surgical resection; radiation has been used in cases not amenable to surgery. Treatment of any precipitant	Systemic therapy, commonly directed at IL-6 inhibition. Chemotherapy may also be used Treatment of any precipitant	Systemic therapy, commonly directed at B-lymphocyte depletion by the monoclonal antibody, rituximab alone or in combination with chemotherapy KSHV-directed therapies effective in some cases
Outcomes	Generally excellent	Generally excellent	Variable but improving with IL-6-directed therapies	Formerly very poor but improving with current regimens. May progress to large cell lymphoma

^aPlasmacytic and plasmablastic variants are distinguished by some pathologists



infected with KSHV showed no clinical symptoms of infection and those that did occur (almost exclusively KS) were mainly in older individuals, presumably as a result of a diminution of the host immune response with advancing age. The introduction into the human species of HIV fundamentally disrupted this relationship, as HIV infects and destroys CD4⁺ T lymphocytes and so impairs the critical role of the host immune system in detecting and controlling KSHV-infected cells and replication. Thus, there is substantially less control of KSHV, leading to increased viral replication and the development of KSHV-associated tumors. Even when treated with antiretroviral therapies and achieving a normal CD4⁺ T cell count, HIV-infected patients continue to exhibit defects in their immune response that permit the development of KSHV-associated tumors, especially KSHV-MCD. Tumor development may be further enhanced by the presence of HIV-associated inflammation and perhaps by direct interactions between HIV and KSHV at the cellular level.

In KSHV-MCD, it appears that the crucial elements leading to the development of the tumor are dysregulation of viral replication and proliferation of KSHV-infected B lymphocytes, with consequent marked elevations of human and viral cytokines. In particular, levels of both viral and human IL-6 are elevated during KSHV-MCD flares, as are KSHV viral loads (at least in part as a result of replication) and several other host cytokines including IL-10 (Aoki et al. 2001). In addition, as noted above, affected lymph nodes show virus in its lytic (replicative) state in a substantial proportion of virally infected cells. Notably, it has been shown that detectable KSHV replication in peripheral blood commonly precedes the development of symptoms in patients later found to have KSHV-MCD.

Interestingly, KSHV-MCD develops most commonly in patients with relatively well-controlled HIV (CD4⁺ T-lymphocyte count above 200 cells/mm³). There is also some evidence that its incidence has increased in recent years, following the widespread adoption in the developed world of highly active antiretroviral therapy for HIV (Powles et al. 2009). Taken together, these observations suggest that the

development of the full clinicopathological syndrome of KSHV-MCD requires a level of preservation of the host immune infrastructure and mechanisms that is absent in patients with the more profound immunodeficiency of uncontrolled AIDS. It is also possible that its development requires longer survival with HIV than was seen early in the epidemic.

It should also be noted that similar inflammatory symptoms to those seen in KSHV-MCD have recently been described in KSHV-infected patients *without* pathological evidence of KSHV-MCD (Uldrick et al. 2010). These patients presented with a constellation of fevers, cachexia, and laboratory abnormalities including cytopenias, hypoalbuminemia, and elevated CRP. However, lymphadenopathy and splenomegaly were not prominent, and the pathognomonic nodal changes of KSHV-MCD were not demonstrable, in some cases even with repeated lymph node biopsies. Patients showed evidence of KSHV replication and disturbances of human and viral IL-6 similar to those seen in KSHV-MCD flares. Many of these patients had other KSHV-associated diseases (KS or PEL), suggesting that these tumors might contribute to or be the major source of the inflammatory abnormalities. This putative syndrome has provisionally been named the KSHV inflammatory cytokine syndrome (KICS) and a working case definition has been proposed (Polizzotto et al. 2012). Its pathophysiology, clinical outcomes, and relationship to KSHV-MCD are now being defined prospectively.

Treatment of KSHV-Associated Multicentric Castleman Disease in HIV-Infected Patients

Partly as a result of its recent recognition as a disease entity, rarity, and consequent difficulty in establishing high-quality clinical studies, there is no standard therapy for KSHV-MCD. This difficulty is further compounded by the complexity of clinical studies in patients who have both HIV and an associated tumor or malignancy, where both life-threatening diseases must be successfully managed. Therefore the majority of articles

describing the therapy of KSHV-MCD have consisted of either retrospective case reports or series or small prospective studies without control arms. Nonetheless, the evolving understanding of the unique aspects of the viral etiology of KSHV-MCD has suggested several novel approaches to therapy, and there is now emerging evidence that these therapies are having a positive impact on patients with KSHV-MCD, improving their symptom control and, importantly, long-term survival when compared to historical controls.

Early treatment strategies for KSHV-MCD reflected its unusual position at the interface of infectious diseases and clonal lymphoid malignancies. The first therapies studied generally approached the disease from one of these perspectives. One approach adapted a commonly used antiviral agent with known activity against herpesviruses, ganciclovir (Casper et al. 2004). As noted above, this agent is activated by phosphorylation by KSHV and then acts on the replicating virus, becoming incorporated by the viral DNA replication enzyme (DNA polymerase) into the DNA chain and arresting further replication by preventing the addition of additional nucleotides to the chain. It can thus reduce the cell-to-cell spread of KSHV. In parallel, other investigators used single chemotherapy agents or combination chemotherapy regimens adapted from successful lymphoma strategies, particularly CHOP (doxorubicin, vincristine, cyclophosphamide, and prednisolone). In both approaches, patients were usually started on antiretroviral therapy to control the associated HIV infection. Each of these approaches showed modest activity in KSHV-MCD but overall outcomes remained suboptimal.

More recently, the development of a humanized monoclonal antibody directed at the CD20 antigen on B lymphocytes has opened a new approach to KSHV-MCD therapy. As noted above, many of the cytokine-producing cells in lymph nodes affected by KSHV-MCD express CD20. CD20-positive lymphocytes are also a major reservoir of KSHV infection in the infected host, potentially providing an ongoing source of replicating virus to perpetuate the disease state. Several groups have now reported good results

with rituximab therapy of KSHV-MCD (alone or following chemotherapy) in patients with KSHV-MCD, including patients who had previously failed other approaches, as measured by the resolution of symptoms, viral replication, and cytokine abnormalities (Bower et al. 2007). On the basis of these initial results and more recently reported long-term outcomes, rituximab has become probably the most commonly used initial therapy for symptomatic KSHV-MCD. Rituximab therapy of KSHV-MCD is associated with the development or progression of KS in a substantial minority of cases, and some approaches therefore use it in combination with chemotherapy agents that have some activity against KS spindle cells, including the liposome-encapsulated formulation of doxorubicin. It may also be that some patients, perhaps those with the most KSHV-MCD, would benefit from a therapeutic approach that combines rituximab with cytotoxic chemotherapy agents directed at B cells. The question of when, how, and for whom to add chemotherapy to a rituximab therapy “backbone” will be a critical issue in KSHV-MCD investigation in the coming years.

An additional novel approach is targeted at KSHV, exploiting the virus’s own enzymatic machinery to deliver a toxin specifically to KSHV-infected cells in which lytic replication is occurring (as it is in KSHV-infected plasmablasts in nodes affected by KSHV-MCD). This approach, called “virus activated cytotoxic therapy,” uses the combination of two agents, high-dose zidovudine (or AZT) and valganciclovir. Each of these is commonly used as a conventional antiviral drug, acting as described above as a chain terminator (zidovudine in the HIV reverse transcriptase and valganciclovir in the herpes DNA polymerase). However, in this approach they function differently. Rather than interrupting DNA replication, each is activated through phosphorylation by two KSHV-encoded enzymes to moieties that are toxic to the infected cells. These enzymes are expressed by KSHV only in its lytic phase, and therefore this approach provides a method to selectively target and kill only those cells which are central to the pathogenesis of KSHV-MCD. This approach has been shown to

be moderately effective in patients with symptomatic KSHV-MCD, again as measured by the resolution of symptoms, viral replication, and cytokine abnormalities (Uldrick et al. 2011). This combination may also have a role in combination with other approaches or in “maintenance” or “consolidation” therapy for patients who have completed therapy with other agents.

Further targets for the therapy of KSHV-MCD are suggested by the critical role of human and viral IL-6 in its pathogenesis and symptomatology. Inhibitors of human IL-6 may not affect KSHV vIL-6, which has little antigenic similarity with hIL-6 and may also signal differently. However, while there has been little economic incentive to develop inhibitors of vIL-6 specifically, as noted above two monoclonal antibodies inhibiting IL-6 activity have been developed (one directed at the molecule itself, the other at its receptor). One, tocilizumab, has been approved by the US Food and Drug Administration and is now in clinical use in several rheumatologic diseases. While rituximab was recently approved for idiopathic castleman’s disease. Alone or in combination with other drugs, these may in the future provide add to the armamentarium against KSHV-MCD.

Prevention of KSHV-Associated Multicentric Castleman Disease and Related Lymphomas in HIV-Infected Patients

The role of the oncogenic virus KSHV in the pathogenesis of KSHV-MCD and other tumors raises the possibility that preventive strategies directed at the virus, over and above the management of immune deficiency through control of the HIV infection, may be of benefit in preventing the development of these tumors. There are currently no vaccines against KSHV, and as our understanding of its transmission remains incomplete, even behavioral risk modification strategies are limited. However, there is at least proof in principle that KSHV-related disorders can be prevented with antiviral agents: ganciclovir, when used for cytomegalovirus retinitis, was incidentally found to

reduce the risk of KS. This observation, together with the fact noted above that asymptomatic KSHV replication commonly precedes the onset of symptomatic KSHV-MCD, suggests that carefully targeted preventive antiviral strategies may be of use in the future. A better understanding of the precipitants of KSHV lytic replication is likely to be a crucial step in this direction.

On a related front, significant progress has been shown in preventing the most feared complication of KSHV-MCD through effective therapy. As noted above, as many as 20% of patients with KSHV-MCD previously progressed to large cell lymphoma. In the majority of lymphomas arising in this setting, the malignant cells show evidence of KSHV infection with plasmacytic features and immunoglobulin expression that distinguish them as the cells of primary effusion lymphoma, the other lymphoma established to be caused by KSHV. Rarely, large cell lymphomas not associated with KSHV are also seen. The lymphomas in the setting of KSHV-MCD carried a poor prognosis and were one of the most common causes of death. Recent studies of patients treated with rituximab, with or without chemotherapy, have shown evidence of reductions in lymphoma incidence as well as good control of the symptoms of KSHV-MCD (Gérard 2012). It remains unclear if this is a specific outcome of CD20⁺ B-lymphocyte depletion by rituximab (since the most common lymphomas arising in KSHV-MCD are B-cell lymphomas) or a general consequence of the reduction of the local and systemic inflammatory milieu with treatment. Further studies to extend this seminal observation will be crucial.

KSHV-Associated Multicentric Castleman Disease in Resource-Limited Countries

As has been discussed elsewhere, the co-occurrence particularly in parts of sub-Saharan Africa of high endemic rates of oncogenic virus infection (including KSHV) and an epidemic of HIV infection (until recently uncontrolled) has led to very high rates of virally associated malignancies. For example, in sub-Saharan Africa KS is

among the most common cancers. Intriguingly though, until recently cases of KSHV-MCD have only rarely been reported from this region. This is particularly notable as KSHV-MCD is reported in immigrants from these regions in the United States and Europe.

There are a number of possible explanations for this apparent discrepancy. Until the widespread rollout of antiretroviral therapy in Africa through the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the US President's Emergency Plan for AIDS Relief (PEPFAR), most HIV in this region was untreated. It may therefore be that many patients succumbed to other sequelae of profound immunodeficiency before the development of KSHV-MCD. Furthermore, as noted above KSHV-MCD commonly occurs in patients with at least relatively well-controlled HIV, and it may be that at least some level of immune competence is required for the full clinicopathological syndrome to manifest. Perhaps the most important contribution to the paucity of reported KSHV-MCD in this region is that the relatively undeveloped and under-resourced state of clinical and pathological services in this region has hampered the recognition of this complex diagnosis. KSHV-MCD clinically may be mistaken for uncontrolled mycobacterial infection, lymphoma, or other chronic infective and inflammatory processes. Given the magnitude of the burden of tuberculosis in Africa, empiric anti-mycobacterial therapy of suspected lymphadenopathic tuberculosis is relatively common throughout this region. Even in cases where lymph node sampling is performed, limited tissue sampling and in some instances inadequate resources to complete a comprehensive pathological assessment likely contribute to missed diagnoses. The very high untreated mortality of KSHV-MCD and the emergence of relatively low-cost therapies that are potentially deliverable in resource-limited settings (such as ganciclovir with or without high-dose zidovudine) suggest that improved surveillance for and recognition of KSHV-MCD has the potential to deliver important health benefits for people with HIV/AIDS in resource-limited settings (Gopal et al. 2012).

Conclusion

The advent of the AIDS epidemic has brought new prominence to this hitherto uncommon lymphoproliferative disorder and further led indirectly to the discovery of a new oncogenic virus, KSHV, and the recognition that MCD caused by KSHV is a distinct entity with unique pathophysiology and therefore additional specific therapeutic targets. Building on this knowledge, new therapies have greatly improved outcomes for patients with HIV and KSHV-MCD, including important improvements in long-term survival and reduction in the risk of lymphomas. There is the prospect of continued improvements as new agents become available. The challenges of preventing this disease in those most susceptible, and of bringing these improvements in outcomes to the resource-limited settings most affected by HIV/AIDS, continue to occupy researchers and clinicians.

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Multilevel Interventions/Structural Approaches to HIV Prevention

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Definition

Multilevel interventions/structural approaches to HIV prevention include structural prevention strategies that, by definition, target the economic,

social, contextual, policy, or organizational levels or factors that have increased risk of or protected against HIV. Multilevel interventions or approaches include strategies that target both individual and structural levels.

Introduction

Structural interventions, including those that either directly target or explicitly incorporate structural factors, are gaining support in both research and practice. HIV-related structural factors are defined as barriers to, or facilitators of, individual HIV prevention behavior that function on an economic, social, contextual, policy, or organizational level (Sumartojo 2000). Further expanding on the structural environment, the United States (US) Centers for Disease Control and Prevention (CDC) suggests a framework of structural factors including economic resources, policy, societal attitudes, and organizational structures and functions associated with governments, service organizations, businesses, workforce organizations, faith communities, justice systems, media organizations, educational systems, and healthcare systems (Sumartojo 2000). The importance of structural factors when considering HIV risk and transmission is based on the premise that behavior is shaped by more distal features of the environment, and failure to carefully consider these features will result in only partial realization of positive health behavior change. In this entry, key examples of prominent US interventions that operate solely at the structural level and/or exist at an individual level but heavily and/or directly impacted by structural factors will be discussed. Relevant HIV-related policy, as well as socio-contextual factors of relevant communities, including high-risk groups, and the neighborhoods within which they live will be emphasized. Finally, multilevel interventions will also be discussed, including intervention approaches that target individual-level factors and at least one macro-level feature. For the purposes of this entry, the macro-level features of multilevel interventions discussed in this entry will be of a structural nature.

Policy Impact on HIV Prevention

Policy can directly impact individual behavior, including health behaviors that increase risk or protect against acquisition of HIV. In the USA, public health policies that have influenced HIV prevention include condom distribution, sterile syringe access, routine HIV testing services, and HIV partner notification. Either led by the federal, state, or local government, policies enacted to help prevent the spread of HIV have shaped the HIV epidemic by creating social norms that have influenced individual risk and protective behaviors.

Condom Distribution

The absence of an HIV vaccine coupled with persistent sexual risk behavior provides more than adequate support for the promotion of condom use as the leading HIV prevention message. Since the start of the HIV epidemic, with little exception over the past three decades of this disease, federal, state, and local health agencies have actively promoted and financially supported condom use through large-scale condom distribution programs for those at risk for HIV (Abstinence, Be Faithful, Condoms (ABC), Prevention of HIV). Promoting condom use through individual-, group-, and community-level interventions has demonstrated positive intervention effects. Interventions that have specifically incorporated features beyond individual-level factors have had a greater impact on condom use (Charania et al. 2011), while HIV intervention strategies exclusively targeting individual behaviors have often failed to demonstrate impact (Wohlfeiler and Ellen 2008). Condom use strategies that consider structural concepts of availability, accessibility, and acceptability are what researchers and public health practitioners consider as an example of a structural intervention approach to promoting condom use. For example, in addition to conveying the importance of using condoms, a structural approach may also provide free condoms in places where high-risk groups frequent (e.g., drug treatment facilities, STI clinics, brothels, etc.) that is financially supported by local government (e.g., city or state health

departments). These activities clearly address challenges to access and availability; however, such structural approaches are unlikely to influence acceptability. Attempts to change social norms by making condom use more normative have been the most common approach for condom acceptability, which has been done through the use of public service announcements, large-scale media, and social network marketing campaigns (Alstead et al. 1999). A recent meta-analysis of 21 US and international studies provides strong evidence of not only the critical importance of structural approaches to increasing condom use and distribution but also the importance of multilevel approaches that target both individual and structural factors which has provided evidence of even greater impact (Charania et al. 2011).

Syringe Access Programs

In the USA, injection drug use has been a driving force of the HIV epidemic since the mid-1980s. At the start of the epidemic, close to half of all new infections were among injection drug users (IDUs) in northeastern cities. The geographic distribution of HIV has closely followed the heroin and cocaine epidemics across the USA, with northeastern cities representing the heaviest drug markets (Department of Health and Human Development 2000) (Substance Use). Two potential structural approaches to preventing HIV among IDUs would be to decrease availability of illicit drugs (e.g., heroin and cocaine) and decreasing demand (e.g., drug treatment). Neither has been successful as exemplified by the failure of the “War on Drugs” campaign (Fields 2009) and the limited, individually targeted treatment modalities which are tainted with high rates of relapse which is particularly problematic among African Americans, who carry the highest risk and burden of HIV and AIDS in the USA. Thus, for those who are unable to stop injecting, the use of sterile hypodermic needle and syringes (“syringes” hereafter) for each injection has been recommended by public health professionals and researchers, since the use of non-sterile syringes was largely responsible for HIV transmission among IDUs at the height of the

epidemic. However, given the illegality of injection drug use, simply providing public health messages emphasizing the importance of using a sterile syringe for each and every injection was ineffective given the extremely limited access and availability of sterile syringes for the purposes of injecting illegal drugs. In response, the USA established its first syringe exchange program (SEP) in Tacoma, Washington, in 1988, which allowed for the legal exchange of used syringes for sterile syringes. Other SEPs were subsequently established in Portland, San Francisco and New York City. However, because of political and economic constraints, success varied. New York City, for example, had one of the highest rates of HIV in the country, but struggled to establish an exchange large enough to meet the needs of the IDUs population. Since 1988 and in the absence of federal government funding, local and private funding supported many SEPs across the country in urban centers of high heroin and cocaine markets. By 1995, access to SEP services had been positively associated with several outcomes including reduced drug use, decreased incidence and prevalence of HIV and other blood-borne infections, improved access to HIV prevention programs, decreased rates of criminal activity, decreased needle sharing and other high-risk injection drug use behaviors, and improved entry and retention in drug treatment programs. These outcomes have been directly linked to subsequent structural changes in service delivery by coupling SEPs with (1) health education; (2) alcohol swabs to prevent abscesses and other bacterial infections; (3) condoms to further prevent the transmission of HIV and other sexually transmitted infections (STIs); (4) on-site medical services including counseling and screening for tuberculosis, hepatitis B, hepatitis C, HIV, and other infections; and (5) referrals to substance abuse treatment and other medical and social services. These additional services continue to be important, as many IDUs have difficulty accessing public health and medical services for fear of being mistreated in the healthcare system because of their drug use (Miller et al. 2001).

While SEPs (Needle Exchange) have been instrumental in reducing HIV transmission

among IDUs (Injection Drug Users), laws and regulations preventing legal possession of syringes for the purposes of injecting illegal drugs has prevented SEPs from reaching their full potential as an HIV prevention strategy. Furthermore, police harassment at SEP sites has been directly associated with decreased use of SEPs (Bluthenthal et al. 1997). Even where SEPs are legal, the criminalization of syringe possession creates a conflict between public health recommendations and law enforcement practices.

A growing number of states permit IDUs to access syringes through pharmacies without requiring a prescription. Structurally, pharmacies provide direct access to syringes, are already established in high-risk communities, have longer and more convenient hours of operation than SEPs, can potentially provide greater anonymity for syringe customers, and may attract those uncomfortable with SEPs or who require syringes outside the hours of SEP operation. Twelve states (Connecticut, Hawaii, Maine, Minnesota, New Hampshire, New Mexico, New York, Oregon, Rhode Island, Washington, Wisconsin, and California) have implemented legislation to permit pharmacy sales of syringes without a prescription (Pouget et al. 2005), plus New Jersey where an SEP pilot program (New Jersey Department of Health 2012) has been recently instituted.

Evaluations of pharmacy syringe access from the New York State's Expanded Syringe Access Program (NYS-ESAP) have provided evidence of impact on decreased syringe sharing (Pouget et al. 2005), ability to reach non-SEP participants (Fuller et al. 2002), lack of increase in needlestick injuries, lack of increase in criminal activity or drug abuse (Vlahov et al. 2003), and increased positive attitudes toward drug users and pharmacy syringe sales specifically for the purposes of preventing HIV among pharmacists (Crawford et al. 2013). Similar to SEPs, tensions between public health recommendations and law enforcement overshadow nonprescription syringe sales in pharmacies due to the criminalization of syringe possession.

Routine HIV Testing

Providing free HIV testing in venues outside of hospital settings and in a confidential and/or

anonymous manner greatly increases both access and availability and, to a lesser extent, acceptability in situations of anonymous testing. Along with the “use condoms” HIV prevention message, “get an HIV test” has risen as an equally critical prevention message particularly since early detection is critical to treatment and prevention. Until recently, HIV testing has only been routinely recommended for those at high risk for HIV (e.g., men who have same sex partners, IDUs, and the sexual partners of these subgroups). But, in 2006, the Centers for Disease Control and Prevention (CDC) recommended routine HIV testing for all Americans 13–64 years of age. The CDC definition of routine testing is that all patients in all types of healthcare facilities be told that HIV testing is a routine part of care and they will be tested unless they decline. Aside from “how” and “where” HIV testing is offered, implementation of routine HIV testing tackles several social, contextual, and structural factors. First, routinely testing everyone essentially removes the responsibility of deciding to get tested from the individual who must perceive themselves to be at risk as a first step in HIV testing decision process (Mimiaga et al. 2007). Second, offering HIV testing to everyone and presenting it as routine without prior discussion of risk behaviors may have potential to ease HIV testing-associated stigma. Third, there is evidence that patients are more likely to agree to HIV testing when it is suggested by a physician (Mimiaga et al. 2007). And fourth, routine testing does not require written informed consent or pretest counseling in an effort to decrease the time involved in providing HIV testing which, in turn, may increase the likelihood of physicians implementing this new recommendation; physicians may not feel comfortable discussing risk behaviors, and eliminating pretest counseling reduces such instances (Freedberg and Samet 1999). As expected, many public health practitioners have not supported removal of written informed consent and pretest counseling, arguing that consent and counseling are essential for educating individuals about risks and also better prepare individuals psychosocially in the event of a HIV-positive result. Furthermore, the CDC recommendation for routine testing is unable to

reconcile individual state laws and regulations requiring informed consent and information related to the pretest counseling session.

While routine HIV testing may overcome many individual and structural barriers to HIV testing, the impact of this structural approach is unknown, likely due to conflicting state policies. Many states have attempted to apply this recommendation in some form or fashion. For example, one hospital emergency department (ED) offered HIV testing to all ED patients and offered HIV informational literature, posted HIV testing posters, and obtained consent (Informed Consent) using a special script with the express goal of reducing the need for questions when interacting with clinical providers (Wolf et al. 2007). In another instance, an adolescent clinic provided physicians with a pocket guide in addition to patient brochures, HIV testing posters, and reduced pretest counseling time to support and increase routine testing in this clinic (Branson et al. 2006). While such modified approaches have not been formally evaluated, attention to reducing time necessary to spend with patients and clinician preparation and education might be structural features to address when attempting to implement a successful routine HIV testing program. For example, the ease and efficiency with which physicians discuss risks related to diabetes, heart disease, or other chronic disease illnesses requiring routine screening should occur similarly with HIV which can further normalize HIV for both patients and providers.

Partner Notification

Since the beginning of the epidemic, partner notification has been recommended as an intervention strategy to help prevent HIV transmission and connect newly identified HIV-positive individuals to care. Also known as contact tracing, partner notification is the process of informing individuals of their potential exposure to an infectious disease (in this case, HIV) and offering HIV testing, counseling, and treatment. The CDC has released recommendations for how to provide partner notification services, also known as “partner counseling and referral services” (PCRS), which can also be applied to the private sector

and nonprofit service organizations where new HIV diagnoses occur (Partner Notification). From a structural perspective, the manner in which partner notification occurs and the level of funding for these services (which is largely dependent upon laws and legislation surrounding the level of obligation to notify partners) is directly related to the success of the program's goals.

Acknowledging the varying state-mandated laws surrounding partner notification, CDC recommends three strategies for notifying partners of HIV-positive individuals: (1) provider referral, (2) patient or client referral, and (3) contract referral. In the case of provider referral, the clinical provider or the health department official obtains partner contact information, locates the partner, informs the partner of potential exposure without disclosing the identity of the HIV patient or client, and provides the partner with a referral to counseling, testing, and support services. In this case, the burden of responsibility is on the provider or health department official. A second strategy involves the burden of responsibility residing with the HIV-positive client or patient known as patient or client referral. Here, the patient is entirely responsible for contacting their partners and referring them to testing. In contract referral, the HIV-positive individual is granted several days to notify their partners, and if these identified partners have not come into the health department for counseling and testing at the end of the 3-day period, the health department initiates contact. A variation to this approach is dual referral, wherein partner notification is a collaborative effort – the HIV-positive client or patient and the provider work together to locate partners and inform partners together. Individual states either require the permission of the HIV-positive individual to contact their partners or not, which is known as “the duty to warn.” The duty to warn is based on the premise that while the confidentiality for HIV-positive individuals is required for autonomy, the protection of third parties who are at heightened risk for serious, potentially lethal harm must be given priority. It is important to note that while all states attempt to maintain confidentiality when notifying third-party individuals, it is not entirely possible to do so in all

situations. For example, some third parties may have had only one partner and would therefore be able to deduce the identity of the HIV-positive individual. Known as the *Tarasoff* doctrine (Partner Notification), duty to warn began to gain support, while the public health and medical communities remained at odds beginning in the early 1990s for a myriad of reasons related to confidentiality and fear that such a policy could cause more harm than good (Bayer and Toomey 1992). For example, could this policy undermine trust between providers and patients, limiting the capacity of providers to encourage their patients to test for HIV, reduce risk and transmission, or volunteer partner information? Regardless of state-specific “duty to warn” policies, the Federal Ryan White CARE Reauthorization Act requires that all health departments receiving Ryan White funds show a “good faith” effort to notify partners of HIV-positive individuals and the overwhelming majority of US states receive these funds.

The structure of partner notification services will likely impact their success. While some clinicians may wish to take on the responsibility of informing partners, one study indicated greater success among health department specialists compared with clinicians, likely due to insufficient time and training among clinicians to effectively locate and properly inform partners (Giesecke et al. 1991). Additionally, health department officials may be more effective than HIV-positive individuals when notifying partners (Landis et al. 1992). A 2009 report using data from Chicago and Los Angeles found that 75% of participants who identified one or more locatable partners used client referral to notify partners. However, many HIV-positive study participants reported not discussing partner notification with their HIV testing counselors (48.8%) and/or medical care providers (33.7%) and were not offered health department partner notification services by their HIV counselors (60.8%) or medical providers (52.8%) (Mackellar et al. 2009). A San Francisco study suggested that once an HIV-positive individual was referred to partner notification services, a vast majority were interviewed (80%) and most partners of the

HIV-positive individuals were located and interviewed (12% not located and 8% refused to talk with health department official) (Ahrens et al. 2007). Regardless of the level of use, partner notification exists in every jurisdiction with varying levels of resources to locate, inform, and test partners. Issues of confidentiality, lack of trust, lack of location information for some short-term partners, and discomfort in disclosing (Mackellar et al. 2009) may limit use of partner notification services. Thus, structurally targeting social normative attitudes toward HIV is critical for greater impact of partner notification services.

Community and Neighborhood Structural Features that Impact HIV Prevention

Governmental policies must function within previously established community and neighborhood structures which can result in differential impact of HIV-related policies and/or policy-related interventions by community. Here, “neighborhood” is defined in terms of geographic location with formal (e.g., zip code or census tract) or informal physical boundaries, as opposed to “community,” which is defined as a specified group delineated by social boundaries (e.g., race/ethnicity, culture, sexual identity, religion, etc.) and may or may not be confined by physical geographic boundaries.

A clear example of the interplay between public health policy and neighborhood and/or community is the implementation of New York State’s Expanded Syringe Access Program, ESAP, in 2001 (Fuller et al. 2007). As discussed earlier, this policy intervention was established to increase syringe access and availability through nonprescription syringe sales in pharmacies to help reduce the use of previously used syringes among IDUs. Overall, ESAP was successful and ultimately became permanent NYS law in 2009 (Fuller et al. 2007); however, early evaluations revealed uneven program uptake, with lower rates of utilization in communities most heavily burdened with HIV, namely, black and Latino

IDUs, and in specific low-income neighborhoods (Fuller et al. 2004). An evaluation in 2003 indicated perceived race discrimination as a significant correlate of self-reported “nonuse” of pharmacies as a syringe source, above and beyond individual characteristics, including knowledge of the new program (Fuller et al. 2004). In response, a large-scale multilevel intervention targeting minority neighborhoods was implemented to increase ESAP participation by targeting individual IDUs with education about HIV prevention services such as ESAP; targeting pharmacy staff with education about ESAP, drug use, and importance of HIV prevention for the IDU community; and targeting community residents with information about the importance of ESAP in reducing community HIV risk and harm reduction approaches to help reduce drug use- and HIV-associated stigma – all of which fulfilled the overarching goal of creating a more receptive social environment for this public health policy. The intervention resulted in significantly higher support toward HIV prevention for IDUs and pharmacy syringe access among community residents and pharmacy staff and a significant increase in IDU self-reported use of pharmacies as a syringe source, particularly among black IDUs (Fuller et al. 2007). This example highlights the importance of implementing policy in tandem with other institutional or community norms and/or practices to not only alleviate existing disparities, as in this case with HIV/AIDS, but also to help prevent new social disparities from arising as a result of a new policy or practice. This is also an example of how and why comprehensive, multi-level approaches that target individual, and structural-level factors, rather than “risk factor-targeted” interventions, are needed to address physical (e.g., syringe access venues) and social (e.g., drug use- and HIV-associated stigma) barriers to HIV prevention services.

There are a host of neighborhood- and community-level points of intervention that can be drawn from the multilevel intervention example described above. Below, a brief discussion follows that includes these features and other salient neighborhood- and community-level targets for HIV prevention in the USA.

Stigma

The effect of social policies and community-level HIV prevention efforts are often dampened among those at the highest risk of HIV such as substance users, men who have sex with men (MSM), and homeless or unstably housed individuals. A growing body of literature suggests this may result from stigmatization and discrimination which may isolate high-risk individuals from critical HIV prevention and treatment resources and may limit the availability of safe/low-risk social relationships, thereby creating “hot spots” of isolated HIV infection (Crawford et al. 2012). Bridges between stigmatized and non-stigmatized individuals often exist in many disadvantaged communities linking those at high risk with those at low risk, making targeted interventions difficult. Recent literature has shown that labeling-induced stigma awareness, stereotype agreement, and anticipated stigma predict lower frequency of reported HIV testing, willingness to test for HIV, and greater HIV test refusal. In such instances, structural interventions that tackle community norms and work to create and reconnect community resources to stigmatized populations by reducing stigma are necessary.

Racial Residential Segregation

The most apparent and recognized explanation for persistent racial and ethnic disparities in HIV on a neighborhood level is racial, ethnic, and social class segregation, which systematically limits critical resources not only for HIV prevention but also several other indicators of health and well-being (White and Borrell 2011). While the evidence of the impact of segregation on health suggests an important effect, many studies fail to examine measures of segregation consistently and use both formal (i.e., dissimilarity index) and proxy (i.e., neighborhood proportion minority) measures of segregation on various scales (e.g., metropolitan statistical areas, census tracts) that limit the interpretability and comparability of the literature in this field (White and Borrell 2011). Despite this, the mechanism through which residential segregation (by race and social class) influences HIV transmission has most often been through access to critical HIV testing

promotion and substance use treatment resources (Discrimination). In fact, studies have shown a relationship between racial residential segregation measured by the dissimilarity index with lower HIV testing behaviors (Ford et al. 2009), access to clean syringes (Cooper et al. 2009), and drug treatment programs (Jacobson et al. 2007) in minority and disadvantaged neighborhoods, where there is clear public health need denoted by high rates of drug-related hospitalizations and high burden of HIV disease.

Gentrification, the process of neighborhood renewal and increasing neighborhood values as a result of the influx of high-income residents and the displacement of low-income residents (Freeman 2009), has been shown to disenfranchise racial and ethnic minorities who are often residents of gentrifying neighborhoods (Freeman 2009). The role of gentrification on previously established networks of HIV prevention and treatment services has not been examined. However, studies have shown that neighborhood change processes, including gentrification and neighborhood revitalization, directly influence increases in HIV incidence and substance use (Rhodes et al. 2005). Therefore, it is plausible that through neighborhood gentrification, prevention and treatment services are broken, barriers to critical resources needed to prevent HIV arise, and social relationships and connections are disrupted, creating several points of opportunity for increases in HIV risk and transmission. Research that focuses on neighborhood gentrification and the mechanisms through which it may affect HIV, and other related health outcomes, is needed to better understand and prevent systematic disadvantage of communities at high risk of and heavily burdened with HIV.

Policing and Incarceration

A critical area of HIV research today investigates the impact of racialized policing and incarceration which, in turn, facilitates a revolving door between jail, prison, and the community on HIV risk in the USA. The criminalization of drug use in the USA results in a fear of being arrested which can prevent drug users from accessing HIV prevention resources (e.g., HIV testing, syringe

exchange, pharmacy-based syringe sources) and toward higher-risk environments (e.g., shooting galleries). Furthermore, criminalization of drug use reduces the availability and promotion of drug abuse treatment therapies, which could reduce HIV transmission while incarcerated or upon release, within the criminal justice system. In many jurisdictions, drug users are often sentenced with incarceration time instead of directed to evidence-based addiction programs, which may disrupt HIV treatment and increase transmissibility of HIV via higher viral loads and ART resistance (Fields 2009).

Investigating the criminal justice system becomes particularly important when addressing racial/ethnic HIV disparities, given the burgeoning incarcerated population over the past several decades, which is overwhelmingly racial/ethnic minorities (Rhodes et al. 2005; Fields 2009; Drucker 2011). The mass incarceration of black and Latino men and women in the USA is fueled by arrests and sentencing for misdemeanor drug offenses with short prison sentences (Drucker 2011). Systematic imprisonment and release of individuals from these communities creates a bridge between high-risk institutionalized and lower-risk noninstitutionalized populations, which lends to a continuum of debilitating social and health consequences, one being facilitation of HIV transmission (Rhodes et al. 2005). While individual-level interventions to reduce HIV transmission within prisons and among the formerly incarcerated populations are important, structural interventions addressing penal policies that disproportionately target minorities for drug offenses are needed.

Conclusion

Structural and multilevel interventions show great promise for optimizing individual behavior change. Targeting structural level factors and/or incorporating structural features in HIV intervention approaches can have significant and far-reaching impacts. Further, multilevel intervention strategies that address both individual-level and structural-level factors have also resulted in

high public health impact. To create an optimal socio-environmental context within which positive health behaviors are more likely to occur, attention to policy, social norms, and relevant systems is essential when designing and implementing effective HIV intervention strategies.

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MX2 and HIV-1 Restriction

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Definition

The human myxovirus resistance 2 protein (MX2, also named MxB) belongs to the dynamin superfamily of high molecular weight guanosine

triphosphatases (GTPases). MX2 is homologous to human MX1 (or MxA) and mouse Mx1 and Mx2 proteins. The Mx genes are inducible by type 1 and type 3 interferons (IFNs). Human MX1 has been long recognized as a potent antiviral factor, capable of preventing replication by numerous RNA viruses, including influenza A and measles viruses, and DNA viruses, such as hepatitis B virus. Human MX2 was recently shown to possess a strong antiviral activity against HIV-1 and other primate lentiviruses. MX2 participates in the type 1 interferon-induced block to HIV-1 infection and acts after reverse transcription, at the level of viral DNA nuclear import and/or integration into the host cell genome.

Introduction

Detection of viruses and other microbes by cellular sensors in infected cells induces the production of type 1 and type 3 interferons (IFNs). These antiviral IFNs in turn induce an “antiviral state” in target cells through the regulation of hundreds of genes, termed interferon-stimulated genes (ISGs). Some of these ISGs, such as *protein kinase R (PKR)*, *2'-5' oligoadenylate synthetase (OAS)*, and *MX1*, are well known to possess antiviral activity. It has been known for decades that the IFN-induced antiviral state efficiently protects some cell types against HIV-1 infection in vitro (Ho et al. 1985). Interestingly, exogenous administration of type 1 IFN is capable of producing a profound, although reversible, decrease in HIV-1 viral load in the majority of infected patients and of allowing control of viral load during antiretroviral treatment interruption in half the patients (Asmuth et al. 2010; Azzoni et al. 2013). Analysis of the kinetics of viral load decline during IFN administration in vivo suggests that IFN acts by blocking the de novo infection of susceptible cells (Neumann et al. 2007). In a model of rhesus macaques infected with SIV, type 1 IFN appears essential in the initial control of viral replication (Sandler et al. 2014).

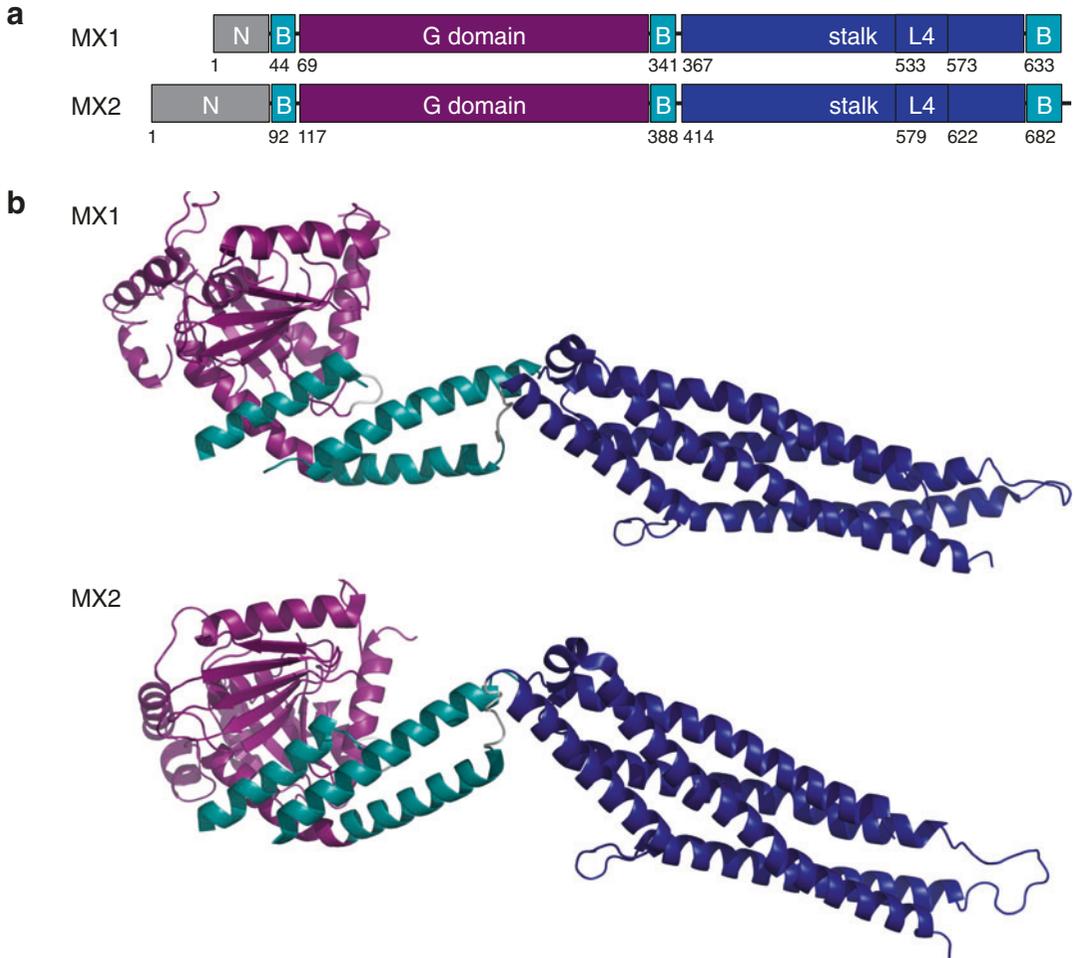
In vitro, the IFN-induced infection block to HIV-1 infection correlates with a strong defect in viral DNA accumulation, and an even stronger

effect is observed on the amount of integrated viral DNA (Goujon and Malim 2010; Cheney and McKnight 2010). Whereas the cellular factors responsible for the block to viral DNA accumulation are yet to be identified, the MX2 GTPase was recently identified as a potent postentry inhibitor of HIV-1, suppressing nuclear import and proviral formation (Goujon et al. 2013; Kane et al. 2013; Liu et al. 2013).

The Mx GTPases

Historically, the Mx genes were discovered in mice: *Mx1* was first identified in a specific mouse strain (A2G) unusually resistant to influenza A virus infection (Lindenmann 1962; Horisberger et al. 1983). Mx genes are highly IFN-inducible, cell-autonomous restriction factors, capable of blocking replication of various viruses. Most mammals possess two Mx genes, which arose by gene duplication and present different antiviral specificities often correlated with their subcellular localization. For instance, nuclear mouse Mx1 prevents influenza A virus and Thogoto virus replication, whereas cytoplasmic mouse Mx2 inhibits vesicular stomatitis virus. Humans also possess two homologous MX genes: *MX1* (the product of which is also called MxA) and *MX2* (MxB). Human MX1 and MX2 share 63% identity at the amino acid level. Despite the nomenclature, human MX1 is actually closer to mouse Mx2 (77% identity) than to mouse Mx1 (67%), and human MX2 and mouse Mx2 share only 62% identity.

Human MX1 is cytoplasmic and has broad antiviral activity against many RNA viruses, such as orthomyxoviruses (e.g., influenza A virus, Thogoto virus), paramyxoviruses (e.g., measles virus), bunyaviruses (e.g., LaCrosse encephalitis virus), and rhabdoviruses (e.g., vesicular stomatitis virus), as well as DNA viruses (e.g., Hepatitis B virus, HBV, and asfar viruses) (reviewed in (Haller et al. 2015)). The antiviral activity of human MX2 was initially tested against the main viral targets of MX1, such as influenza A virus. As MX2 had no effect on the targets of MX1, it was thought for a long time that MX2



MX2 and HIV-1 Restriction, Fig. 1 Structure of human MX1 and MX2 proteins. (a) Domain organization of human MX1 and MX2. The amino-terminal domain (N) of the proteins is represented in *gray*, the GTPase domain (G domain) in *purple*, the bundle-signaling element (B) in *light blue*, and the stalk domain (with the

position of L4 indicated) in *dark blue*. (b) Crystal structures of human MX1 and MX2 without their amino-terminal domains (Adapted from Gao et al. (2011) and Fribourgh et al. (2014) (PDB 4WHJ and 3SZR, respectively)

lacked the antiviral activity possessed by its homologous human or murine proteins.

Human MX1 and MX2 proteins share a similar domain organization and structure, as shown by their recently solved structures that are almost completely superimposable (Gao et al. 2011; Fribourgh et al. 2014; Haller et al. 2015; Fig. 1). These proteins are composed of an amino-terminal domain of different lengths (43 and 91 amino acids for MX1 and MX2, respectively) of which the structure has not been solved, a

globular head containing the GTPase domain (that binds and hydrolyzes GTP), connected to a stalk domain (important for multimerization and viral target recognition in the case of MX1 (Gao et al. 2011; Mitchell et al. 2012), see below) via a tripartite bundle-signaling element (BSE), which transfers conformational changes between the GTPase domain and the stalk, upon GTP binding and hydrolysis.

Two isoforms of human MX2 are co-expressed in human cells following IFN

treatment, due to alternative translation initiation: a long isoform of 78 KDa corresponding to the full-length protein of 715 amino acids and a shorter one of 76 KDa, lacking the first 25 amino acids (Melen et al. 1996; Melen and Julkunen 1997). The latter is dominant (representing about 80% of the total MX2 protein in the cell) and is cytoplasmic. The long isoform is mainly localized at the nuclear envelope, as well as in cytoplasmic granules or bodies of unknown composition (Melen et al. 1996; Kane et al. 2013; Goujon et al. 2014; Matreyek et al. 2014). A sequence enriched in arginine and lysine present in the first 25 amino acids of MX2 was initially thought to act as a nuclear localization signal (NLS) (Melen et al. 1996; Melen and Julkunen 1997; King et al. 2004). However, a more recent study has shown that the 91 amino acids of MX2 function as a nuclear envelope-targeting domain and not an NLS per se as they are able to relocate the turbo-red fluorescent protein at the nuclear envelope (and not inside the nucleus) when appended to its amino-terminus (Goujon et al. 2014). The binding partners of this amino-terminal domain of MX2 and the mechanism-conferring nuclear envelope localization are currently unknown.

Based on the subcellular localization of MX2 at the nuclear envelope and its apparent constitutive expression in some cell types, a potential physiological role for MX2 has been explored (King et al. 2004). It was proposed then that MX2 may regulate the nuclear import and/or cell cycle progression, as the expression of GTPase mutants (potentially acting as dominant negatives) or MX2 depletion disturbed these processes (King et al. 2004).

Three recent studies have shown that similarly to its human and murine homologous counterparts, MX2 is actually an antiviral effector of the IFN response. MX2 is able to act against HIV-1 and other primate lentiviruses. Indeed, the silencing of MX2 in the context of the IFN-induced antiviral state significantly rescues HIV-1 infection, and conversely, the overexpression of MX2 induces a potent block to HIV-1 infection (Goujon et al. 2013; Kane et al. 2013; Liu et al. 2013).

MX2 Blocks the Viral Life Cycle After Viral DNA Accumulation

When overexpressed, MX2 prevents HIV-1 DNA integration into the host cell genome as well as the formation of 2-LTR circles, an episomal form of viral DNA generated only in the nucleus and often used as a surrogate for viral DNA nuclear entry (Goujon et al. 2013; Kane et al. 2013; Matreyek et al. 2014). This suggests that MX2 may act at the level of viral DNA nuclear entry. Interestingly, MX2 is mainly localized at the nuclear envelope (Melen et al. 1996; King et al. 2004; Kane et al. 2013; Goujon et al. 2014) and may have a role in regulating nucleocytoplasmic trafficking, as aforementioned (King et al. 2004).

Capsid Determines the Susceptibility to MX2

The overexpression of human MX2 is sufficient to confer resistance to the infection with most lab-adapted and primary strains of HIV-1 (Goujon et al. 2013). Whereas some SIVs are sensitive to the antiviral activity of MX2, HIV-2 and SIV_{MAC} are partially resistant (Goujon et al. 2013, 2014; Matreyek et al. 2014), and non-primate retroviruses, such as the feline immunodeficiency virus (FIV), the equine infectious anemia virus (EIAV), and the murine leukemia virus (MLV), are insensitive to MX2 (Goujon et al. 2013; Busnadiago et al. 2014; Matreyek et al. 2014). Interestingly, some transmitted/founder HIV-1 strains have recently been shown to be resistant to MX2 (Liu et al. 2015). This might partially explain why transmitted/founder viruses are less sensitive to the IFN-induced antiviral state (Fenton-May et al. 2013; Parrish et al. 2013).

Initial studies have shown that HIV-1 Capsid determines the sensitivity to MX2, with some amino-terminal mutants of Capsid being able to escape antiviral activity, such as the P90A and G89V mutants (Goujon et al. 2013; Kane et al. 2013; Liu et al. 2013). These Capsid mutants are unable to bind to Cyclophilin A (CypA), a

known cofactor of HIV-1 (Zhou et al. 2012). Extended mutagenesis analyses have confirmed the importance of the CypA-binding loop of Capsid for MX2 restriction (Busnadiego et al. 2014; Matreyek et al. 2014; Liu et al. 2015). Moreover, CypA has been suggested to play a role in the restriction as MX2 may interact with CypA (as shown in a co-immunoprecipitation experiment) and cyclosporin A (CSA), a drug inhibiting CypA binding to Capsid, may rescue HIV-1 from MX2 restriction (Liu et al. 2013). However, the ability of CSA to alleviate MX2 restriction has been questioned (Busnadiego et al. 2014). Therefore, the effect of CSA and the importance of CypA in MX2 restriction will require further investigation. Other determinants of MX2 sensitivity have been mapped in the carboxy-terminal domain of Capsid, with mutants P207S, G208R, and T210K being less sensitive or completely resistant to MX2 activity (Busnadiego et al. 2014), confirming the importance of Capsid in the MX2-mediated restriction.

MX2 Is Able to Physically Interact with Capsid

MX1 is well known to recognize and interact with some components of viral nucleoprotein complexes such as Thogoto virus Nucleoprotein (Haller et al. 2015), and HIV-1 Capsid determines the sensitivity to MX2. Taken together, this strongly suggested that MX2 may be able to physically interact with HIV-1 Capsid. A clear physical interaction of MX2 with assembled HIV-1 Capsid has indeed been reported, using systems of recombinant Capsid or Capsid-Nucleocapsid nanotubes, which are known to mimic the tridimensional structures of HIV-1 cores (Fribourgh et al. 2014; Fricke et al. 2014). However, the relevance of this interaction is currently unclear as MX2 interacts as efficiently with wild-type Capsid as with Capsid mutants that are able to escape from the restriction, such as the aforementioned P90A mutant (Fribourgh et al. 2014; Fricke et al. 2014).

The Amino-Terminal Domain of MX2 Specifies the Anti-HIV-1 Activity

A 40-amino acid loop present in the stalk domain, the L4 loop, has been shown to be essential for viral target recognition by MX1 (Mitchell et al. 2012; Patzina et al. 2014). Indeed, sequence analyses of MX1 orthologs in primates reveal strong signatures of positive selection in L4 during primate evolution, and a particular amino acid in L4 largely determines antiviral specificity against orthomyxoviruses (Mitchell et al. 2012). Moreover, L4 can be swapped between orthologous proteins and confer the antiviral specificity of the parental protein, showing that it can act as an autonomous module (Mitchell et al. 2012; Patzina et al. 2014). Additional sites of positive selection have been identified in the flexible amino-terminal part of MX1, suggesting alternative interfaces that might be used to recognize other viruses (Mitchell et al. 2012).

Contrary to what is observed for MX1 and orthomyxoviruses, an approach using engineered chimeric MX1/MX2 proteins has shown that L4 was not important for HIV-1 recognition by MX2 (Goujon et al. 2014). Instead, the 91-amino acid-long amino-terminal domain of MX2 confers antiviral specificity in the case of HIV-1 restriction. Indeed, only the long isoform of MX2, containing the full-length amino-terminal domain, is active against HIV-1 (Kane et al. 2013; Goujon et al. 2014). In addition, the amino-terminal domain contains positively selected sites within primates and seems to dictate antiviral specificity between primate MX2s (Busnadiego et al. 2014). Strikingly, a human MX1 chimeric protein containing the first 91 amino acids of MX2 becomes fully active against HIV-1 (Goujon et al. 2014). And this MX1 chimeric protein containing the amino-terminal domain of MX2 is able to bind to HIV-1 Capsid, whereas MX1 is not (Fricke et al. 2014). Moreover, the transfer of anti-HIV-1 activity to completely unrelated proteins with the transfer of MX2 amino-terminal domain (Goujon et al. 2015) (see below) further demonstrates its essential role in HIV-1 restriction.

An alanine-scanning mutagenesis approach has shown that the amino-terminal domain of MX2 contains a crucial triple arginine motif at positions 11–13 (Goujon et al. 2015). Surprisingly, mutating this domain completely abrogates antiviral activity without changing subcellular localization to the nuclear envelope and cytoplasmic granules. Canine MX2 is not active against HIV-1, and the transfer of either the first 27 amino acids of human MX2 to this homologous protein (Busnadiego et al. 2014) or only the missing third arginine in the triple arginine motif (Goujon et al. 2015) completely rescues anti-HIV-1 activity, highlighting the importance of this motif. Determining the cellular binding partners of this specific motif will certainly shed light onto the mechanism of action of MX2.

MX2 Dimerization, but Not GTPase Activity, Seems Essential for Antiviral Activity

Antiviral activity of human MX1 and mouse Mx1 proteins generally requires GTP binding and hydrolysis, an intact BSE and oligomerization via the stalk (Pitossi et al. 1993; Ponten et al. 1997; Gao et al. 2010, 2011). However, there are exceptions, and, for instance, restriction of HBV infection by MX1 does not rely on GTPase activity (Yu et al. 2008). Similarly, MX2 GTPase mutants partially or fully retain anti-HIV-1 activity (Goujon et al. 2013, 2014; Kane et al. 2013; Matreyek et al. 2014). This possibly underlies a different mechanism of action for MX1 and MX2, which may also depend on the viral target.

High-order homo-multimerization is essential for the activity of MX1 against influenza A virus (Ponten et al. 1997; Kochs et al. 2002; Gao et al. 2010, 2011), and the current model for viral restriction proposes that MX1 may form oligomeric rings around viral nucleoprotein complexes upon GTP hydrolysis. But recent analyses have shown that contrary to MX1, dimerization of MX2, but not oligomerization beyond dimers,

appears to be necessary for viral suppression (Fribourgh et al. 2014; Buffone et al. 2015), once again evoking different mechanisms of action for the 2 homologous proteins.

Interestingly, fusion of the 91 amino-terminal amino acids of MX2 confers potent anti-HIV-1 activity to Fv1, an unrelated protein and known restriction factor of some strains of MLV, which naturally forms oligomers (Bishop et al. 2006; Goujon et al. 2015). In addition, partial and significant anti-HIV-1 activity could be conferred with fusion of this domain to a dimeric leucine zipper but not to a monomeric mutant (Goujon et al. 2015), emphasizing the importance of dimerization for MX2 activity.

Conclusion

MX2 appears as a potent inhibitor of HIV-1 infection, acting on the particularly vulnerable post-entry steps of the viral life cycle. MX2 does not have a described viral antagonist (as yet); therefore, this protein cannot be strictly referred as a restriction factor in the current definition used in the retrovirology field. It has nonetheless become clear from a number of recent studies that MX2 can be added to the list of HIV-1 resistance factors, which are able to efficiently limit HIV-1 replication. MX2 is clearly one of the major effectors of the IFN-induced antiviral state against HIV-1 in vitro (Goujon et al. 2013; Liu et al. 2013). The importance of MX2 in HIV-1 control in vivo remains to be analyzed, but it is tempting to speculate MX2 certainly plays a role in the positive effect of type 1 IFN observed in HIV-1-infected patients (Asmuth et al. 2010; Azzoni et al. 2013) and on SIV in infected rhesus macaques (Sandler et al. 2014). In agreement with this, two recent studies have shown a correlation between MX2 expression levels and resistance to HIV-1 infection in highly exposed seronegative individuals, possibly highlighting a protective role of MX2 (Sironi et al. 2014; Stein et al. 2015).

Further work is now needed to fully understand the mechanism of action of MX2. For instance, it

will certainly be helpful to understand why some Capsid mutants are able to escape from MX2 antiviral activity, whereas the latter is able to interact with them. Understanding the mechanism of MX2 restriction will certainly bring light to some of the postentry events of HIV-1 replication, which remains poorly understood.

Cross-References

- ▶ [APOBEC3F/G and Vif: Action and Counteractions](#)
- ▶ [Cellular Cofactors of HIV as Drug Targets](#)
- ▶ [Cyclophilin A and HIV-1 Replication](#)
- ▶ [Counteraction of SAMHD1 by Vpx](#)
- ▶ [Nef/Env/Vpu/Tetherin](#)
- ▶ [TRIM5alpha](#)

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Natural History and Clinical Features of HIV-2 Infection

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Definition

A summary of the key literature on the virological, immunological, and clinical progression of HIV-2-infected individuals, with emphasis on the contrast between HIV-2 and HIV-1.

Natural History and Viral Load

The single most distinguishing feature of HIV-2 compared to HIV-1 is its much lower viral load set point. There are no data on the viral loads achieved during the acute phase of HIV-2 infection and less data than for HIV-1 on the viral loads maintained during chronic infection, limiting the precision of viral load estimates at a population level. The viral loads of individuals with untreated chronic HIV-2 infection show considerable variability. While a minority of those infected with HIV-2 have viral burdens typical of progressive HIV-1 infections, in approximately 80% of

HIV-2-infected individuals, viral loads remain very low or, in up to perhaps a third of cases, undetectable (Grant et al. 1997; van der Loeff et al. 2010). This is in contrast to HIV-1, where only 1% of those infected have a similar level of control without treatment (Saksena et al. 2007).

The lower viral set point typically seen with HIV-2 has profound implications for its progression and transmission. It makes HIV-2 much less readily transmissible than HIV-1 (Gottlieb et al. 2006). Disease progression is also related to viral load and is typically slower with HIV-2, with greater diversity in outcomes (Ariyoshi et al. 2000). As a result, most individuals with HIV-2 experience a much longer delay before developing severe symptoms related to their immune compromise. HIV-2-infected patients thus tend to present with higher CD4⁺ T-cell counts and later in life.

Mortality rates are lower with HIV-2 than HIV-1. Compared to HIV-uninfected controls, mortality rate ratios of approximately 2–3 have been reported for individuals infected with HIV-2. The mortality rate ratios for HIV-1 infection, by contrast, are approximately 10–15 (Jaffar et al. 2004). For those infected with HIV-2 having undetectable viral loads, mortality is probably equivalent to HIV-negative controls; this was most convincingly demonstrated in an 18-year follow-up of a community cohort in Guinea-Bissau with serial viral load measurements (van der Loeff et al. 2010).

While HIV-2 shares the same modes of transmission as HIV-1, its prevalence in endemic areas is dropping even as that of HIV-1 rises. This appears to be the result of competitive exclusion of HIV-2 by HIV-1 as a consequence of the lower viral loads in the former (de Silva et al. 2013).

Mechanism of Viral Suppression

The lower viral set point in HIV-2 is a combination of multiple virus- and host immune-related factors. HIV-2 successfully infects CD4+ T-cells, integrating into host cell genomes as well as HIV-1 does. Thereafter, HIV-2 generates less mRNA and fewer viral particles (MacNeil et al. 2007). It induces less immune activation in the host and less apoptosis of infected cells. HIV-2 also appears more susceptible to host immune responses. Most individuals infected with HIV-2 generate greater CD4+, CD8+, NK cell, and antibody responses, and exert greater control over their virus, than people infected with HIV-1 (Leligowicz and Rowland-Jones 2008). Variation in HIV-2 viral control is more a function of differences in host immune response than differences in the viral genome (de Silva et al. 2013), however differences between viruses appear to be important determinants of immune control. The key finding linking the structure of the virus and the effectiveness of the immune control in HIV-2 may be the prevalence of proline-rich P-26 capsid proteins in the virus, which make viral particles less stable intracellularly, facilitating both their breakdown by TRIM5 α and the presentation of their antigens to other elements of the immune response (reviewed in de Silva et al. (2008)).

CD4+ T-Cell Decline

Immunologic decline in HIV infection is represented by falling CD4+ T-cell counts. CD4+ T-cell decline is a function of viral load in both HIV-1 and HIV-2. In modal HIV-2-infected individuals with low viral loads, this decline is more gradual than that typically seen in HIV-1 infections, and those with undetectable viral loads may experience essentially no progression at all (van der Loeff et al. 2010). By contrast, among the minority of HIV-2-infected individuals with viral loads equivalent to those seen with progressive

HIV-1 infections, the rate of CD4+ T-cell count decline is roughly the same (Gottlieb et al. 2002).

Dual Infection with HIV-1

Dual infection with both HIV-1 and HIV-2 occurs routinely in settings where both viruses are common. Rate estimates of dual infection vary by setting and testing method, subjects which lie outside the scope of this entry. Immunologic and clinical deterioration in dual infection are driven by viremia. As is the case in mono-infection, HIV-2 viral loads in dually infected individuals are generally lower than their HIV-1 viral loads; they may be very low or undetectable while HIV-1 viral loads are high in the same individuals. The clinical course of dually infected individuals is therefore typically determined by their HIV-1 infection (Jaffar et al. 2004). The existence of a meaningful interaction between the two infections has long been debated. Recent research suggests that prior infection with HIV-2 may modulate early responses to HIV-1 infection, with a possible effect on viral set point and progression, though this remains contested (de Silva et al. 2012; Esbjornsson et al. 2012).

Clinical Features

The spectrum of clinical disease in both HIV-1 and HIV-2 is dominated by the degree of immune impairment, generally measured by CD4+ T-cell counts and/or CD4 percents. Opportunistic diseases in HIV infection, primarily infectious, are frequently associated with specific CD4+ T-cell counts below which their incidence increases substantially. Furthermore, local epidemiology, particularly of infectious diseases, plays a crucial role in determining the clinical presentation of HIV-infected people.

Opportunistic Infections

In general, the opportunistic infections associated with HIV-2 are the same as those associated with HIV-1, after controlling for the effect of CD4+ T-cell count (Grant et al. 1997; Martinez-Steele et al. 2007). The relative paucity of data on the clinical presentation of HIV-2 however limits the

certainty with which differences in clinical associations between the two forms of HIV can be asserted or excluded.

Several retrospective studies have suggested specific differences between the opportunistic infections associated with HIV-1 and HIV-2. In the most conclusive of the studies, Kaposi's sarcoma was found to be 12 times more common in individuals infected with HIV-1 than in those infected with HIV-2 (Ariyoshi et al. 1998). In this study population, asymptomatic human herpes virus 8 coinfection was equally common among those with HIV-1 and HIV-2 infections, and sex and degree of immune suppression were both controlled for. It has been proposed that this difference may be the result of HIV-1 Tat proteins being more potent tumor inducers than HIV-2 Tat proteins (Weiss and Boshoff 2000).

In a study of 790 hospitalizations, presentation with either bacterial or chronic diarrhea was more common among those infected with HIV-2, while oral candidiasis, meningitis, and chronic fevers were more common in HIV-1-infected patients (Ndour et al. 2000). The authors of this study are nonetheless reluctant to assert this represents a true clinical difference between HIV-1 and HIV-2, as only 17% of the subjects had CD4+ T-cell counts done and these were not controlled for in the analysis. Furthermore, no statistical correction was made for multiple comparisons, and 21% of the subjects were repeat admissions.

In a landmark autopsy series from the Ivory Coast, CMV, HIV encephalitis, and cholangitis were more frequently found among the 40 people who had died with HIV-2 and undergone autopsy compared to the 154 who had died with HIV-1. For those people undergoing autopsy for whom a CD4+ T-cell count was available, the median of HIV-2-infected subjects was $50 \times 10^6/l$, compared to $101 \times 10^6/l$ for those with HIV-1 infection. Taking into account the natural history of the diagnoses at autopsy, the authors conclude that the differences between HIV-2 and HIV-1 in their series can be explained by the lower CD4+ T-cell counts combined with a slower disease progression in the terminal phase of the illness among their HIV-2 subjects, rather than being a

consequence of the virus type itself (Lucas et al. 1993).

Results from a cross-sectional study published in 2003 suggested a greater risk of high-grade squamous intraepithelial cervical cancer lesions with HIV-2 infection compared to HIV-1 infection. This association was however not confirmed in a longitudinal prospective study, controlling for the duration of infection, carried out by many of the same investigators at the same study site (Hawes et al. 2006).

Response to Antiretroviral Therapy

A substantial body of literature has developed around antiretroviral therapy for HIV-2. Diminished sensitivity to a number of agents, as well as different resistance pathways, complicates HIV-2 therapy. This is particularly problematic for second-line and salvage therapy. For treatment-naïve individuals, standard combination therapy with boosted lopinavir has the best clinical data supporting it, though selected other boosted protease inhibitors (saquinavir, darunavir, and possibly indinavir) may also be used (Peterson et al. 2011b; Camacho 2012). With appropriate antiretroviral therapy, immunologic recovery appears comparable in HIV-2 and HIV-1 infections, although recovery may initially be slower in HIV-2 (Drylewicz et al. 2010; Peterson et al. 2011a).

Conclusion

HIV-2 infection is mechanistically very similar to HIV-1 infection. It shares the same modes of transmission and generally infects the same cells. HIV-2 is a less fit virus in humans than HIV-1, and the immune system is better able to exert some control over it. Only a minority of HIV-2-infected individuals generate high levels of viremia. As a consequence of this diminished viremia, most people infected with HIV-2 will experience a much slower rate of immunologic decline, present with HIV-related symptoms later and at an older age, and have a lower mortality rate than those infected with HIV-1. For a given CD4+ T-cell count, the clinical manifestations of HIV-2 are

indistinguishable from those of HIV-1, although the data in HIV-2 are much more limited. The only opportunistic condition for which strong evidence of a difference between HIV-1 and HIV-2 exists is Kaposi's sarcoma. This appears to be much less common in HIV-2-infected individuals after controlling for CD4+ T-cell count.

Causal inferences about differences in clinical presentation between HIV-2 and HIV-1 are limited by a paucity of data and epidemiological confounders. As HIV-2 is endemic to West Africa, regional disease epidemiology is a potential confounder, both in studies conducted in West Africa and in studies conducted elsewhere with emigrants and travelers from West Africa. And, as demonstrated by the two high-quality studies on cervical cancer mentioned above, prospective studies are needed to minimize the effects of subject age and length of HIV infection on differences in clinical presentation between HIV-2 and HIV-1.

Clinical management of individuals infected with HIV-2 differs from HIV-1. While issues with HIV-2 testing, treatment, and monitoring are covered in depth elsewhere, the natural history of HIV-2 bears on its clinical management in several ways. The threshold for HIV testing needs to remain low in HIV-2 endemic areas or where there is a plausible risk of HIV-2 infection, even among those with no HIV-related symptoms and no recent history of potential HIV-2 exposure, as infections may only manifest themselves many years after initial infection. The variable course of HIV-2 infection argues for effective monitoring, in particular the use of viral loads to determine prognosis. Slower declines in CD4+ T-cell counts and the more limited antiretroviral armamentarium could be taken as arguments for deferred antiretroviral treatment of HIV-2. On the other hand, deferring therapy prolongs the risk of transmission, and recovery of CD4+ T-cell counts on therapy is blunted at lower CD4+ T-cell counts, suggesting earlier initiation may be needed in order to achieve a satisfactory level of immune reconstitution.

Although its prevalence appears to be dropping and it is less rapidly progressive than HIV-1, HIV-2 continues to be transmitted and its variable course includes aggressive disease indistinguishable from

HIV-1. Testing, monitoring, and treatment of HIV-2 infection also have specific challenges, discussed in detail elsewhere. For these reasons, public health authorities and treating physicians cannot afford to be sanguine about HIV-2.

Cross-References

- ▶ [Antibody Response to HIV-2](#)
- ▶ [Cellular Immune Response to HIV-2 Infection](#)
- ▶ [Cervical Cancer and HIV](#)
- ▶ [Community Viral Load](#)
- ▶ [Epidemic Kaposi Sarcoma, Pathogenesis and Presentation](#)
- ▶ [Epidemiology of HIV-2 Infection in West Africa](#)
- ▶ [HIV-2 Diagnosis and Viral Load Measurements](#)
- ▶ [HIV-2 Infection in Europe, Epidemiology of](#)
- ▶ [HIV-2 Infection: The Role of Immune Activation in Pathogenesis](#)
- ▶ [HIV-2 Transmission](#)
- ▶ [Immune Activation and HIV Transmission](#)
- ▶ [Immunological Responses to Antiretroviral Therapy](#)
- ▶ [Interactions Between HIV-2 and Host Restriction Factors](#)
- ▶ [Long-Term Nonprogressors and Elite Controllers](#)
- ▶ [Natural Killer Cells Function and Innate Immunity in HIV-2 Infection](#)
- ▶ [Overview: Immunopathogenesis](#)
- ▶ [Tat Expression and Function](#)
- ▶ [Transmission HIV-2: Origin, Epidemiology, and Phenotype](#)
- ▶ [TRIM5 Alpha and HIV-2 Infection](#)
- ▶ [Update on HIV-1 and HIV-2 Dual Infection](#)

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Natural Killer Cells and Their Role in Preventing HIV-1 Transmission

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Definition

Despite the enormous efficacy of HIV-1 transmission resulting in a worldwide epidemic, the virus is extremely vulnerable in the brief period between initial HIV-1 exposure and establishment of systemic infection with most HIV-1 infections being prematurely abrogated by the innate immune defenses of the human host. Evidence is accumulating that natural killer (NK) cells play an important role in mediating this innate resistance to HIV-1 infection. NK cells are innate lymphocytes in the first line of defense against viruses capable of rapidly killing virus-infected cells without prior sensitization. NK cells also modulate the ensuing adaptive immune responses by releasing cytokines and promoting dendritic cell maturation. Genetic studies of highly HIV-exposed but seronegative (HESN) subjects consistently showed that these individuals are enriched for NK cell receptor repertoires with an NK cell-activating profile. While functional studies of NK cells are starting to unravel the mechanisms behind these genetic associations, ex vivo analyses frequently identified signatures of

activated NK cells in HESN individuals. Recent investigations of the mucosal genital immune environment, however, show that protection against HIV-1 infection depends on a delicate balance of protective and detrimental consequences of innate immune activation and of NK cells in particular. Further research will have to unravel the specific components of this balance before seeking to apply the HIV-1 preventive potential of NK cells in immune-based medical interventions.

Introduction

HIV-1 has been remarkably successful since it was first transmitted from a SIV-infected chimpanzee to a human host in West-Central Africa around the beginning of the twentieth century. By the 1960s, the virus had steadily made its way to the local urban centers from where it started seeding the worldwide HIV-1 epidemic. To date, HIV-1 has infected approximately 60 million people of whom 30 million have died, making it the most devastating infectious disease of the recent times. The successful spreading of HIV-1 around the world is remarkable given its actually rather low transmission efficiency per unit of exposure. Sexual exposure to HIV-1, which accounts for the large majority of HIV-1 infections, has a transmission probability between 1/100 and 1/1,000. Although several factors are known to increase the risk of “► [HIV-1 sexual transmission](#)” like a high viral load of the index partner, concurrent sexually transmitted infections, anal vs. vaginal and receptive vs. insertive intercourse, and lack of male circumcision, the prevailing view is that the defense mechanisms of the human genital tract are very effective most of the time in preventing HIV-1 infection (Haase 2011). This is supported by single-genome-amplification studies showing that the majority of acute HIV-1 infections start with only a single HIV-1 virus clone that was capable of breaking through the genital defense barriers.

Detailed investigations of the first events following HIV-1 exposure are difficult to perform in humans, but SIV infection experiments of

nonhuman primate species like rhesus macaques have been highly informative in that regard (Haase 2011; Keele and Estes 2011) (also see “► [Nonhuman Primate Models of HIV Transmission](#)”). Both cell-free virus particles and virus-infected cells present in genital secretions contribute to HIV-1 transmission. Infected cells could be notably efficient mediators of transmission because they can continue releasing virus while penetrating deeply into the recipient's tissues. In case the virus succeeds in crossing the mucosal barriers of the genital tract, its next challenge is to get access to suitable submucosal target cells that support viral replication and dissemination in the recipient host (see “► [Cellular Cofactors for HIV-1 Transcription](#)”). Mucosal Langerhans cells, dendritic cells, and macrophages take up viral particles, which are either processed endogenously for the stimulation of the innate and adaptive immune responses or passed on to infect other target cells, but they cannot be productively infected themselves. Reproductive tract histological studies of acutely infected rhesus macaques show that the first productively infected target cells are CD4+ T cells residing in the submucosal layers, a finding that is consistent with the obligate CD4+ T cell/CCR5-tropic nature of isolated transmitted/founder viruses. Sufficient numbers of HIV-1-permissive CD4+ T cells have to be present in the submucosa to sustain a beginning infection, which can either result from prior mucosal immune inflammation for instance due to another sexually transmitted infection or from newly arriving activated CD4+ T cells in response to the developing innate immune response (see “► [Immune Activation and HIV Transmission](#)”). The virus first replicates in mucosal CD4+ T cells for about a week, establishing small founder populations of infected cells which progressively expand in size and number. After that, the virus spreads to the local lymph nodes where it suddenly has access to large numbers of permissive CD4+ T cell targets which go on to traffic through the blood circulation and into the tissues thereby causing an irreversible systemic infection.

Accumulating evidence suggests that adaptive immune responses like B cell responses, CD4+

T cell responses, or CD8+ T cell responses cannot prevent infection because they require priming and proliferation and only develop well after HIV-1 has established itself as an irreversible systemic infection (Picker et al. 2012). Even anamnestic HIV-1-specific T cell responses which develop more rapidly after a prior exposure to the virus require days to expand and come too late to prevent infection. Although the adaptive immune responses eventually reduce the HIV-1 viral load levels in the blood and contribute to viral control in at least some individuals (see “► Long-Term Nonprogressors and Elite Controllers”), they are incapable of clearing the virus from the body because of viral immune escape. In fact, error-prone HIV-1 replication rapidly results in the establishment of a swarm of viral variants soon after systemic infection from which new escape mutants are perpetually selected. In addition, HIV-1 has evolved numerous mechanisms that actively deregulate the antiviral immune responses (see “► Overview: Immunopathogenesis”). Together with a progressive decline in CD4+ T cells, this contributes to the exhaustion of the adaptive immune responses eventually leading to immunodeficiency and death (see “► HIV-Associated Immune Exhaustion”).

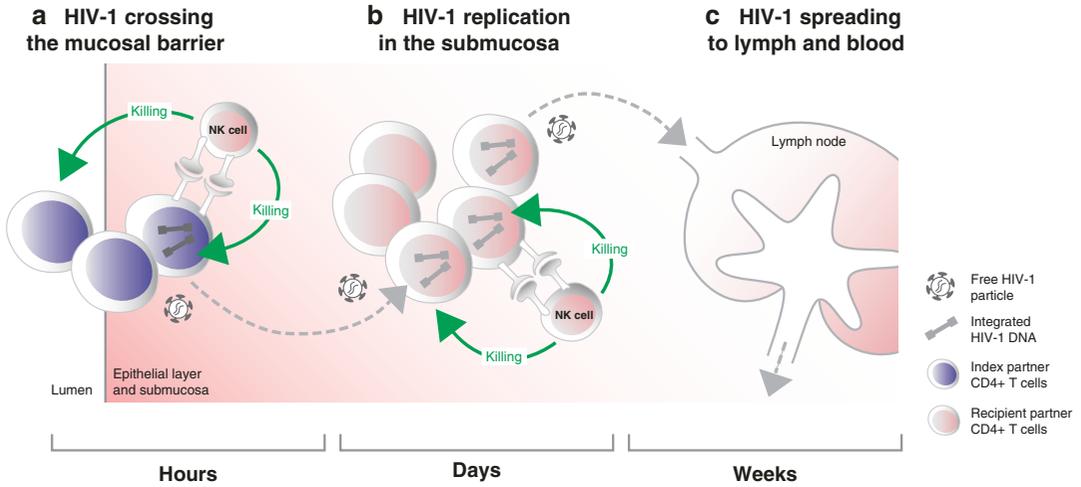
The innate immune response to HIV-1 likely plays a much more important role in determining the outcome of HIV-1 exposure because of its capacity to respond immediately without requiring priming or proliferation (Tomescu et al. 2011; Ploquin et al. 2012). Mucosal genital secretions contain several low-molecular-weight innate proteins with potent antiviral activity, e.g., defensins, elafin/trappin-2, and several protease inhibitors (see “► Mucosal Immunity to HIV-1”). Mucosal plasmacytoid dendritic cells that recognize incoming HIV-1 particles via Toll-like receptor-7 (TLR-7) and TLR-9 rapidly respond by secreting innate cytokines like IFN-alpha, IL-15, and IL-18. IFN-alpha induces the expression of several innate cellular factors like APOBEC3G, TRIM-5alpha, and tetherin that restrict HIV-1 replication in potential target cells. The innate cytokines also result in the activation of a specific innate cytotoxic cell subset, the natural killer (NK) cells. NK cells can directly kill virus-infected cells, and they secrete cytokines like IFN-gamma and TNF-alpha

that in turn induce the maturation of mucosal dendritic cells, thereby regulating their functions towards adaptive immunity. At the same time, however, IFN-alpha starts a cascade of local immune inflammation resulting in a mucosal influx of immune mediators including activated CD4+ T cells which can subsequently become the target cells for HIV-1 propagation (see “► Immune Activation and HIV Transmission” and “► Inflammatory Cytokines”). This duality can best be illustrated by comparing pathogenic SIV infections in rhesus macaques with ► nonpathogenic SIV infections in African green monkeys. Although acute SIV infection results in a strong upregulation of IFN-alpha-stimulated genes in both species, the expression levels rapidly return to basal levels only in African green monkeys thereby preventing pathogenic ► chronic immune activation. Thus, the right balance of protective and detrimental effects of the innate immune response appears crucial towards preventing HIV-1 infection and disease pathogenesis.

Two early steps of the HIV-1 transmission process are likely extremely vulnerable to the innate immune defenses and to NK cells in particular (Fig. 1). These viral Achilles' heels include the initial transfer of free virus or virus-infected cells across the mucosal epithelial barrier and the subsequent viral replication in submucosal founder populations of infected cells before spreading to the local lymph nodes. During these steps, the number of virus-infected cells is still sufficiently low to be efficiently targeted by the innate immune responses. Knowing that the physical barriers of the human genital tract are already very effective in preventing HIV-1 infection, a potent innate immune defense could certainly tip the balance in favor of the host to significantly reduce incidence (Keele and Estes 2011). In this chapter we focus on NK cells and the existing evidence regarding their role in preventing HIV-1 transmission.

Receptor/Ligand Regulation of NK Cells

NK cells play an important role in the innate immune response against virus infections, either



Natural Killer Cells and Their Role in Preventing HIV-1 Transmission, Fig. 1 Opportunities for NK cells to prevent HIV-1 transmission early after exposure. Two early steps of the HIV-1 transmission process are likely extremely vulnerable to NK cells. These include the initial transfer of free virus or virus-infected cells over the mucosal epithelial barrier (a) and the subsequent viral replication in submucosal founder populations of infected cells (b).

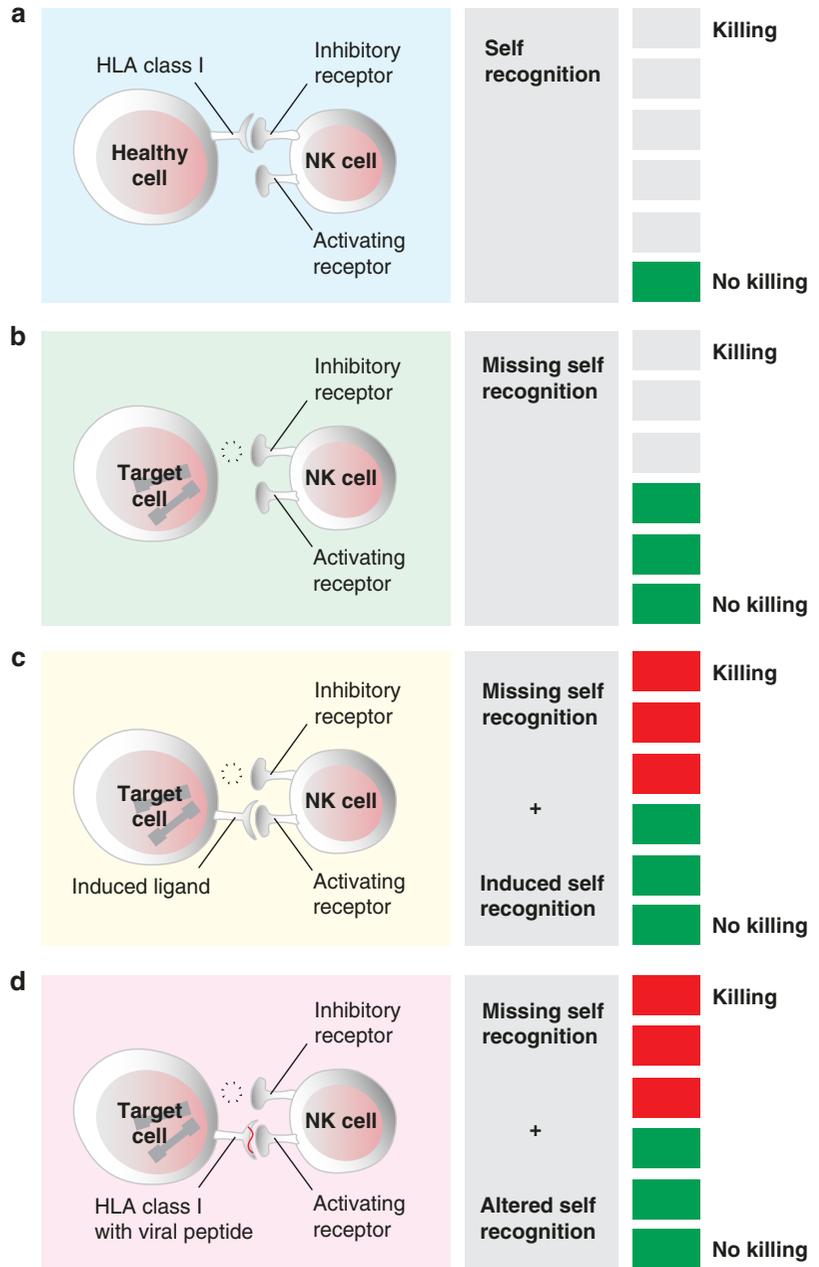
During these steps, the number of virus-infected cells is still sufficiently low to be efficiently targeted by NK cell responses. This is no longer true when the virus reaches the local lymph node (c), where it has access to large numbers of permissive CD4+ T cell targets which go on to traffic through the blood circulation and into the tissues thereby causing an irreversible systemic infection

by directly killing infected cells or by modulating the other arms of the innate and adaptive immune response. NK cells are regulated by complex sets of activating and inhibitory cell surface receptors that interact with ligands on potential target cells and the integration of all signals determines whether the target cell will be killed or not (Lanier 2005) (Fig. 2). NK cell receptors belong to different receptor families with different genetic and structural characteristics. The best studied NK cell receptors are the killer immunoglobulin-like receptors (KIR) which belong to the immunoglobulin superfamily and consist of 15 different inhibitory and activating genes (see “► [KIR Locus Variation](#)”). The number and kind of KIR genes vary between individuals, individual KIR genes themselves are polymorphic, and KIR genes are randomly expressed on the surface of a given NK cell, resulting in enormous variation in KIR expression both at the individual and population level. Several inhibitory KIR recognize classical HLA class I molecules (see “► [MHC Locus Variation](#)”) generally expressed on healthy cells. This interaction

results in NK cell sparing of healthy tissue but in loss of NK cell inhibition towards cells that have downregulated HLA class I expression, e.g., on virus-infected cells, a process termed “missing self-recognition” (Fig. 2). The ligands of the activating KIR are less well characterized but are often stress molecules expressed on aberrant or virus-infected target cells thereby inducing NK cell activation in a process termed “induced self-recognition” (Fig. 2). Activating KIR were also shown to recognize HLA class I molecules presenting viral peptides, termed “altered self-recognition” (Fig. 2). A second important set of NK cell receptors belong to the C-type lectin-like family and include heterodimers of CD94 with either an inhibitory receptor, NKG2A, or an activating receptor, NKG2C or NKG2E. Unlike the KIR, NKG2 genes are present in most individuals and display limited polymorphism. NKG2 receptors recognize nonclassical class I HLA-E molecules which bind peptides derived from classical HLA class I molecules, allowing them to monitor classical HLA class I expression on potential target cells in parallel with the inhibitory KIR.

Natural Killer Cells and Their Role in Preventing HIV-1 Transmission, Fig. 2

Receptor/ligand regulation of NK cell killing. NK cells are regulated by activating and inhibitory receptors that interact with ligands on potential target cells and the integration of all signals determines whether the target cell will be killed or not. Interaction of inhibitory receptors with HLA class I molecules on healthy target cells delivers strong inhibitory signals to the NK cell (a). This inhibition is lost when NK cells encounter cells that have lost HLA class I expression, e.g., as a result of viral infection. However, such “missing self-recognition” is not sufficient to trigger NK cell killing (b). NK cells require additional activating signals to induce target cell killing. These signals can come from activating receptors interacting with induced self, e.g., virus-induced stress molecules (c), or altered self, e.g., HLA class I molecules presenting viral peptides (d)



Genetic Evidence for NK Cell Protection Against HIV-1 Transmission

Because of their extreme population variability and crucial roles in innate and adaptive immunity, KIR and HLA class I genes are believed to be important determinants of disease outcome at the individual level. This is supported by several

epidemiologic studies showing associations between KIR/HLA genotype and susceptibility to infection, autoimmune and inflammatory conditions, and cancer (Kulkarni et al. 2008). A series of studies of a large North American cohort of HIV-1 infected patients showed that certain gene combinations of activating KIR3DS1, high-expressing inhibitory KIR3DL1 (KIR3DL1^{hi})

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and HLA-Bw4, the HLA class I ligand for KIR3DL1, contribute to improved control of HIV-1 disease (Bashirova et al. 2011). Although it has remained unclear how exactly these gene combinations translate into protective NK cell responses, the KIR3DS1/HLA-Bw4 combination was suggested to induce stronger NK cell killing of HIV-1 infected target cells presenting HIV-1 peptides in the context of HLA-Bw4 (“altered self-recognition”), while the KIR3DL1^{hi}/HLA-Bw4 combination was suggested to better “educate” NK cells for the killing of HIV-1-infected target cells with downregulated HLA-Bw4 (“missing self-recognition”) (further discussed in “► [NKT Cells: Bridging Innate and Adaptive Immunity](#)”). Studies of the role of KIR3DS1, KIR3DL1, and HLA-Bw4 genes in resistance to HIV-1 acquisition made comparable observations (Bashirova et al. 2011; Tomescu et al. 2011). HIV-exposed seronegative (HESN) intravenous drug users and HESN partners in HIV-1 discordant couples from European or Euro-American populations showed increased frequencies of KIR3DL1^{hi}/HLA-Bw4, similar to what was observed in HIV-1 controller patients, as well as of homozygous KIR3DS1, either in the presence or absence of HLA-Bw4. A series of studies analyzing all known activating and inhibitory KIR genes in two African populations of HESN subjects, female sex workers from Côte d’Ivoire and HIV-negative partners in HIV-1 discordant couples from Senegal, could not confirm a direct role for KIR3DS1, probably because of the low frequency of this gene in African populations (Tomescu et al. 2011; Jennes et al. 2006, 2013). These studies, however, found other group B haplotype genes than KIR3DS1 to be enriched in HESN subjects, i.e., KIR2DS2, KIR2DS3, KIR2DL2, and KIR2DL5, at the expense of typical group A haplotype genes like KIR2DL3. Because of extensive linkage disequilibrium in the KIR locus, KIR genes are inherited in two broad haplotypes termed A and B (see “► [KIR Locus Variation](#)”). Group A haplotypes contain fewer and mostly inhibitory KIR genes, while group B haplotypes contain more activating KIR genes, implying that HESN subjects have a more NK cell-activating genetic profile. In

addition, both African HESN populations showed increased frequencies of inhibitory KIR genes in the absence of their cognate HLA ligands, i.e., KIR2DL2 or KIR2DL3 in the absence of HLA-C1 and KIR3DL1 in the absence of HLA-Bw4, combinations that could decrease the threshold for NK cell activation (Jennes et al. 2006, 2013). Polymorphisms in HLA-G and HLA-E, nonclassical HLA class I ligands for KIR2DL4 and NKG2 receptors, respectively, have also been implicated in NK cell-mediated resistance to HIV-1 infection in ways that are similar to those described above. HIV-seronegative African women showed increased frequencies of an HLA-G*0105N null variant, resulting in abrogated KIR2DL4 inhibition and a lowering of the NK cell activation threshold, and of a HLA-E^G variant with higher affinity for viral peptides facilitating “altered self-recognition” (Tomescu et al. 2011). In summary, the available genetic studies so far collectively suggest that resistance to HIV-1 infection is associated with carriage of an NK cell-activating genetic profile. Different NK cell receptor/ligand systems and mechanisms are implicated, possibly reflecting the different ethnic origins and routes of HIV-1 exposure of the studied HESN cohorts. Independent confirmatory studies of the observed genetic associations as well as functional studies investigating their precise mechanisms are required to move this field forward.

Functional Evidence for NK Cell Protection Against HIV-1 Transmission

The first functional data which suggested that NK cells play a role in resistance to HIV-1 infection came from a study of Vietnamese HESN intravenous drug users showing increased levels of NK cell activation, cytokine production, and cytotoxicity when stimulated with NK-sensitive cancer cell lines (Tomescu et al. 2011). Several studies since then have contributed to our further understanding of the functional mechanisms of NK cell-mediated protection against HIV-1 infection. NK cells of HESN subjects consistently displayed increased levels of cellular activation and

degranulation, after *in vitro* stimulation but also when directly tested *ex vivo*, possibly reflecting ongoing innate immune reactivity in response to frequent episodes of HIV-1 exposure. In one study, HESN subjects specifically showed increased mRNA expression ratios of activating KIR3DS1 relative to inhibitory KIR3DL1, and of activating NKG2C relative to inhibitory NKG2A, in line with the activating NK cell receptor genotypes discussed above. *In vitro* studies have started to unravel the functional consequences of the different NK cell-activating genetic profiles that are observed in HESN subjects (Bashirova et al. 2011). Notably, the protective KIR3DS1/HLA-Bw4 genotype was shown to effectively result in increased NK cell killing of HIV-1 infected CD4⁺ T cells, and the protective KIR3DL1^{hi}/HLA-Bw4 genotype was shown to display increased NK functionality towards NK-sensitive cancer cell lines. Another study found that NK cells of HESN subjects make stronger responses towards exogenously HIV-1 infected autologous CD4⁺ T cells, but here KIR/HLA genotype was not taken into account. Thus, in order to unravel the precise functional mechanisms of KIR/HLA genotype in resistance to HIV-1 infection in HESN subjects, comprehensive studies of NK cell-mediated killing of HIV-1 infected CD4⁺ T cells in the context of KIR/HLA genotype are required.

NK cell activation and cytotoxicity depend in part on virus recognition by mucosal plasmacytoid dendritic cells which secrete innate cytokines like IFN- α that stimulate the antiviral innate immune responses. Evidence is accumulating that plasmacytoid dendritic cell functionality and the NK cell-dendritic cell cross talk are important additional correlates of resistance to HIV-1 infection (Ploquin et al. 2012). Plasmacytoid dendritic cells in HESN subjects showed increased levels of maturation and activation and they secreted more cytokines upon specific TLR triggering. In turn, NK cells more efficiently killed the immature dendritic cells, improving the capacity of the dendritic cell compartment to regulate the adaptive immune responses. Interestingly, the increased dendritic cell responses upon TLR triggering in HESN

subjects coincided with lower baseline levels of inflammatory cytokines and CD4⁺ T cell activation in the genital mucosa, illustrating the importance of a precisely tuned and narrowly targeted innate immune response in resistance to HIV-1 infection (Yao et al. 2013). These findings are in agreement with the strong but rapidly controlled IFN- α responses seen during nonpathogenic SIV infection of African green monkeys, like discussed above. Along the same lines, pre-treatment of macaques with IFN- α increased NK cell frequencies and protected against intrarectal SIV transmission, but accelerated SIV disease progression once infected (Sandler et al. 2014). Further investigations at the level of the genital mucosa are warranted to identify all components of a successful antiviral innate immune response.

Most of the evidence supporting a role for NK cells in mediating protection against HIV-1 infection is derived from observational studies of at-risk populations while precisely designed clinical trials confirming their function are lacking. A recent HIV-1 vaccine trial in Thailand, termed RV144, demonstrated for the first time that a vaccine can provide protection against HIV-1 infection (see “► [HIV-1 Transmission Blocking Microbicides](#)”). Although the protection was rather modest, a 31% reduction in new HIV infections among trial participants, the results provided an unprecedented opportunity to investigate the immune mechanisms of vaccine protection. A detailed investigation of immune mechanisms revealed an important role for NK cells and antibody-dependent cellular cytotoxicity (ADCC) in vaccine protection (Haynes et al. 2012). During ADCC, Fc γ receptors (Fc γ RIII also known as CD16) on NK cells bind to the constant domains (Fc) of HIV-specific IgG antibodies that coat the surface of infected cells, thereby triggering a cytotoxic NK cell response. These findings have invigorated HIV-1 vaccine research now focusing on antibody vaccines that are capable of inducing strong ADCC responses. However, the contribution of ADCC to protection against HIV-1 infection in HESN populations is unclear, as HIV-specific IgG antibodies are not expected to be present in these populations.

Alloreactive NK Cells in HIV-1 Transmission

Although NK cells are best known for their potential to lyse virus-infected and tumor cells, they were first discovered because of their cytotoxic activity against allogeneic target cells in the context of transplantation. Recently, the important role of NK cells in killing allogeneic target cells came to the fore again in studies of haploidentical hematopoietic stem cell transplantation as a treatment for acute myeloid leukemia (Moretta et al. 2011). It was found that ► [stem cell transplantation](#) from a haploidentical donor, i.e., matched for only half of the leukemia patient's HLA, often resulted in the emergence of an alloreactive NK cell subset with strong cytotoxic activity against residual leukemia cells, thereby greatly reducing the risk of leukemia relapse. Alloreactivity of NK cells was found to specifically depend on "missing self" KIR/HLA combinations consisting of inhibitory KIR in the donor that recognize a self HLA in the donor but that is missing in the recipient. The requirement for a KIR-specific self HLA in the donor reflects the need for proper NK cell education towards recognition of aberrant or missing HLA on potential target cells. A recent study investigated whether comparable allogeneic KIR/HLA combinations and NK cell alloreactivity play a role in HIV-1 transmission and lack thereof among African couples who are either HIV-1 concordant (phylogenetically confirmed HIV-1 transmission from one partner to the other) or HIV-1 discordant (one partner HIV-1 positive, the other HIV-negative) (Jennes et al. 2013). Such an effect would be plausible, given that incoming HIV-1 infected cells are effective mediators of HIV-1 transmission as well as strong inducers of innate immune activation. HIV-1 discordant couples indeed showed an increased frequency of a specific "missing self" KIR/HLA combination, while HIV-1 concordant couples showed an increased frequency of a specific "matched" KIR/HLA combination. Functional experiments confirmed the involvement of "missing self" as opposed to "matched" KIR/HLA combinations in provoking alloreactive NK cell killing of HIV-1 patient-derived CD4+

T cells. These findings could have important implications for our understanding of NK cell protection against HIV-1 transmission and urgently require confirmation in other cohorts of HIV-1 discordant couples, in the setting of mother-to-child transmission, and by SIV transmission experiments using simian models. These data may also help to understand recent attempts to cure HIV infection in a number of patients by allogeneic stem cell transplantation (Cannon et al. 2014). One patient, referred to as the "Berlin patient", received a CCR5-delta32 homozygous allogeneic stem cell transplant and is HIV free now 6 years after stopping antiretroviral therapy. Two other patients, referred to as the "Boston patients", received CCR5-wild type allogeneic stem cell transplants resulting in temporary viral remissions lasting 3 and 8 months off therapy, respectively. Although the exact mechanisms contributing to HIV control are unknown, the possibility that alloreactive NK cells as part of the graft versus host reaction have contributed to HIV remission certainly warrants further investigation.

Conclusions

Despite the enormous efficacy of HIV-1 transmission resulting in a worldwide epidemic, the virus is highly vulnerable during sexual acquisition and the first few days thereafter with most HIV-1 infections actually being prematurely abrogated by the innate immune defenses. Evidence is accumulating that NK cells play an important role in mediating this innate resistance to HIV-1 infection. Genetic studies of HESN subjects consistently show that these individuals are enriched for NK cell receptor repertoires with an NK cell-activating profile and functional studies are starting to unravel the precise mechanistic implications of these associations. Promising new findings are pointing towards antibody-dependent cell-mediated cytotoxicity and NK cell alloreactivity as additional mechanisms of NK cell protection against HIV-1. Based on these findings and on investigations of the mucosal genital immune environment of HESN subjects and nonhuman primate species, it is becoming

increasingly clear that resistance to HIV-1 infection depends on a delicate balance of protective and detrimental consequences of the innate immune defenses and of NK cell responses in particular. Further research will need to understand the specific components of this balance before seeking to apply the HIV-1 preventive potential of NK cells in immune-based medical interventions.

Cross-References

- ▶ [Cellular Cofactors for HIV-1 Transcription](#)
- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [HIV-1 Mutational Escape from Host Immunity](#)
- ▶ [HIV-1 Transmission Blocking Microbicides](#)
- ▶ [HIV-1 Transmission: Influence of Bodily Secretions](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [Immune Activation and HIV Transmission](#)
- ▶ [Inflammatory Cytokines](#)
- ▶ [KIR Locus Variation](#)
- ▶ [Long-Term Nonprogressors and Elite Controllers](#)
- ▶ [MHC Locus Variation](#)
- ▶ [Mucosal Immunity to HIV-1](#)
- ▶ [NKT Cells: Bridging Innate and Adaptive Immunity](#)
- ▶ [Nonhuman Primate Models of HIV Transmission](#)
- ▶ [Nonpathogenic SIV Infection of Sooty Mangabeys](#)
- ▶ [Overview: Immunopathogenesis](#)
- ▶ [Stem Cell Transplantation](#)

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Natural Killer Cells Function and Innate Immunity in HIV-2 Infection

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Definition

This entry describes what is known about the function and activity of the innate immune cell populations natural killer (NK) cells and natural killer T cells (NKT) in HIV-2 infection.

Introduction

In contrast to the extensive efforts made to learn more about HIV-1 in the fields of epidemiology, geographical distribution, and viral pathogenesis, very little work has been carried out to understand the interactions between the HIV-2 and the host immune system. Although both cellular and humoral immune responses to HIV-1 have been shown to play a part during acute infection, these activities are impaired during chronic infection, leading to disease progression and eventually death (Zhang et al. 2003).

Studies of HIV-2-infected individuals have shown that the majority experiences a slower and more prolonged disease course than seen in HIV-1 infection: therefore, a better understanding of the interactions between HIV-2 and the host immune response may be useful in providing insights for therapeutic and vaccine strategies. One way viruses are attacked early in infection is through the action of natural killer (NK) cells, which are one of the key components of the innate immune response to infections (Biron and Brossay 2001). These cells are naturally activated in response to infection without the requirement for prior sensitization and may play an important role in the outcome of viral infections. NK cells provide the first line of defense in the early stage of many infections, including HIV, and continue

to render a critical service in nonspecific host defense mechanisms. However, little is known about NK cell function in HIV-2 infection.

Natural Killer Cells

Natural killer (NK) cells are large granular lymphocytes forming about 15% of the total lymphocyte population. There are two main subpopulations of NK cells. These are CD56^{bright}, which produce cytokines and form about 10% of the NK cell population (Cooper et al. 2001), and CD56^{dim}, which exhibit cytotoxicity and form about 90% of the NK cell population. They respond to bacterial, parasite, and viral infection, as well as to tumor cells, without the need for prior antigen sensitization. Early studies showed evidence that the nonadaptive immune response mediated by NK cells played a role in bacterial, parasite, tumor, and viral immunity (Fehniger et al. 1998). NK cells exert their activities by the use of both inhibiting and activating receptors expressed on their surface. In addition to direct stimulation through activating receptors, NK cells are indirectly activated during the initial stages of viral infection by cytokines secreted by various cells of the immune system (Biron and Brossay 2001). Infection stimulates the direct production of innate cytokines such as interferon (IFN)- α , IFN- β , interleukin-2, interleukin-12, and interleukin-15. In response to these cytokines, NK cells release cytolytic granules that directly lyse infected cells. They also rapidly produce IFN- γ , tumor necrosis factor alpha (TNF- α), granulocyte-monocyte colony-stimulating factor (GM-CSF), and chemokines such as macrophage inflammatory protein-1 α (MIP-1 α), MIP-1 β , and RANTES to prevent further infection of new cells and also suppress viral replication in already infected cells (Scott-Algara and Paul 2002). As a result, the cytokines and chemokines produced during viral infection in vivo may serve not only to induce an antiviral state in nearby cells but also to enhance NK cell activity within circulating leukocytes. In addition, the activated NK cells play a role in modulating hematopoietic cell growth, reviewed in Cooper et al. (2001), and their functions further enhance the adaptive immune response.

Natural Killer Cell Development

Natural killer cells develop within the microenvironment of the bone marrow (BM) with CD34+ hematopoietic stem cells expressing growth factors (c-KIT and FLT31), cytokine receptors (IL2R β and IL-15R α), as well as other lymphoid development transcriptional factors (Yu et al. 1998).

Numerous studies have identified IL-15 as the most important soluble factor for the development of human and murine NK cells (Cooper et al. 2001). Interleukin-15 induces differentiation of human hematopoietic progenitor cells (HPCs) into NK cells in the absence of other cytokines *in vitro*. Interleukin-2 was also identified to play a role with IL-15 in inducing the differentiation of bipotential cells into NK cells, whereas IL-7 and stem cell factor (SCF) support further maturation and differentiation of NK cells. The recently described cytokine, IL-21, in combination with IL-15, was also reported to induce the development of CD56+16+ NK cells from BM HPCs (Parrish-Novak et al. 2000). The induction of IL-15 results in the development of NK cells that are CD56^{bright} cells (Cooper et al. 2001). These produce immunoregulatory cytokines or chemokines upon stimulation. The development of CD56^{dim} cells might have a distinct precursor that has not yet been identified.

Location and Subsets of Natural Killer Cells

As well as forming about 15% of circulating lymphocytes, NK cells have a similar frequency in the spleen. Natural killer cells have also been identified in the lungs, gastrointestinal tract, and liver, together with populations of NKT cells at these sites (Norris et al. 1999). They are however not commonly found in unstimulated lymph nodes and do not circulate through the lymphatic system.

The cell surface density of the CD56 receptor allows classification of NK cells into two functionally distinct NK cell subsets. About 10% of NK cells are CD56^{bright} CD16+ (Cooper et al. 2001) and the majority (90%) are CD56^{dim}CD16+, exhibiting high cytotoxic activity.

The CD56^{bright} NK cells secrete high levels of cytokines that are useful in preventing viral

infection after stimulation with monokines (Cooper et al. 2001). They show low-density expression of CD16 and are of low natural cytotoxicity as well as low antibody-dependent cellular cytotoxicity (ADCC) (Cooper et al. 2001). They also exhibit potent lymphokine-activated killer (LAK) activity. The expression of inhibitory receptors also varies in this subset of NK cells. The inhibitory CD94/NKG2A C-type lectin NK receptors (NKR) are highly expressed but they have low expression of killer immunoglobulin-like receptors (KIR). The cytokine and chemokine receptors expressed include high-affinity interleukin-2 receptors (IL-2R $\alpha\beta\gamma$), c-kit, and CC-chemokine receptor 7 (CCR7) (Andre et al. 2000). The adhesion molecule L-selectin, in combination with CCR7, is involved in trafficking to secondary lymph nodes and is predominantly found on CD56^{bright} NK cells.

In contrast CD56^{dim} NK cells have a more granular morphological structure than CD56^{bright} NK cells and produce lower levels of NK-derived cytokines (Cooper et al. 2001). They are potent mediators of ADCC, LAK activity, and natural cytotoxicity. The CD56^{dim} NK cell subsets have low levels of expression of cytokine receptors (e.g., IL-2R $\beta\gamma$) and chemokine receptors (e.g., CXCR1 and CX3CR1) (Campbell et al. 2001). They however lack L-selectin but highly express PEN5-P-selectin glycoprotein ligand-1 (PSGK-1) adhesion molecules.

Natural Cytotoxicity Receptors

There are other distinct receptors that have been identified on the surface of NK cells. They allow NK cells to recognize their target cells in a manner that is quite different from that of T cells and B cells, which depend on the presence of foreign antigen. These include the natural cytotoxicity receptors (NCRs): NKp30, NKp44, and NKp46. NKp 30 and NKp46 are uniquely expressed on resting NK cells, and their blockage impairs lysis of target cells by NK cells (De Maria et al. 2001). Their ligands, however, have not yet been identified, although it has recently been suggested NKp30 and NKp46 interact with dendritic cells and hemagglutinin, respectively (Ferlazzo et al. 2002). NKp44 is expressed when NK cells are

activated and facilitates killing of target cells during NK cell activation.

Other receptors called killer cell immunoglobulin-like short-tail (KIR-S) activating receptors in humans also contribute to the cytolytic activity of NK cells. Most activating NK cell receptors are transmembrane molecules with short cytoplasmic domains that lack intrinsic signaling features. They are associated with adaptor signaling proteins through charged amino acid residues of their transmembrane domains. The activating receptors KIR2DS, Ly49D/H, NKp44, and CD94/NKG2C associate with KARAP/DAP12 signal-transducing adaptor proteins (LaBonte et al. 2006), whereas CD16, NKp30, and NKp46 couple to the Fc ϵ R1 γ and CD3 ζ transmembrane adaptor proteins. The activating receptors recognize both MHC Class I and non-MHC Class I ligands. There are also a number of other coreceptors, which are implicated in the activation of NK cells. These include 2B4 (CD244) NKp80 and NTB-A (Bianconi et al. 2001) and CD48, CD58, and CD84 and SLAM/CD150 belonging to the CD2 subfamily of Ig-SF. They work in combination with NCRs in mediating NK cytotoxicity.

Natural Killer Inhibitory Receptors

Natural killer cells use a set of activating and inhibitory receptors to identify cells that lack MHC Class I expression. The balance between the engagements of these receptors by the target cells may result in cytolysis. Thus, NK cells do not lyse healthy MHC Class I positive cells because the presence of MHC Class I molecules usually engages inhibitory receptors on NK cells. The upregulation or downregulation of MHC Class I molecules, as may occur in tumors or infected cells, plays a major role in the ability of NK cells to lyse infected or target cells or become inhibited from cytolysis, depending on which type of these molecules is engaged by NK cells.

There are three different groups of inhibitory receptors identified for classical MHC Class I molecules on target cells. The first family of the inhibitory receptors is the killer cell immunoglobulin-like receptors (KIRs) with different numbers of immunoglobulin-like domains

(Borrego et al. 2002). They include CD158a (KIR2DL1), CD158b (KIR2DL2), CD158e (KIR3DL1), and CD158k, which recognize different alleles of HLA-A, HLA-B, and HLA-C molecules. The second is the leucocytes Ig-like receptor (ILT2/LIR1/CD85j) family found in human cells. The third family of inhibitory receptors is the lectin-like heterodimers, represented by CD94/NKG2, which are found in both rodents and human.

Natural Killer Cell Activity in Viral Infection

Natural killer cells are important in controlling intracellular pathogens. In viral infection, NK cells provide an important early defense against pathogens, while T cells, which are responsible for adaptive immunity, are undergoing a process of activation. The importance of NK cell functions in the defense against pathogens was appreciated from observations such as in an adolescent case of a selective NK cell defect which led to recurrent herpes virus infection in the patient (Biron and Brossay 2001). Loss of NK cell function also resulted in severe infection by cytomegalovirus (MCMV) in mice (Biron and Brossay 2001). Thus, NK cells play a major role in controlling microbial infections at a very early stage in infection before the adaptive immune response mediated by T lymphocytes sets in.

One way in which NK cells control infection is by direct lysis of infected cells. This involves the release of cytolytic granules, perforin, and granzyme A in close contact with the infected cells. However, it has been shown in CMV infection that NK cells could function even in the absence of perforin to mediate cytotoxicity (Seaman 2000), acting through the secretion of soluble factors which enhance the immune response of the host. These factors include cytokines such as IFN- γ , TNF- α , IL-10, IL-13, and GM-CSF. The production of such effector cytokines by NK cells is initially induced by early innate cytokine secretion in response to infection, such as IL-12, IL-15, and IL-21 (Cooper et al. 2001), and plays a major role in the early development of antiviral immune responses by recruiting other cells of the immune system to the sites of infection. These early innate-induced

cytokines not only activate NK cells but also stimulate their development and differentiation.

Natural killer cells also produce substantial amounts of IFN- γ in response to pathogenic/viral infections (He et al. 2004). This cytokine promotes a Th1 response, which in turn promotes adaptive cellular immunity as well as the effective production of antibodies that fix complement, all of which are important in host defense against intracellular infections. Moreover, NK cells are also known to support the production of cytokines that promote Th2 responses, which include the production of IgE and IgG4 and the activation of eosinophils. The capacity of NK cells to secrete IL-5 has been shown to be important in the eosinophilic response to allergen in the peritoneal cavity. Allergen-induced eosinophilic airways inflammation in the mice is also under the influence of IL-5 produced by NK cells (Kiessling et al. 1977). Thus the promotion of Th2 responses by NK cells contributes to host defense against extracellular parasites. NK cells also attack viral-infected cells by the use of fas ligands and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors as well as soluble TRAIL (Hayakawa et al. 2004).

The extent of NK cell activation is known to vary in different infections. Infection of mice with murine CMV promotes the production of IL-12, IFN- $\alpha\beta$, and IL-18 by dendritic cells (DC) that enhances IFN- γ secretion by NK cells to defend the host (Andrews et al. 2003), with IFN- $\alpha\beta$ mainly required for cytotoxicity. In contrast, infection with lymphocytic choriomeningitis virus (LCMV) activates NK cells through the release of IFN- $\alpha\beta$ mainly from DC and other monocytes which promote natural cytotoxicity. Infection with this virus actually suppresses the production of IL-12 by DC and subsequently the secretion of IFN- γ by NK cells respectively (Biron and Brossay 2001), making the role of adaptive cytokine production less important in the control of LCMV.

Type I interferons are the primary cytokines that provide the first line of immune defense against viral infections and are known to enhance the activity of NK cells during infection. They are encoded by 13 α -genes that are responsible for the

secretion of 12 IFN- α subtypes and one β -gene for the IFN- β cytokine (Smith et al. 2005). Infection of mice with murine cytomegalovirus (MCMV) induces innate cytokine IFN- $\alpha\beta$ secretion within 2 days of infection that stimulates NK cell function by promoting cytolytic activity as well as IFN- γ secretion. IFN- γ secretion in turn enhances T-cell polarization in the lymph node leading to an expansion of CD4⁺ T cells during infection. This demonstrates the cascade effect of the innate immune response in the early control of viral infection.

NK Cell Function in HIV-1 Infection

HIV-1 infection is associated with significant changes in the distribution of NK subsets in the circulation, together with the appearance of a dysfunctional subset of NK cells that are CD3-CD56-CD16⁺ and are rarely found in healthy individuals (Alter and Altfeld 2009). It is thought that accumulation of this aberrant subset accounts for the decline in NK function observed with HIV-1 disease progression. Generally, HIV-1-infected individuals with long-term nonprogression exhibit normal values of NK cells as well as normal NK functional activity (Scott-Algara and Paul 2002). A restoration of NK cell numbers has also been noted in individuals on highly active antiretroviral therapy (HAART) (Parato et al. 2002), which may support the hypothesis that continued HIV-1 replication plays a role in impaired NK cell function with disease progression. It has been suggested that infection of NK cells by the virus may favor the persistence of the virus in the host (Valentin et al. 2002). Viral proteins may also contribute to the inhibition of NK cell activities. Whereas the *env*, *nef*, and *vpu* proteins induce a strong reduction in the lytic activity of NK cells (Kottlilil et al. 2006), the *tat* protein blocked L-type calcium channels, thus interfering with NK cell activation. High viremia has been associated with the upregulation of inhibitory and downregulation of activating receptor expressions on the surface of NK cells (Kottlilil et al. 2006). Nevertheless, the mechanisms underlying the low activity and the deregulation of receptor expression of NK cells may be complex, as cytolytic activity has also been observed to correlate with low plasma viral load in some cases (Ahmad et al. 2001).

Enhanced cytolytic activity by NK cells with increased levels of perforin and granzyme A expression has been demonstrated in HIV-1-infected patients following IFN- α administration (Portales et al. 2003). The administration of IL-12 has also been used in early HIV-1 infection to increase the number of NK cells and their activity in patients. A reduced level of IL-15 may contribute to the impaired function of NK cells during chronic infection. However, in vitro costimulation of infected cells with IL-12 and IL-15 resulted in suppression of viral replication by NK cells that produce CC chemokines (Ferlazzo and Munz 2004). Interleukin-18 is produced by macrophages and has an antiviral effect, which is mainly mediated by inducing IFN- γ secretion from NK cells (Pien et al. 2000).

HIV-2 Infection

There are close similarities between the genetic structure of HIV-1 and HIV-2, the main difference being that HIV-2 expresses a *vpx* gene which in HIV-1 is *vpu* (Guyader et al. 1987). HIV-2 is closely related to SIV_{sm} which infects sooty mangabeys in West Africa, overlapping with the distribution of the HIV-2 epidemic. Currently, eight HIV-2 groups, A–H, have been reported, thought to have arisen from eight different cross-species transmissions from sooty mangabeys to humans (Chen et al. 1997), analogous to the HIV-1 groups. Unlike HIV-1 with various epidemic subtypes and circulating recombinant forms (CRFs), HIV-2 is characterized by an epidemic of only two groups (A and B) and six non-epidemic groups (C–H) (van der Loeff et al. 2006).

The Epidemiology of HIV-2 Infection

HIV-2 was first isolated from three AIDS patients from West Africa (Guinea Bissau, Senegal, and Cape Verde) in 1986 (Brun-Vezinet et al. 1987) and has subsequently been found predominantly in heterosexual populations in West Africa. Outside West Africa, HIV-2 infection also occurs in Angola and Mozambique (De Cock and Brun-Vezinet 1989). Sporadic cases of HIV-2 infection have also been identified in Europe, mainly

Portugal, in the Americas, and in India, but also in other places such as Brazil and South Korea.

In recent years, several surveillance studies have revealed a declining trend in HIV-2 prevalence in the region of origin. In West Africa from 1985 to 1996, HIV-2 infection in blood donors has decreased in nine out of ten nations (Bouckennooghe and Shandera 1999). There was also decline in HIV-2 infection rates in a survey carried out from 1988 to 2003 in 23,363 patients aged 15 years or older in the Gambia from 7.0% to 4.0% (van der Loeff et al. 2006). Between 1988 and 1992, Ivory Coast witnessed a drop in HIV-2 infection rates from 2.6% to 1.5% during a survey of 19,701 women of reproductive age (Eholie and Anglaret 2006). Similar declines have been reported in men in Guinea Bissau, with a decrease in HIV-2 prevalence from 9.1% to 4.7% in a period of 9 years from 1987, accompanied by a decline among pregnant women from 8.3% to 3.3% in 14 years from 1987 (van der Loeff et al. 2006).

Transmission of HIV-2 Infection

Although HIV-2 can be transmitted by the same routes as HIV-1, transmission usually occur through heterosexual contact. HIV-2 can also be vertically transmitted from mother to child as well as through blood transfusions, but transmissions through homosexual contact and in intravenous drug users (IVDU) are not common (van der Loeff et al. 2006). Human immunodeficiency virus type 2-infected patients show lower rates of transmission and lower plasma viral burdens than HIV-1 in infected subjects in asymptomatic stage of the disease. The mortality rate is also significantly lower than HIV-1 in subjects with CD4 counts >500 cells/ μ l but similar at lower CD4 counts (van der Loeff et al. 2006). The plasma viral load is much lower in HIV-2, but the proviral load is similar to patients with HIV-1 at the same stage of disease. This may be due to the increased sensitivity of HIV-2 to the recently described host restriction factor TRIM-5 α (Ylinen et al. 2005), which restricts the activity of retroviruses after cell entry.

HIV-2 Pathogenesis and Progression to Disease

HIV-2 infects cells of the immune system and the central nervous system, resulting in disease that occurs in very similar stages to those found in HIV-1 infection. However, HIV-2-infected individuals have a much slower disease progression and longer survival, with some individuals never progressing to AIDS (Mota-Miranda et al. 2001). The asymptomatic phase lasts for much longer periods in the majority of HIV-2-infected individuals, sometimes more than 27 years. Longitudinal studies in Caio, Guinea Bissau, have indicated that the majority of HIV-2-infected people do not progress to AIDS (van der Loeff et al. 2006).

Toward the last stage of the disease, defined as AIDS, HIV-2-infected individuals show the same clinical manifestations as seen in HIV-1 infection, with a few exceptions. Researchers in Ivory Coast found that extrapulmonary tuberculosis (TB) was less frequent in HIV-2 infection, whereas multi-organ cytomegalovirus (CMV) infections, HIV encephalitis, and cholangitis were more frequent in HIV-2 compared with HIV-1 infection (Abouya et al. 1995). In an 11-year study in Senegal, researchers found that oral candidiasis and chronic fever were more frequent in HIV-1 infection and that bacterial and cryptococcal meningitis was found only in 59 HIV-2-infected patients (Ndour et al. 2000). On the other hand, chronic diarrhea, especially those caused by bacterial infections, were observed more frequently HIV-2 AIDS patients (Ndour et al. 2000). Studies in the Gambia showed that Kaposi's sarcoma (KS) is less frequent in HIV-2 than in HIV-1 infection, but that wasting syndrome was more frequent in HIV-2 (Martinez-Steele et al. 2007).

Natural Killer Cell Function in HIV-2 Infection

Previous studies of adaptive responses in HIV-2 infection have highlighted the strong broadly neutralizing antibody responses in HIV-2-infected subjects (Rodriguez et al. 2007) and the preservation of HIV-2-specific CD4⁺ T-cell function in subjects with a normal CD4⁺ T-cell count

(Duvall et al. 2006), both of which contrast with findings in HIV-1 infection.

Only one study comparing NK cell numbers and function between HIV-1 and HIV-2 infection has been reported. In a cross-sectional study of 30 HIV-1- and 30 HIV-2-infected patients at different stages of disease recruited from clinics in the Gambia, there were similar frequencies of NK cells in both asymptomatic HIV-1- and HIV-2-infected individuals. However, in subjects with a CD4 count in the normal range, NK cell cytolytic activity was significantly higher in HIV-2-infected individuals, in whom it was comparable to that seen in seronegative controls (Nuvor et al. 2006). These differences between HIV-1 and HIV-2 infection were lost in the groups with disease progression as evidenced by lower CD4 counts: this suggests that preserved NK function may play a part in viral control in the long asymptomatic period of HIV-2 infection.

Further studies in these subjects demonstrated higher levels of expression of activating NKp44 and NKp46 receptors on NK cells from HIV-2-compared with HIV-1-infected individuals, while the expression of NKp30 was similar between the groups (V. Nuvor, personal communication). The levels of expression of the inhibitory receptor CD158a were similar between HIV-1- and HIV-2-infected subjects, but there was significantly higher expression of inhibitory receptors p70 and CD158b in HIV-1+ compared to HIV-2+ subjects. The expression levels of the NKG2D receptor and coreceptors, 2B4 and NTBA, were similar in both infections. The upregulation of activating receptors on the NK cells in HIV-2-infected subjects and the absence of the increased expression of some inhibitory receptors seen in HIV-1-infected subjects may explain our previous observations of enhanced NK-mediated cytotoxicity in HIV-2 asymptomatic infection compared to HIV-1-infected donors at the same stage of disease (Nuvor et al. 2006).

NKT Cells in HIV-2 Infection

Another lymphocyte subset, natural killer T (NKT) cells, which express invariant $\alpha\beta$ T-cell

receptors, may also play a role in HIV infection. These cells express NK cell receptors as well as the CD3 marker characteristic of T cells. Natural killer T cells form part of the early response to infection but can also enhance adaptive immunity by activating cytotoxic T lymphocytes through the secretion of Th1/2 cytokines such as IFN- γ , TNF- α , IL-4, IL-10, and IL-13 upon activation (Kotsianidis et al. 2006). These cytokines play a critical role in regulating the immune response: secretion of IL-4 in particular inhibits Th1 responses by inducing a Th2 response, whereas the production of IFN- γ enhances Th1 responses, resulting in an effective adaptive immune response. However, NKT cells are susceptible to HIV-1 infection and are significantly reduced in the peripheral blood of HIV-1-infected individuals with high levels of viremia (Mureithi et al. 2011). CD4⁺ NKT cells appear to be even more susceptible to infection than conventional CD4⁺ T cells. Thus, they are rapidly depleted during disease progression when the viral load is high, whereas CD4⁻ NKT cells are much less affected by HIV-1 infection.

It was noted that a population of NKT cells that express CD4 but are impaired in IFN- γ production was expanded in asymptomatic HIV-1- compared to HIV-2-infected individuals (Nuvor et al. 2012). These cells may serve as target cells for virus infection and replication in HIV-1-infected individuals leading to their subsequent depletion during chronic infection.

HLA-KIR Genotypes in Resistance and Susceptibility to HIV-2 Infection and Disease Progression

The function of individual NK cells is tightly regulated by the combination of inhibitory and activating receptors expressed on their surfaces, which interact with specific MHC Class I molecules and other unidentified ligands. Among these receptors are the killer cell immunoglobulin-like receptor (KIR) groups, which are categorized according to the number of immunoglobulin-like domains they encode (Borrego et al. 2002; Moretta et al. 2001). The binding of these receptors usually transmits inhibitory signals to NK cells, preventing their activity. However, when

there is downregulation of MHC or altered MHC Class I expression, as observed during HIV-1 infection (Hultstrom et al. 2004), the inhibitory receptors are not engaged by their MHC ligands, allowing the activation of NK cells and subsequent lysis of their target cells. Certain KIR gene products have been strongly implicated in the control of HIV-1 (Carrington et al. 2008), for example, KIR3DS1 in combination with ligands HLA-B Bw4-80 has been shown to confer protection against rapid progression to AIDS in HIV-1 infection (Martin et al. 2002). A recent report from Yindom et al. analyzed the relationship between KIR and HLA alleles with susceptibility and resistance to HIV-2 infection in a community cohort in Caio, Guinea Bissau (Yindom et al. 2010). This cohort was of Manjako ethnicity and was found to have more activating KIRs than seen in other W. African populations, particularly KIR3DS1. Although, in this cohort, there were no significant associations between KIR genotypes and HIV-2 disease progression, certain compound genotypes of KIR and HLA (KIR2DS2 and KIR2DL2 with at least one HLA C allele of the C1 group) were with resistance to HIV-2 infection, raising the possibility of a contribution of NK cell activity to preventing HIV-2 infection.

Conclusion

Taken together, the published data suggest that the early innate immune function of NK cells may contribute to both resistance to HIV-2 infection and long-term viral control. Adaptive cellular responses are much better preserved in asymptomatic HIV-2-infected compared to HIV-1-infected subjects, and it may be that the interactions between NK, NKT, and T cells make an important contribution to this phenomenon.

Although the NK cell function appears to be preserved in asymptomatic HIV-2-infected individuals, further studies to explore the contribution of NK cells to immune control and determine the underlying mechanisms. These efforts will complement the current search for effective vaccines and immunotherapy for HIV-1 infection.

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Nef/Env/Vpu/Tetherin

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Definition

The interferon-inducible protein tetherin (also called BST2, CD317, or HM1.24) is a key component of the innate immunity against retroviral infections. Tetherin was named after its unique ability to restrict the release of enveloped viruses by physically tethering them to the plasma membrane of infected cells. Notably, tetherin also acts as a pattern recognition receptor inducing NF- κ B-dependent expression of antiviral genes upon sensing of budding virions.

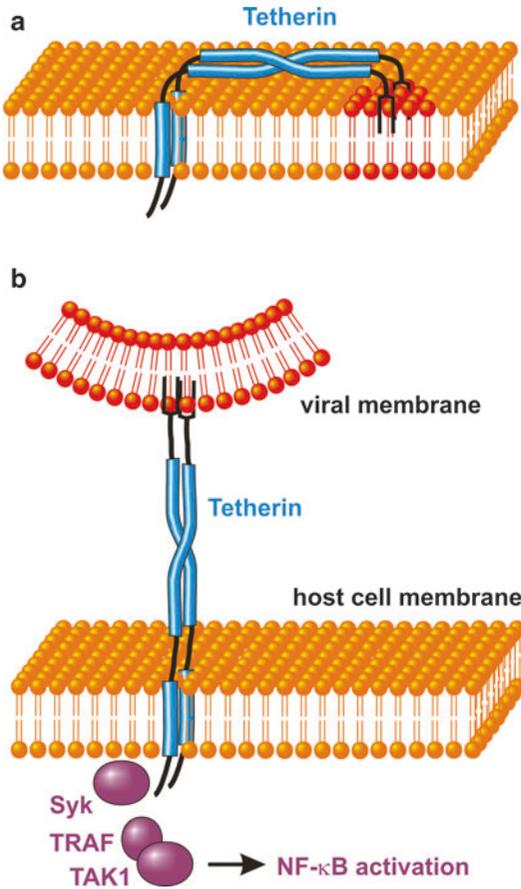
At least three primate lentiviral proteins (Vpu, Nef, and Env) have evolved the ability to counteract tetherin and enable the virus to evade this restriction. Due to a constant evolutionary arms race between virus and host, antagonization of tetherin by viral proteins is frequently species-specific. Human tetherin, for example, is resistant against counteraction by many simian immunodeficiency viruses (SIV) and represents a significant hurdle for successful zoonotic transmissions of SIV to humans. Thus, the evolution of a potent antagonist of the human tetherin ortholog may have been a prerequisite for the efficient spread of HIV in the human population.

Discovery of Tetherin

Although tetherin was already described as an interferon (IFN)-inducible gene in the 1990s, it was initially thought to be involved in the maturation of B cells since it is constitutively expressed at high levels on the surface of bone marrow stromal cell lines (“BST2”) and (plasma) B cells. Various types of cancer cells also express high levels of tetherin, and it has been hypothesized that this may constitute an evasion mechanism to inhibit the immune response against tumor cells. In agreement with this hypothesis, tetherin was shown to inhibit the secretion of type I IFN and other cytokines by interacting with immunoglobulin-like transcript 7 (ILT7) on plasmacytoid dendritic cells (pDC). A recent study, however, challenged this finding and suggested that the interaction of tetherin with ILT7 serves as a homeostatic regulation loop on immature pDCs rather than a negative feedback signal for IFN secretion (Tavano et al. 2013). The observation that the K5 protein of the Kaposi sarcoma-associated herpesvirus (KSHV) induces the ubiquitination and subsequent degradation of tetherin gave a first hint that tetherin may also be involved in the antiviral immune response. Although it had long been known that the HIV-1 accessory protein U (Vpu) counteracts an interferon-inducible restriction to enable the efficient release of progeny virions, it took until 2008 to identify tetherin as the restriction factor that blocks the release of budding HIV particles and is antagonized by HIV-1 Vpu (Neil et al. 2008; Van Damme et al. 2008).

Inhibition of HIV Release

The ability of tetherin to restrict the release of budding virions is based on its unusual topology: Tetherin has a length of about 180 amino acids and is a type II transmembrane protein with an N-terminal alpha-helical transmembrane domain that is followed by a flexible coiled-coil ectodomain and a C-terminal glycosylphosphatidylinositol (GPI) anchor (Fig. 1a) (Kupzig et al. 2003). This combination of an N-terminal



Nef/Env/Vpu/Tetherin, Fig. 1 Topology and antiviral activity of tetherin. (a) Tetherin consists of a short N-terminal intracellular tail that is followed by an α -helical transmembrane domain, an extracellular coiled-coil domain, and a C-terminal GPI anchor that is localized in lipid rafts. Three disulfide bonds mediate the formation of parallel homodimers. (b) In the presence of tetherin, newly formed virions are trapped at the surface of an infected cell since they incorporate the GPI anchor into their membrane, whereas the transmembrane domain of tetherin remains attached to the plasma membrane of the cell. The intracellular tail of tetherin is phosphorylated upon binding of budding virions and induces an NF- κ B-dependent antiviral immune response via the recruitment of Syk, TRAFs, and TAK1

transmembrane domain with a C-terminal GPI anchor is almost unique among vertebrate proteins and probably only shared with an isoform of the prion protein. Mature tetherin is glycosylated at two asparagine residues in its ectodomain and recycles between the plasma membrane, endosomes, and the trans-Golgi

network (TGN). Clathrin-mediated endocytosis depends on recruitment of AP2 to a noncanonical tyrosine motif in its intracellular N-terminal tail. Three conserved cysteine residues in the extracellular domain mediate the formation of parallel homodimers and stabilize the ectodomain of tetherin. Ultimately, antiparallel four-helix bundles may form between two homodimers. The C-terminal GPI anchor targets tetherin to cholesterol-rich microdomains (lipid rafts) which have been suggested to represent the main budding sites of HIV. As a result, budding virions predominantly incorporate the GPI anchor of tetherin into their membrane, whereas the N-terminal transmembrane domain that is located outside the lipid raft remains attached to the plasma membrane of the cell (Fig. 1b). Thus, tetherin serves as a physical bridge preventing the release of newly formed progeny virus from infected cells. This process requires a structural flexibility of the ectodomain, which is achieved by alternating stretches of stabilizing and destabilizing residues. Structural analyses revealed a distance of around 170 Å between the viral and the cellular membrane. Tethered virions are endocytosed and ultimately probably degraded in intracellular compartments.

The functional characterization of a chimeric protein with a tetherin-like topology revealed that the combination of an N-terminal transmembrane domain, a flexible ectodomain, and a C-terminal GPI anchor rather than a specific primary sequence is required for restriction of viral release (Perez-Caballero et al. 2009). In agreement with an unspecific targeting of the viral membrane, tetherin does not only inhibit the egress of lentiviruses but also that of many other enveloped viruses including filo-, arena-, herpes-, paramyxo-, or rhabdoviruses (Neil 2013; Swiecki et al. 2013; Sauter 2014).

Sensing of HIV Infection

In 2003, tetherin was identified in a screening for proteins inducing the activation of NF- κ B (Matsuda et al. 2003). Meanwhile, it has been shown that this activation of NF- κ B signaling is

linked to the ability of tetherin to act as an innate sensor. Tetherin is a classical pattern recognition receptor (PRR) and contains a hemi-immunoreceptor tyrosine-based activation motif (hemITAM) that is phosphorylated upon binding of budding virions. Tyrosine phosphorylation depends on the connection of tetherin to the cortical actin network and induces the subsequent recruitment of the tyrosine kinase Syk (Galão et al. 2014). This in turn promotes the assembly of TRAF2 and/or TRAF6, which mediates the activation of the kinase TAK1 and activates the canonical NF- κ B signaling cascade to induce the expression of antiviral genes (Fig. 1b).

Tetherin orthologs have been identified in many mammalian and some reptile species. Whereas the ability to restrict virion release is highly conserved among different species, only human and to a lesser extent chimpanzee tetherin are able to sense viral infections in human cells. Thus, innate sensing seems to be an evolutionarily novel function that tetherin has acquired rather recently.

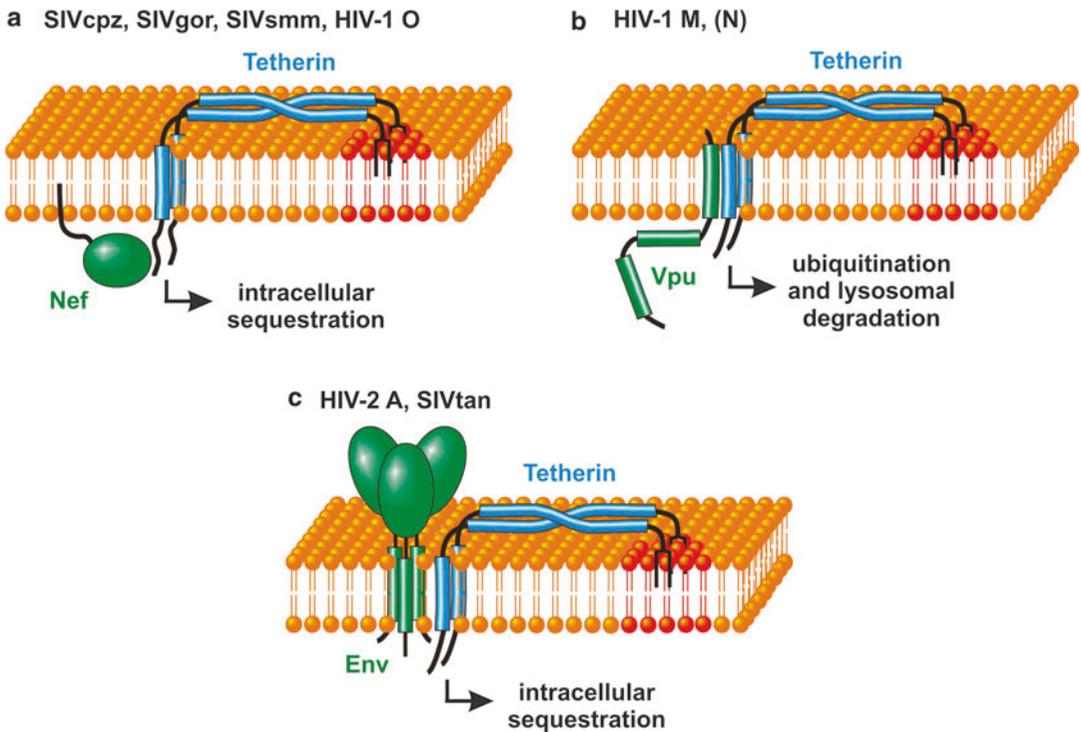
In summary, the antiviral activity of tetherin is not limited to its ability to trap budding virions at the surface of infected cells but also involves the induction of an antiviral immune response (Hotter et al. 2013).

Retroviral Countermeasures

Since tetherin does not target a specific viral protein but rather nonspecifically interferes with the budding process by targeting the viral envelope, viruses cannot simply acquire evasion mutations that render them resistant against tetherin. Instead, many enveloped viruses have evolved direct *trans*-acting antagonists that remove tetherin from the sites of budding and/or target it for degradation. Among the tetherin antagonists identified until today are three primate lentiviral proteins: Nef, Vpu, and Env. Most simian immunodeficiency viruses (SIV) including SIVcpz and SIVgor – the direct chimpanzee and gorilla precursors of HIV-1 – use their accessory protein Nef to counteract tetherin in their respective host species (Zhang et al. 2009; Sauter et al. 2009). The

myristoyl-anchored SIV Nef protein targets the N-terminal cytoplasmic tail of monkey or ape tetherin to enhance its AP2- and clathrin-mediated endocytosis (Fig. 2a). Notably, total cellular levels of tetherin are not altered in the presence of Nef since it does not target the restriction factor for degradation but rather sequesters it in intracellular compartments. Residues within and adjacent to a conserved di leucine motif (EXXXLL) in the C-terminal loop of Nef are crucial for tetherin counteraction. These amino acids may either directly interact with the cytoplasmic domain of tetherin or affect the affinity for specific adaptor proteins that are involved in cellular trafficking pathways. Interestingly, human tetherin is resistant against SIV Nef due to a deletion of five amino acids in its cytoplasmic tail that evolved after the divergence of chimpanzees and humans. As a consequence, tetherin constitutes a significant barrier for successful zoonotic transmissions of SIV to humans (Sauter et al. 2010).

HIV-1 groups M, N, O, and P are the results of four independent cross-species transmissions of SIVcpz or SIVgor and have evolved different means to overcome this hurdle. HIV-1 group M, which is largely responsible for the global AIDS pandemic, has switched from Nef to Vpu to antagonize tetherin in humans (Fig. 2b). The ~16 kDa protein Vpu interacts with tetherin in the trans-Golgi network via its transmembrane domain and inhibits the anterograde transport of the restriction factor. Although this sequestration of tetherin in the TGN may already enhance virion release to a certain extent, full anti-tetherin activity depends on a conserved diserine motif (DSGXXS) in the cytoplasmic part of Vpu that binds the adaptor protein β -TrCP to recruit an E3 ubiquitin ligase complex. As a result, tetherin is ubiquitinated and ultimately degraded in endolysosomal compartments. Vpu proteins of HIV-1 group N strains have also evolved the ability to directly interact with human tetherin. Many HIV-1 group N Vpus however lack the β -TrCP-binding motif and/or additional functional domains. Therefore, most of them are only poor tetherin antagonists and have lost their second function: the ability to decrease the surface levels of CD4 (Sauter et al. 2009). The poor anti-tetherin activity



Nef/Env/Vpu/Tetherin, Fig. 2 Viral counteraction of tetherin. (a) Nef proteins of HIV-1 group O and many simian immunodeficiency viruses target the N-terminal tail of tetherin and sequester it in intracellular compartments by inhibiting its anterograde transport or accelerating its endocytosis from the plasma membrane, respectively. (b) Vpu proteins of HIV-1 group M inhibit the anterograde transport of tetherin and recruit an E3 ubiquitin ligase complex to induce its ubiquitination and subsequent endolysosomal

degradation. HIV-1 group N Vpus have only weak anti-tetherin activity since most of them fail to recruit the ubiquitin ligase complex. Vpu and tetherin interact via their transmembrane domains. (c) HIV-2 Env enhances clathrin-mediated endocytosis of tetherin and relocates it to intracellular compartments. The extracellular gp120 subunit of Env probably interacts with the ectodomain of tetherin

and loss of Vpu-mediated CD4 down-modulation may be one plausible explanation why HIV-1 group N strains did not spread significantly in the human population. Only about 17 patients infected with HIV-1 group N have been identified until today. HIV-1 group O has spread epidemically in Africa and infected around 100,000 individuals, although it has not evolved a Vpu-mediated counteraction of tetherin. Instead, HIV-1 O Nef acquired the ability to target a motif adjacent to the protective deletion in the intracellular tail of human tetherin to enable efficient egress of progeny virions (Kluge et al. 2014). Similar to SIV Nef, HIV-1 O Nef does not induce the degradation of tetherin but sequesters it in intracellular compartments. This sequestration depends on amino acid residues in the

C-terminal loop of Nef and involves the inhibition of the anterograde transport of newly synthesized tetherin (Fig. 2a). With only two cases described, HIV-1 group P is the rarest of all four known groups of HIV-1. In agreement with a poor adaptation to the new human host, no anti-tetherin antagonist has been described in this group so far.

The direct precursor of HIV-2, the second human immunodeficiency virus, is SIVsmm naturally infecting sooty mangabeys. At least nine independent cross-species transmissions of SIVsmm to humans occurred and gave rise to HIV-2 groups A-I. Similar to SIVcpz and SIVgor, SIVsmm Nef efficiently antagonizes tetherin in its natural host (Fig. 2a) but fails to counteract the human ortholog due to the protective five-amino-acid deletion in its cytoplasmic tail. HIV-2 does

not encode a *vpu* gene and mastered the tetherin hurdle by switching from Nef- to Env-mediated antagonism (Le Tortorec and Neil 2009). The HIV-2 envelope protein probably interacts with the extracellular domain of human tetherin and accelerates its clathrin-mediated endocytosis (Fig. 2c). The latter effect depends on an endocytosis signal in the cytoplasmic gp41 subunit of Env and results in an intracellular sequestration of tetherin. Interestingly, the envelope protein of a distantly related virus from tantalus monkeys (SIVtan) employs a similar mechanism to antagonize tetherin (Fig. 2c). However, it remains to be determined whether this is a real and general ability of SIVtan Env proteins since the only isolate that was analyzed so far had been passaged in a human T cell line before cloning. In contrast to HIV-1 group M Vpu, HIV-2 and SIVtan Env do not induce the degradation of tetherin but only interfere with its subcellular trafficking. The nine groups of HIV-2 differ substantially in their prevalence in the human population: Whereas groups A and B spread to a certain extent in Western Africa, groups C to I have only been isolated in single patients and may represent dead-end zoonoses. It should be noted that only envelope proteins of HIV-2 group A have been characterized for their anti-tetherin activity. Thus, it remains unclear if and how HIV-2 groups B-I counteract human tetherin.

Interestingly, two different isoforms of human tetherin exist that differ in their sensitivity against Vpu- and Nef-mediated counteraction (Cocka and Bates 2012). The shorter isoform lacks twelve amino acids at its N-terminus since translation is initiated from an alternative start codon. This N-terminal truncation involves the loss of the dual tyrosine motif that is essential for activation of NF- κ B signaling, binding to the cortical actin network and endocytosis. Although the short isoform does not act as an innate sensor, it may still confer a selective advantage against viral proteins that target residues in the intracellular N-terminal tail of tetherin. HIV-1 O Nef, for example, sequesters only the long isoform of human tetherin in intracellular compartments but fails to antagonize the short isoform due to the lack of its target sequence. Similarly, the short isoform is largely

resistant against HIV-1 group M Vpu since full anti-tetherin activity of Vpu depends on the presence of the endocytosis signal and N-terminal ubiquitination sites.

In addition to the evolution of direct antagonists, primate lentiviruses may also employ alternative strategies to evade restriction imposed by tetherin. One such strategy is to inhibit the expression of tetherin on a transcriptional level. Vpu proteins of most primate lentiviruses, for example, have been shown to inhibit the activation of the transcription factor NF- κ B that regulates the expression of interferons and interferon-stimulated genes (ISGs). As a result, Vpu prevents the induction of tetherin and several additional antiviral genes in SIV- or HIV-infected cells. Notably, this effect of Vpu on NF- κ B is independent of its anti-tetherin activity and involves the stabilization of I κ B (Sauter et al. 2015).

It has also been suggested that tetherin restriction may be saturated by large amounts of budding virions and (at least partially) be overcome by direct cell-to-cell spread, which constitutes a major route of transmission in an HIV-infected individual. All in all, primate lentiviruses have evolved various independent sophisticated means to counteract or evade the host restriction factor tetherin (Neil 2013; Swiecki et al. 2013; Sauter 2014).

Role in Pathogenesis and Spread

Several mouse models confirmed a significant role of tetherin in restricting retroviral infections *in vivo* and revealed that lack of tetherin is associated with increased viral loads and faster disease progression. For example, Moloney murine leukemia virus infections were more pathogenic in tetherin knockout mice than in their wild-type littermates. Similarly, Friend murine leukemia virus replication was inhibited by increased tetherin surface expression levels in another mouse model. In agreement with this, Vpu boosted the initial phase of HIV-1 M replication in humanized mouse models, although it seemed less important for viral dissemination during later stages of infection. The role of tetherin in the

pathogenesis of AIDS is underscored by an association study that identified a 19-base-pair insertion/deletion polymorphism in the promoter of human tetherin that is associated with disease progression, potentially due to differences in total tetherin expression levels (Laplana et al. 2013). Although HIV-1 groups M, N, O, and P differ in their ability to antagonize human tetherin, all four groups are pathogenic, and infected patients may ultimately progress to AIDS. Noteworthy, however, the pathogenic potential of these viruses has never been compared systematically, and it remains unclear whether the rates of disease progression are comparable. The investigation of longitudinal samples from HIV-1 M-infected individuals revealed that Vpu-mediated anti-tetherin activity is highly conserved throughout the course of infection, arguing for a constant selection pressure exerted by tetherin.

Notably, tetherin may not only limit viral dissemination and pathogenesis within an infected individual but also reduce transmission of the virus and inhibit its spread in the human population. The fact that only HIV-1 groups M and O spread significantly after zoonotic transmission from apes to humans suggests that the evolution of a potent tetherin antagonist may have been a prerequisite for the efficient spread of primate lentiviruses in the human population. In contrast, HIV-1 groups P and N, which do not or only poorly antagonize tetherin, respectively, have remained rare and largely restricted to Cameroon. Thus, the host restriction factor tetherin has clearly shaped the evolution of HIV, and the current AIDS pandemic may be a sinister example for the striking ability of primate lentiviruses to quickly adapt to new hosts.

Conclusion

Tetherin unifies all characteristics of a classical antiretroviral restriction factor. As a first line of defense against viral pathogens, it is constantly expressed on many cells including activated

CD4⁺ T cells and macrophages, the main target cells of HIV. Furthermore, tetherin is interferon-inducible, and its expression is upregulated upon viral infection. Similar to other host restriction factors such as TRIM5 α , SAMHD1, or APOBEC3G, tetherin also inhibits a specific step of the viral replication cycle, and many retroviruses have evolved means to antagonize or evade this restriction. Due to the strong and constant selection pressure exerted by viral antagonists, tetherin shows signatures of site-specific positive selection, another feature of prototypic host restriction factors. As a consequence of the rapid coevolution between viruses and their respective hosts, tetherin antagonism is often species-specific. A significant example with probably far-reaching implications for the evolution of HIV is the deletion of five amino acids in the N-terminal cytoplasmic tail of human tetherin that rendered it resistant against Nef-mediated counteraction by many simian immunodeficiency viruses. Similar species-specific effects have also been described for Vpr-/Vpx-mediated counteraction of SAMHD1 and the interaction of TRIM5 α with lentiviral capsid proteins. Thus, tetherin and other host restriction factors do frequently not only inhibit the spread of viral pathogens within a population but may also represent significant hurdles for successful (zoonotic) cross-species transmissions.

In addition to these classical features of antiretroviral restriction factors, tetherin also exhibits a unique characteristic: Unlike TRIM5 α and APOBEC3G, which are largely retrovirus-specific, tetherin only unspecifically targets the viral budding process and has broad activity against diverse enveloped viruses. In this regard, tetherin resembles pattern recognition receptors of the innate immune system because it senses a conserved pattern (i.e., budding of progeny virions) of only distantly related pathogens and induces the activation of NF- κ B to trigger an antiviral immune response. Since tetherin senses the very last step of the viral replication cycle, this alert signal certainly comes too late for the infected cell. Nevertheless, tetherin-mediated release of

interferons and other antiviral cytokines triggers the induction of ISGs and the establishment of an antiviral state in neighboring cells that are at risk for infection.

In summary, the coevolution of primate lentiviruses and the tetherin orthologs of their respective host species is a vivid example of the Red Queen hypothesis proposing that organisms must constantly evolve and adapt to not become extinct in an ever-changing environment. Tetherin combines all features of a classical restriction factor with those of a pattern recognition receptor. Its potent and broad antiviral activity has driven the evolution of at least three different antagonists in primate lentiviruses, and the degree of adaptation to human tetherin may be an important determinant of the spread and potentially pathogenesis of HIV in humans.

Cross-References

- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [Cellular Cofactors of HIV as Drug Targets](#)

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Network Interventions

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Definition

Social networks refer to the web of social ties or relationships that exist among individuals. Some common examples of social network members include family, friends, sex partners, drug partners, neighbors, etc. Social network members influence each other's behaviors because individuals often compare themselves to others (Latkin and Knowlton 2005).

This entry describes network-level interventions which are designed to change the knowledge, skills, norms, and behaviors of a social network rather than a specific person. These interventions capitalize on naturally occurring relationships among individuals.

HIV and Social Networks

The behaviors that increase the risk for HIV, such as having unprotected intercourse and sharing needles, are social behaviors. This means that each behavior involved is often done in the presence of one or more people. HIV is transmitted through risk networks including sex partners or drug partners (Neaigus et al. 1994).

Social network members' characteristics and behaviors have been linked to a variety of HIV risk behaviors such as frequency of drug use, sharing injection equipment, multiple partners, condom use, and exchanging sex for money or drugs (Davey-Rothwell et al. 2010; Latkin et al. 1995; Neblett et al. 2011). For example, individuals are more likely to engage in unprotected sex or share needles if they have social network members who also do these risky behaviors. In addition, social network characteristics are associated with health-promoting behaviors such as HIV

medication adherence and HIV testing (Downing et al. 2001; Simoni et al. 2007). If a family member or friend encourages or supports a person living with HIV/AIDS (PLHA) to take HIV medications, that PLHA will be more likely to take medications due to the social support provided in their social network.

Because HIV risk may be shared among social network members, interventionists have capitalized on social network dynamics as a vehicle to disseminate HIV risk reduction. By engaging a relatively small number of individuals in a social network, social network interventions are a viable method to reach hidden populations that may not access prevention services or participate in interventions.

In network-level interventions, social network members may provide support to each other, establish and enforce norms about appropriate behaviors, and share information and skills.

Social Networks and Diffusion

Social networks are a vehicle through which HIV prevention information, resources, social norms, skills, and behaviors can be diffused from a few people to a larger number of individuals. Network structures may also influence the ability for intervention diffusion and sustainability. Behavior change may diffuse more quickly in dense (i.e., close) networks. Isolated networks (i.e., small networks, with few connections to other individuals) may be more likely to sustain behavior change, yet there is likely to be less diffusion of behavior change from these networks to other networks (Latkin and Knowlton 2005). More than one distinct behavior may be diffused in HIV prevention interventions. Given the diversities of network structures and social positions or statuses, providing individuals and communities with a variety of risk-reduction options is more likely to succeed compared to promoting only one risk-reduction option.

Social Support and Social Networks

Social network members provide resources to each other in the form of social support. Social

support may include tangible support, such as lending money, or intangible support such as offering advice. Common types of social support include emotional support (i.e., sharing advice), informational support (i.e., sharing information and resources), financial support (i.e., lending money), and material support (i.e., sharing goods). Social support may be perceived or enacted. *Perceived support* is an individual's perception that she could obtain support from social network members if needed. For example, perceived support could be measured by the question "Do I have someone in my social network that could lend me money (financial support)?" while *enacted support* is support that has actually been provided, such as "Who has given me a loan in the past?" In social network interventions, individuals learn skills to influence network members as well as provide emotional, informational, and even material support for behavior change.

Social Norms and Social Networks

Social norms serve as guides for which behaviors are common in a social network. Social networks establish and proliferate norms about which behaviors network members should practice as well as behaviors that network members disapprove of. Norms have consistently been found to predict HIV risk behaviors. Specifically, if a person believes that members of their social network, such as sex partners or friends, share needles, that person is more inclined to share needles, too.

Social norms are created by observing others and comparing behaviors to others as well as verbal and nonverbal communication. Through observance of others' behaviors and comparing actions, norms about which behaviors are appropriate for a given social environment may emerge. Research has shown that people are more likely to be influenced by others that they perceive as similar to them (Festinger 1954). Thus, a person who uses drugs may be more likely to utilize a needle-exchange program if they believe that other social network members use them too or if a network member encourages them to use the program.

Social network interventions are designed to promote HIV risk-reduction behaviors as socially acceptable and socially approved behaviors. For example, as participants learn risk-reduction techniques such as how to clean injection equipment, they begin to model their behaviors among their drug partners. By observing this risk-reduction behavior among network members, drug users may be persuaded to change their own risky behaviors. As more and more people practice risk reduction, cleaning injection equipment becomes a normative behavior. Some behaviors, however, are not readily observable among network members. For sex behaviors such as condom use, network members may change acceptable communication norms by discussing safer sex. Consequently, these discussions may lead to changes in perceived norms that endorse safer sex and changes in risk behaviors.

Examples of Network-Level Interventions

Two models of HIV prevention network interventions that have been shown to lead to behavior change and have been widely implemented in both the USA and international settings are presented in the next section. Both of these interventions have been disseminated by the Centers for Disease Control and Prevention to community-based organizations and public health departments across the USA (www.effectiveinterventions.org).

Network-Oriented Peer Education Interventions

One type of network intervention model is peer education. Network-oriented peer education programs train a small group of individuals to educate members of their social networks. Through peer education, individuals serve as a vehicle to share information and exert social influence through persuasion and modeling to members of their social network. Any individual in a social network may be trained as a peer educator. Peer education has been implemented in both the USA and international settings within a variety of populations such as college students, commercial sex workers,

people living with HIV/AIDS, and people who use drugs.

During a peer education intervention, peer educators acquire knowledge, skills, and resources such as condoms and information about local HIV testing or addiction services. Skills are built through hands-on activities such as role-playing and problem-solving exercises. In addition to learning about risk reduction, participants are also taught communication and outreach skills. Peer educators use communication and outreach skills to engage their peers and disseminate the intervention material. Thus, peer education interventions have the potential to reach a wider range of individuals than just those who participate in the intervention. In addition to diffusing information, peer educators also model risk-reduction behaviors (Davey-Rothwell et al. 2011).

As network members observe peer educators and increase discussion about safer behaviors, network members may perceive risk reduction as normative and begin to change their behaviors. Likewise, as peer educators share information and resources with people in their network, they may also begin to change their own behavior. Thus, peer education interventions have been shown to lead to behavior change among peer educators and the recipients of peer outreach (Tobin et al. 2011).

The Self-Health in Eliminating Life-Threatening Diseases (SHIELD) intervention was developed in Baltimore, MD, through a research study funded by the National Institutes of Health (Latkin et al. 2003). In the SHIELD intervention, people who used drugs were trained to be peer educators. The peer educators attended several group sessions led by two facilitators. Through intervention activities, participants learned HIV risk-reduction information and options; practiced safer sex, safer drug use, and peer outreach skills; and reached risk-reduction materials. After attending each session, participants were asked to share risk-reduction information and materials with members of their social network through outreach and modeling of safer behaviors. A 6-month evaluation showed that peer educators reduced their needle sharing and drug injection frequency and increased condom use with casual partners as

compared to the comparison condition. Additional analyses from this study showed that this approach changed the norms of network members, which could lead to changes in behavior. This model has been tailored and implemented in a variety of other locations including Philadelphia, Pennsylvania; Vietnam; St. Petersburg, Russia; Chiang Mai, Thailand; and Chennai, India.

Popular Opinion Model

Another network-level intervention implemented in HIV prevention is the popular opinion model. While peer educator interventions are guided by the premise that any member of a social network can affect another member's behavior, the popular opinion model emphasizes the importance of identifying key (i.e., popular) social network members to promote risk reduction. Key network members often have high centrality (i.e., high number of ties with other members of the network) or information social roles within their social network. Through formative work, researchers identify individuals in a social network who interact with a large number of people and are considered influential or popular and may be able to have an influence on other people's behavior. These individuals participate in training and then are asked to share information and model behavior with people they know such as close friends and acquaintances.

The popular opinion model was first introduced in the early 1990s as part of an HIV prevention intervention for men who have sex with men (Kelly et al. 1991). In this intervention, bartenders were asked to identify popular individuals, who were then trained to endorse and promote risk reduction among their bar-attending friends. These key network members, called "opinion leaders," were trained in HIV risk reduction and communication skills, such as practicing HIV-related conversations that they could then repeat with their peers. This intervention model has been widely applied in the USA and in international settings. Initial studies of the "popular opinion leader" model showed decreases in unprotected anal sex, decreased sex partners, and increased condom use (NIMH Collaborative HIV/STD Prevention Trial Group 2010).

Conclusion

Network-level interventions can have a broad impact and therefore play an important role in HIV prevention. Social network interventions are cost-effective because they serve participants in the interventions as well as the hidden populations who are not directly trained by the program staff. Through the diffusion of information, social support, and norms, network-level interventions have great potential for sustainability and change in a given population.

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NeuroAIDS in Resource-Poor Settings, Assessment, and Treatment of

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Introduction

In 2013, an estimated 35 million people (0.8%) were infected with human immunodeficiency virus (HIV) globally, although the burden of the epidemic is disparately distributed, with over two-thirds of the world's HIV-infected individuals residing in sub-Saharan Africa where the adult prevalence is as high as 4.7% (UNAIDS 2014). Thus, the majority of individuals affected by HIV who require treatment reside in resource-poor settings. With increased dissemination of, and access to, combined antiretroviral therapy (cART), the profile of HIV infection is changing to that of a chronic disease, with the cause of death more likely to be from an HIV-associated non-AIDS condition. This has implications for clinicians

and other health-care providers in terms of the goals of care and the approach to management.

In low-resource settings, many individuals with HIV will develop some form of peripheral or central nervous system (PNS or CNS) disease. Neurological disorders most often associated with HIV can include opportunistic infections (e.g., tuberculosis, cryptococcal meningitis, toxoplasmosis, and cytomegalovirus encephalitis), CNS neoplasias (e.g., Kaposi's sarcoma, primary CNS lymphoma), progressive multifocal leukoencephalopathy (PML), and CNS manifestations that are the adverse effects of cART (Singer et al. 2010). Seizures, myelopathies, aseptic meningitis, neuropathies, and myopathies occur less commonly. Similar to developed regions, however, HIV-associated neurocognitive disorders (HAND) are the most prevalent neuroAIDS disorder in low- and middle-income countries (LMICs) (Chibanda et al. 2014), including Africa, the region with the highest HIV burden (Alkali et al. 2013). Since most of the CNS disorders develop in the advanced or acquired immunodeficiency syndrome (AIDS) stage, when CD4 (cluster of differentiation 4) cell counts fall below 200 cells/mm³, their prevalence has decreased with increased access to cART; however, opportunistic infections still pose a major health problem in developing countries where patients often present late with advanced immunosuppression (Wright 2014). In Africa, stigma remains a major problem and can delay HIV testing and seeking help, and even then, the diagnosis of opportunistic infections is often delayed due to a lack of neuroimaging and laboratory facilities, and appropriate treatment for cryptococcal meningitis is often not available or is unaffordable (Alkali et al. 2013). Furthermore, neuro-IRIS (immune reconstitution inflammatory syndrome) is also a common condition in resource-poor settings where cART is delayed and often initiated at advanced stages of HIV (e.g., CD4 cell counts below 50 cells/mm³) (Alkali et al. 2013), resulting in clinical deterioration that occurs shortly after cART initiation due to a dysregulated inflammatory response to underlying infections (commonly CNS tuberculosis, toxoplasmosis, and cryptococcal meningitis). Thus in low-resource settings, the

aforementioned conditions remain major causes of morbidity and/or mortality due to the many challenges that can hamper their timely identification and management.

HAND, one of the major causes of dementia worldwide, remains the most prevalent neuroAIDS disorder in all settings and a primary challenge for clinicians, and will thus be the focus of this review. Although the burden of disease is higher in resource-poor settings, much of the research on HAND has been conducted in developed regions. In low-resource settings, already burdened by delayed initiation of cART and fewer treatment options, there is often a dearth of expertly trained personnel, while existing personnel are overburdened by high patient throughput, limiting early diagnosis.

Classification

The neurocognitive impairments associated with HIV were previously known by various terms, such as "AIDS dementia complex," "HIV dementia," "HIV encephalopathy," and "minor cognitive motor disorder," with more recent consensus criteria now commonly employed (Antinori et al. 2007). HAND is subclassified into asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). Both ANI and MND involve a minor decline in cognition, but MND is diagnosed when there is mild impairment in more difficult activities of daily living (ADL), such as work, financial planning, and driving. In contrast, in HAD there is evidence of a significant decline in cognition, as well as considerable impairments in more routine ADL, such as daily errands, housekeeping, and self-care. Due to decreased availability of formal neuropsychological testing in resource-poor settings, it is likely that ANI goes largely undetected.

Pathophysiology

HIV invades the CNS shortly after initial infection as leucocytes infected with HIV cross the blood-

brain barrier (BBB). The virus then continues to replicate and infect cells in the CNS, such as microglia, macrophages, and astrocytes. HIV infection in the CNS leads to the release of neurotoxic viral proteins and host inflammatory molecules, leading to neuronal cell damage, breakdown of the BBB, and decreased immune function. Higher viral loads in the CNS are associated with increased neurological sequelae (Singer et al. 2010). The main areas demonstrating degenerative changes are the basal ganglia and the frontal white matter tracts. Although limited pathology studies have been conducted in resource-poor settings, these studies support similar pathological changes (Robertson et al. 2010).

Epidemiology

HAND is prevalent across settings internationally; however, varying approaches to neurocognitive assessment make comparison difficult. The wide range in prevalence may be attributable to differences in measurement, ART treatment status, HIV 1 subtypes, comorbidities, or demographic features (e.g., age, education, gender distribution). The prevalence of HAND has remained fairly unchanged even since improved treatment, with about half of individuals stable on cART demonstrating some form of HAND, although the presentation is generally more mild (Clifford and Ances 2013). A systematic review and meta-analysis assessing HAND in sub-Saharan Africa, the region with the highest prevalence of HIV, reported similarly high prevalence of HAND as in developed settings (Habib et al. 2013). The authors estimate that eight million HIV-infected individuals in sub-Saharan Africa are likely to be affected by HAND. While HAND is probably the most common cause of cognitive dysfunction in young adults in LMICs (Sacktor et al. 2007), the burden is likely to be underappreciated in resource-poor settings where HAND may be overshadowed by other serious medical conditions and under recognized by health-care workers. The

persistence of HAND in the current era may be attributed to continued CNS HIV infection and inflammation, despite peripheral viral suppression with cART, as well as late initiation of cART and potential ART neurotoxicity (Clifford and Ances 2013).

Risk factors for HAND include factors related to the virus, treatment, host-related factors, as well as comorbid disorders. Cognitive reserve (premorbid cognitive capacity) is fostered by enriched environments, improved education, and employment, and high reserve can delay or reduce the impact of cognitive disorders. Reserve, however, can be impeded by CNS insults, trauma, and stress, and lower cognitive reserve is a risk factor for the development of dementia, including HAND. In resource-constrained environments, individuals often have barriers to attaining higher cognitive reserve, such as lower levels of poorer quality education and higher unemployment rates, alongside increased medical comorbidity and trauma exposure, leaving these individuals at higher risk for HAND (Tedaldi et al. 2015; Vance et al. 2014). Due to variable quality of education in resource-poor settings, reading ability or literacy may be a better indicator of underlying cognitive functioning (Tedaldi et al. 2015). In these settings, HAND generally appears to be unrelated to HIV 1 subtype (clade), although some studies in African countries have demonstrated differential risk for HAND according to HIV subtype (Robertson et al. 2010). A number of mental and substance use disorders, in particular depression and alcohol use disorders, are frequently comorbid with HAND in LMICs (Chibanda et al. 2014). Similar to developed countries, in sub-Saharan Africa, psychiatric comorbidity is associated with an increased risk of HAND, while the use of cART reduces risk. In a meta-analysis of studies in sub-Saharan Africa, HAND was not associated with age, gender, CD4 cell count, or years of education, risk factors commonly observed in developed settings (Habib et al. 2013). However some studies in Africa have demonstrated associations between anemia, low CD4 cell count, and advanced clinical stage and HAND (Robertson et al. 2010).

Clinical Features

HAND usually develops over a period of months, and symptom severity can fluctuate over time. The pattern of cognitive impairment is typically that of a subcortical dementia, with early impairments in working memory and executive functioning. These impairments correlate with brain regions primarily affected by HIV, such as the striatum and subcortical white matter tracts. Individuals with HAND generally display impairments in three domains: cognition, behavior, and motor functioning (Alfahad and Nath 2013). Studies from Africa similarly demonstrate impairments in executive functioning, verbal fluency, processing speed, memory, and motor speed (Robertson et al. 2010). The majority of individuals with HAND have ANI, and even though impairments are found on testing, these individuals display no symptoms or difficulties with daily functioning. Individuals with MND typically have milder neurocognitive symptoms, with deterioration and slowed performance in complex tasks such as problem solving, comprehension, and mild memory difficulties. Patients with HAD typically have pronounced slowing in thinking and movement, with prominent apathy and mood changes (Alfahad and Nath 2013). Mania and psychosis can develop in patients with HAD with advanced immunosuppression (CD4 count falls below 200 cells/mm³). In individuals stable on cART, HAD can emerge at higher CD4 counts. Cortical impairments characteristic of other dementias, such as episodic memory, language, and visuospatial decline, are seen more frequently in those who develop dementia on cART, which can increase diagnostic complexity (Clifford and Ances 2013).

Screening

Screening may identify individuals who need further evaluation and more formalized testing, particularly if functional impairment is also reported. Ideal screening tests for HAND should be brief, easy to administer, and able to reliably identify individuals with HAND, with annual screening.

Screening shortly after HIV diagnosis and prior to cART initiation can provide a baseline estimate with which to compare future testing. Routine screening for HAND, however, is still not the norm in LMICs (Chibanda et al. 2014), and many effective screening tools in existence have not been culturally and linguistically validated in most LMICs. Of several common pen and paper bedside tests, the International HIV Dementia Scale (IHDS) (Sacktor et al. 2005) is predominantly used in LMICs as it is brief, relies less on language proficiency, and can be administered by health-care workers with varying levels of expertise. The IHDS involves three subtests, a non-dominant finger tapping test, a non-dominant hand position sequence, and a four word recall test (4 points for each test, a total score of 10 or less out of 12 indicates possible HAND). In resource-poor settings, however, patients who screen negative on the IHDS often display impairments of executive functioning on formal testing. Furthermore, the IHDS is best suited for detecting possible HAD than milder forms of HAND, limiting its utility. Adding tests of executive functioning to a screener such as the IHDS may improve sensitivity (Habib et al. 2013). Screens that use a combination of three to five neuropsychological tests (tests of verbal learning, attention/working memory, and processing speed) appear to perform better than paper and pen screens such as the IHDS (Kamminga et al. 2013). However these tests require administration and interpretation by a qualified psychologist which diminishes the practicality of their use. A Swiss study identified three screening questions that can effectively identify symptomatic HAND in individuals stable on cART and can easily be applied in low-resource contexts (Simioni et al. 2010). The three questions focus on difficulties in concentrating, remembering, and reasoning and planning abilities and should preferably be combined with a brief bedside test, such as the IHDS.

As depression and alcohol abuse are the most prevalent mental and substance use disorders in LMICs and are commonly comorbid with HAND, integrated screening approaches that combine screening for cognition, depression, and alcohol

use may work best (Chibanda et al. 2014). ANI and MND can predict the future development of HAD but are often only identified following a full neuropsychological assessment (Alfahad and Nath 2013). Early detection of these milder forms of HAND can help identify individuals at risk allowing for regular monitoring and earlier access to treatment. Future efforts at developing improved screening approaches should ideally take place in resource-poor settings to ensure broader applicability.

Diagnosis

HAND is diagnosed when there is evidence of cognitive deterioration and impairment in functioning, and alternative causes have been excluded or addressed (Antinori et al. 2007), although increased comorbidity of other medical and mental disorders may complicate diagnosis (Chibanda et al. 2014). There are no pathognomonic laboratory or imaging investigations for HAND, and milder symptoms of HAND can easily be ascribed to other disorders. Individuals who have HAND may benefit from earlier ART initiation and require additional planning and support to ensure adequate adherence.

Official diagnosis of HAND requires formal neuropsychological testing, which often is not available in resource-poor settings in part owing to the lack of expertise and personnel shortages (Alkali et al. 2013). Neurocognitive testing is mainly utilized in research, and its use in clinical settings is fraught with difficulties. Determination of cognitive impairment requires the use of population-specific reference norms which are lacking in most low-resource settings (Clifford and Ances 2013). Translated versions of the tests also require validation prior to their use. Increased efforts need to be directed at developing local norms for low-resource settings with a high burden of HIV. Even so, neuropsychological testing may fail to differentiate underlying cognitive limitations from that caused by HAND (Chibanda et al. 2014). Guidelines suggest that if neuropsychological testing is not available, clinicians should depend on clinical assessments, such as

bedside testing, and their professional judgment to diagnose HAND (Antinori et al. 2007).

Self-report can be used to assess functioning, but patients often have limited insight and are not aware of their deficits. Collateral information can be sourced from family and friends but relies on their perceptiveness and acceptance of the situation. More objective measures can also be used, such as pill counts and reports from employers. Formal scales designed to assess ADL in other dementias can be employed but generally do not appear to accurately detect the impairments found in HAND (Clifford and Ances 2013). Functional assessments also need to be culturally appropriate, for instance, in certain parts of Africa, individuals often do not drive and may engage in less complex daily activities (Robertson et al. 2010).

Additional imaging and laboratory investigations are directed toward excluding alternative causes and identifying comorbid disorders that need to be managed. Imaging modalities, such as brain computerized tomography (CT) and magnetic resonance imaging (MRI), may reveal atrophy and white matter changes in some cases of advanced HAD (Singer et al. 2010). Sophisticated imaging techniques can demonstrate changes earlier in the course of HAND but are largely inaccessible in LMICs. Cerebrospinal fluid (CSF) examination is also generally employed to eliminate alternative causes in HAND and may show a raised HIV viral load and slightly raised protein levels, immunoglobulin G (IgG), and lymphocytes (Singer et al. 2010). However, these imaging and laboratory findings are relatively nonspecific for HAND and must be interpreted alongside clinical findings. The search for potential biomarkers may assist in more reliably identifying HAND.

Differential Diagnosis

A diagnosis of HAND requires that alternative causes of cognitive impairment be excluded. Comorbid medical, psychiatric, and substance use disorders must be adequately treated before a diagnosis of HAND can be confirmed.

Delirium entails an acute confusional state that develops rapidly and has a fluctuating course.

Prior to diagnosing HAND, delirium must be excluded, as it indicates a serious underlying medical cause requiring urgent identification and treatment. With advanced immunosuppression, CNS opportunistic infections, such as PML, toxoplasmosis, and cryptococcal meningitis, can present with features similar to that of HAD. While these can usually be distinguished from HAD by diagnostic testing, such as imaging and CSF analyses, in resource-poor settings with limited access to diagnostic testing, these treatable conditions can often be missed (Wright 2014). The dementia developing in individuals stable on cART often involves a wider variety of deficits than those typically encountered in HAD. Other causes of dementia, such as Alzheimer's dementia and vascular dementia, may thus be difficult to distinguish from HAD (Clifford and Ances 2013). Vitamin deficiencies and endocrine disorders are potentially reversible causes of cognitive impairment and should be evaluated for in all individuals presenting with cognitive deficits.

Common mental disorders, particularly depression, occur at increased rates in HIV in developed and in LMICs and must also be differentiated from HAND (Chibanda et al. 2014). The apathy that commonly occurs in HAND can be misdiagnosed as depression; therefore symptoms such as sadness and tearfulness are required to discern major depressive disorder (MDD). Individuals with MDD often report cognitive problems and can have marked functional impairment. These symptoms tend to improve, however, once depression is properly treated. The psychosis and mania developing in the context of HAND can be differentiated from primary psychiatric disorders, such as schizophrenia and bipolar disorder, based on the course and clinical features. Personal or family history of serious mental illness is typically lacking in HAND, and atypical features are observed. The psychosis may have a predominance of visual, and other nonauditory hallucinations and delusions of being cured of HIV frequently arise. AIDS mania is typified by irritability rather than euphoria, psychomotor slowing instead of hyperactivity and prominent aggression. They also tend to have a greater number of symptoms, have a protracted course, respond

inadequately to psychotropic treatments, and have a poorer prognosis. Substance use is frequently comorbid in HIV and can present with a clinical picture suggestive of HAND. In LMICs alcohol use disorders are also more common in HIV-infected individuals than in the general population (Chibanda et al. 2014). Use of certain substances, such as methamphetamine and cocaine, is associated with increased CNS damage and faster HIV disease progression. Substance use disorders must be addressed antecedent to diagnosing HAND and to improve outcomes in HIV. The possibility of symptoms being due to neuropsychiatric side effects of ART should also be explored, such as efavirenz which can cause dizziness, impairments in concentration, and, less commonly, more severe adverse effects such as psychosis and depression (Kenedi and Goforth 2011).

Course and Prognosis

Although the situation is improving globally, individuals in resource-poor settings have decreased access to medical care, including cART, and to sufficient screening and diagnostic approaches for cognitive impairment. Obstacles to care can include limited time available to attend clinics, a lack of transport, and an inability to afford appropriate medical care. Lower health literacy also contributes to increased HIV morbidity, as individuals may not be aware of preventative measures and treatment options available and may only seek help when stigmata of advanced HIV are present (Vance et al. 2014). HAD (HIV encephalopathy) is a WHO clinical stage 4 (AIDS-defining) disease, and without treatment, the disorder generally leads to death within a year (Singer et al. 2010). Untreated individuals with HAND initiated on cART tend to improve; however some exhibit progressive cognitive deterioration despite adequate viral suppression on cART. The course of HAND in individuals on cART tends to be more unpredictable (Clifford and Ances 2013). Some individuals with milder HAND subtypes progress to HAD, while many remain stable on cART. Nonetheless milder forms

of HAND predict progression compared to HIV-positive individuals with no HAND. Prognosis is worsened by the presence of increased risk factors and sustained immunosuppression even with cART. HAND is associated with various detrimental consequences for the individual (Alfahad and Nath 2013). Impairments related to HAND can diminish an individual's ability to work and thus negatively influence their socioeconomic status. Individuals with HAND have increased psychiatric comorbidity, relationship difficulties, and a reduced quality of life. Cognitive impairments and apathy contribute to adherence difficulties, such as forgetting to take medication and missing appointments. Very few studies have evaluated the impact of HAND on adherence in resource-poor settings; however, depression and alcohol use disorders have been associated with reduced cART adherence in LMICs (Mayston et al. 2012). HAND also increases a person's vulnerability to sexual abuse, decreases their likelihood of using HIV preventive measures, and lowers survival rate (Alfahad and Nath 2013). The relationship between HAND and outcomes is less well explored in resource-poor settings (Chibanda et al. 2014).

Management

The first step in the management of HAND is early detection of individuals at risk through screening. Individuals with suspected HAND should then be referred to existing services for testing and diagnosis according to availability. If HAND is diagnosed in ART-naïve individuals, it is advisable to initiate cART. Risk factors and comorbid psychiatric and physical conditions need to be identified and treated to improve outcomes.

The only treatment that has demonstrated some benefit in HAND is cART, moderating the morbidity and mortality associated with HIV in both high-income and LMICs (Chibanda et al. 2014). Response may be variable and unpredictable as some individuals remain stable, some improve, and others continue to deteriorate. Not all ARVs penetrate the CNS equally, and studies examining

whether cART combinations with better penetration improve outcomes have delivered inconsistent results (Clifford and Ances 2013). The optimal cART regimen for HAND remains to be established. Maximally suppressive ART regimens should be used to accomplish peripheral viral suppression and prevent resistance. Tolerability, cost, and ease of dosing need to be considered when determining which cART regimen to use. Medication burden is reduced by fixed-dose combination (FDC) formulations containing all the ARVs in a regimen in a single pill. Efavirenz is regularly avoided due to its neuropsychiatric adverse effects; however, WHO guidelines advise using regimens with lower toxicity and increased convenience, and their recommended first-line regimen is a FDC containing efavirenz (WHO 2013).

Patients with HAND and their caregivers need to be educated about the diagnosis and the steps that need to be taken to improve outcomes. Psychosocial problems, such as bereavement, stress, and trauma, common comorbidities in LMICs, can contribute to a poorer prognosis in HIV and thus also need to be identified and addressed (Seedat 2012). Increased social support slows HIV disease progression and improves adherence. Caregivers such as family and friends should ideally be engaged and involved in treatment planning and providing support. Patients with HAND may require assistance in remembering their appointments as well as taking their medication and ought to be directed to treatment support groups, or practical tools such as diaries, alarms, or pillboxes can be utilized. In more advanced HAND, it may be advisable for caregivers to manage the patient's medication directly. Dementia is best managed by multidisciplinary teams including social workers, occupational therapists, and specialized nursing care. Close collaboration and good communication are required between all individuals involved in the person's care. The safety of the patient and the community should be assessed throughout, and if increased supervision is necessitated, the patient can be referred to a residential care facility. Psychoeducation regarding mental illness and HIV is required to improve insight.

Psychiatric disorders are major causes of poor adherence in developed and resource-poor settings and lead to HIV disease progression (Chibanda et al. 2014). Individuals with HAND should be screened for symptoms of mental illness. If mental illness is suspected to be secondary to or aggravated by HIV, cART must be initiated in addition to appropriate psychotropic medications. Individuals with HIV are generally more sensitive to medication side effects, and thus it is advisable to initiate psychotropic medication at a low dose and gradually increase up to a therapeutic level. As far as possible, treatment regimens should be simplified, with the minimum required medications prescribed and less-frequent dosing used when possible, prescribers should be cognizant of potential drug interactions between psychotropic drugs and ARVs. Interventions for mental illness in resource-poor settings need to be cost-effective, culturally acceptable, and integrated into HIV services (Mayston et al. 2012). As there is a critical shortage of specialists in resource-poor settings, interventions may need to be provided in innovative ways such as training community health workers to provide interventions.

Conclusions and Future Directions

The pathways leading to the development of HAND are complex, and an increased understanding of social and economic contextual factors is required. Of the 35 million individuals infected with HIV, around 37% are on antiretroviral treatment (ART), with regional variability in access to treatment (UNAIDS 2014). In the North American and European regions, one in two individuals are on ART, whereas in the Middle East and North Africa region, only one in ten individuals are on ART. Increasing HIV testing, earlier diagnosis and access to cART, and cART adherence support all contribute to decreasing the frequency and severity of HAND. However despite increased dissemination of cART, HAND remains a significant problem, yielding unfavorable outcomes. There is an ongoing search for treatments that can improve consequences in

HAND. Trials investigating neuroprotective and anti-inflammatory drugs have largely been unsuccessful (Alfahad and Nath 2013). Research into the use of nanotechnology to increase ART delivery to the CNS is expanding and holds promise for improved treatments in the future.

There are many challenges surrounding HAND, diagnosis is difficult, and the identification of biomarkers in low-resource settings still requires investigation. In Africa, the region with the highest disease burden, there are many obstacles such as poor health-care infrastructure, a lack of trained staff, and increased comorbidity, such as tuberculosis and malaria (Robertson et al. 2010). In resource-poor settings, screening tools (e.g., the IHDS) are more frequently employed than neuropsychological test batteries with less severe forms of HAND likely to be missed and the burden of HAND likely to be underestimated (Habib et al. 2013). There is, therefore, a need in low-resource settings to validate screening and neuropsychological assessments that can be administered by nonspecialists, are culturally appropriate and time efficient, and incorporate biomarkers of HAND. There is also a need for well-powered trials to assess the efficacy of adjunctive pharmacological agents (e.g., neuroprotective, anti-inflammatory agents) in reducing the considerable clinical and economic burden associated with HAND.

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Neuro-AIDS, Immunopathogenesis of

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Definition

The immune pathogenesis of Neuro-AIDS entails immune responses inside and outside the central nervous system (CNS) that begin immediately with infection in the periphery and occur throughout infection. Immune pathogenesis of Neuro-AIDS is a result of histopathogenesis. Histopathogenesis manifests in neurological symptoms of AIDS under the umbrella term HIV-associated neurocognitive disorders (HAND). Therefore, components of immune pathogenesis include early viral entry to the CNS, viral replication in inflammatory cells and resident CNS cells, release of toxic proteins by inflammatory and resident CNS cells, and perhaps viral proteins, glial cell responses, perivascular macrophage cuffing, the formation of HIV syncytia, and macrophage nodules. Hallmarks of immune pathogenesis of HIV include the presence of viral RNA, DNA, and proteins in myeloid lineage cells and nonproductive infection of astrocytes. Additionally, there are perivascular macrophage accumulations around CNS blood vessels (cuffing,) reactive astrocytes (astrogliosis), and elevated proteins including sCD14, sCD163, neopterin, and neurofilament light (NFL) shed from activated myeloid and glial cells and nerve cells.

Elevated levels of biomarkers shed from activated and infected myeloid cells, activated glia, or damaged neurons/axons are found in the plasma and cerebral spinal fluid (CSF) and are considered markers of active disease as well as viral replication.

Early Virus Entry Into The CNS

The immune pathogenesis of Neuro-AIDS, by definition, is comprised of the immune responses within the CNS that occur with HIV infection. The immune responses are a result of both virus entering the CNS and cells responding to CNS infection. In simian immunodeficiency virus (SIV) infection of rhesus monkeys, the virus is detected as early as 3 days postinfection (Williams and Burdo 2012). In human iatrogenic infection, virus was detected 24 h postinfection. HIV infection is found as early as 8 days postinfection in the CSF of normally infected individuals (Zayyad and Spudich 2015).

Mechanisms of Viral Entry and Establishment of CNS Reservoir

Mechanisms by which the CNS is infected are not clear. Virus can theoretically enter as free virus or cell-associated virus within CD4⁺ T cells or monocytes programmed to become CNS and perivascular macrophages (Campbell et al. 2014a). In experimental infection of monkeys, SIV DNA and RNA are found within cuffs of macrophages 1–3 days postinfection (Williams and Burdo 2012). SIV DNA is then found throughout the course of infection, but viral RNA and protein are not detected after primary infection, until animals develop AIDS. Whether virus reemergence with AIDS represents recrudescence of virus that entered during primary infection or new waves of entry with immune activation and development of AIDS is not clear. Emerging viral sequence data support the later contention (Zayyad and Spudich 2015). In addition to the presence of SIV and

HIV DNA and RNA early after infection and perivascular cuffs of myeloid cells including perivascular macrophages and inflammatory MAC387⁺ macrophages, CD8⁺ T lymphocytes are evident in perivascular cuffs, with SIV encephalitic (SIVE) and HIV encephalitic (HIVE) lesion. CD8⁺ T lymphocytes comprise approximately 5% or less of the cells in the lesions, where the remaining cells in the lesions are inflammatory macrophages, parenchymal macrophages (microglia), and recruited CD163⁺ perivascular macrophages. Experimental depletion of CD8⁺ lymphocytes in monkeys results in rapid AIDS and severe SIVE. Studies using SIV-gag tetramer complexes and immunohistochemistry demonstrate the presence of SIV-gag-specific CD8 T lymphocytes and CD8 T lymphocytes in the CNS juxtaposed with SIV-p28⁺ macrophages. Depletion of CD4⁺ T cells in SIV-infected monkeys also results in severe CNS infection with numerous multinucleated giant cells (MNGCs) and infected macrophages and microglia, underscoring the importance of CD4 T cell immunity, CD8 T cell immunity, and immune deficiency, in the development of Neuro-AIDS (Micci et al. 2014).

Peripheral Immune Correlates of Brain Inflammation

Early infection (primary infection) and accumulation of inflammatory cells in late infection (chronic infection) correlate with white matter changes measured by MR spectroscopy (MRS) and diffusion tensor imaging (DTI) in humans and monkeys (Ances and Hammoud 2014). Activated monocytes in blood correlate with decreased NAA/Cr in monkeys, which is a measurement of neuronal injury (Crews et al. 2008). Elevation in the numbers of activated monocytes and CD8 lymphocytes correlates with decreased NAA/Cr in HIV infection (Crews et al. 2008). DTI demonstrates early white matter changes that correlate with blood-brain barrier (BBB) disruption that is an early hallmark of CNS pathogenesis and likely subsequent monocyte/

macrophage recruitment (Ances and Hammoud 2014).

Brain Macrophages Are Primary Targets of HIV and SIV Infection

Cells of the myeloid lineage are the most common cell type found latently and productively infected in the CNS (Williams and Hickey 2002; Williams et al. 2011). The CD14⁺ CD163⁺ perivascular macrophages are the most commonly identified infected cell. These cells are continually replaced from the bone marrow normally at a rate that is accelerated with inflammation and AIDS (Williams and Burdo 2012). Perivascular macrophages are repopulated in the CNS from bone marrow-derived monocyte precursors and CD34⁺ stem cells (Williams and Burdo 2012). The resident CNS macrophage, parenchymal microglia, can be infected *in vivo*, but the prevalence of these infected cells is much lower than perivascular macrophages (Williams et al. 2001). Astrocytes were demonstrated to be latently HIV and SIV infected but do not appear to replicate the virus. Prior to combination antiretroviral therapy, multinucleated giant cells (MNGCs) were found in human CNS with fulminant disease. They are routinely found in the CNS of SIV-infected monkeys with AIDS and are in fact a hallmark of SIVE. These cells replicate high levels of HIV and SIV RNA and proteins. They share CD14, CD16, and CD163 immune markers found on perivascular macrophages, have numerous nuclei, and likely arise from a fusion of perivascular macrophages when immune dysregulation of macrophages occur with AIDS (Williams et al. 2001).

Activation of Peripheral Monocytes and Trafficking to the Brain

Outside the CNS, monocyte activation occurs with HIV and SIV infection early and then again late in infection, in a biphasic pattern. Interestingly, this biphasic pattern of monocyte activation

occurs whether infection results in rapid progression and disease, slow progression, and regardless of the rate of development of AIDS. The absolute number or relative percentage of activated CD14⁺ CD16⁺ monocytes correlate with the development of HIV-associated dementia (HAD) in humans and neuronal injury and development of SIVE in SIV-infected monkeys (Campbell et al. 2014a). CD14⁺ CD16⁺ monocytes in humans and monkeys have been demonstrated that are HIV and SIV infected, even in humans on anti retroviral therapy (ART). CD14⁺ CD16⁺ monocytes have varying levels of CD163 and are immune phenotypically similar to CNS perivascular macrophages. Bacterial translocation from the gut is suggested to correlate with monocyte activation and HAND. Bromodeoxyuridine (BrdU) label studies find the magnitude of BrdU⁺ monocytes in blood, which are derived from BrdU-labeled monocyte precursors in the bone marrow and blood, and correlate with the rate of development of AIDS and level of CNS tissue pathogenesis in SIV-infected monkeys (Williams and Burdo 2012). Experimental studies in SIV-infected monkeys, using an antibody against alpha-4 integrin at the time of SIV infection, result in lack of productive and latent SIV infection of the CNS (Campbell et al. 2014b). Using the same experimental paradigm, administering anti-alpha-4 integrin antibody at a time where fulminant CNS has occurred and productively infected macrophages are present in the CNS with neuronal damage results in reversal of neuronal damage, non-detectable SIV DNA or RNA in the brain, and resolution of macrophage nodules (Campbell et al. 2014b). These results underscore the role of monocyte/macrophage infiltration of the CNS in Neuro-AIDS. Different immune fluorescent-tagged dextran dyes experimentally injected into the CSF and taken up by perivascular macrophages at early infection, asymptomatic infection, and AIDS demonstrate turnover of perivascular macrophages at a rate that accelerates with the severity of and rate of development of Neuro-AIDS. Perivascular macrophages labeled with dextran dyes early, prior to the development of SIVE lesions, make up the majority of cells in

SIVE lesions demonstrating perivascular macrophage traffic from justavascular sites to CNS lesions (Williams and Burdo 2012; Williams et al. 2012). Despite this, the role of inflammatory macrophages in the CNS with Neuro-AIDS is critical.

Accumulation of Monocyte/Macrophage and Histopathological Changes in the Brain

In patients with HIV and SIV infection, perivascular macrophages accumulate in CNS vessels and some are infected. Recently recruited MAC387⁺ monocytes/macrophages are also present and are only found in the CNS with inflammation (Williams and Burdo 2012; Williams et al. 2012). The ratio of the number of MAC387⁺ macrophages that are of M1 pro-inflammatory immune phenotype and CD163⁺ perivascular macrophages that are of M2 anti-inflammatory repair phenotype is >1 in early lesions that are active and forming. The ratio of MAC387⁺ macrophages to CD163⁺ macrophages is <1 in established, chronic lesions, consistent with CD163⁺ macrophages functioning to down-regulate CNS and MAC387⁺ macrophages amplifying antiretroviral responses. Overall, the number of inflammatory macrophages is the histopathologic correlate of HAND as well as the degree of neuronal injury (Williams and Burdo 2012; Williams et al. 2012).

Plasma and CSF Biomarkers of CNS Inflammation

A series of proteins including chemoattractants, immune molecules, and in some cases viral proteins are secreted into the plasma or CSF where they are considered biomarkers of CNS Neuro-AIDS disease. Numerous studies support the examination of CSF HIV RNA, soluble CSF and plasma markers of immune activation (MCP-1, sCD14, sCD163, osteopontin (OPN), neopterin), markers of neuronal injury (NFL), and

neuroimaging markers as biomarkers of HAND (Letendre and Ellis 2012). The majority of these markers are shed from activated or infected myeloid cells or are involved in the chemoattraction of myeloid cell traffic to their retention in the CNS. In plasma, soluble CD14 (sCD14) and CD163 (sCD163) are elevated in SIV- and HIV-infected monkeys as well as humans prior to and on durable ART (Williams and Burdo 2012). sCD163 levels in plasma correlate with HIV RNA in acute and chronically infected individuals prior to and after effective ART. Plasma neopterin, an indicator of macrophage activation, is also elevated in untreated HIV⁺ patients. Plasma sCD163 levels correlate with the degree of neurologic deficits in HAND. Plasma LPS that can activate innate immune responses including monocytes and macrophages correlates with the prevalence of HAND (Williams and Burdo 2012; Williams et al. 2012). Plasma OPN (secreted by macrophages) levels are significantly increased in individuals with HIV-associated dementia and increase prior to SIV-induced neurological and clinical abnormalities.

Neopterin, β 2-microglobulin, macrophage chemoattractant protein-1 (MCP-1), quinolinic acid, neurofilament light (NFL), TNF- α and soluble TNF receptor, chemokine receptor-5 (CCR5) ligands (MIP-1 α , MIP-1 β , and RANTES), S100 β , and glutamate are all elevated in CSF with HIV infection and correlate with HAND (Letendre and Ellis 2012). Many of these correlations were found in the pre-ART era. With ART to date, none of these markers are used for clinical studies. But sCD163 in plasma still functions. Neurofilament protein (NFL) levels are elevated in HAD patients. NFL levels predict the onset of HAD, and effective ART reduces NFL CSF levels (Zayyad and Spudich 2015). Measurement of single-copy HIV RNA shows a low level of viral persistence in some ART patients with persistent neuroimpairment. Macrophage activation, reflected by increased levels of CSF neopterin, is also an important factor in neuropathogenesis. In untreated HIV-infected patients, CSF neopterin is elevated and increases as suppression worsens and CD4⁺ T cell counts decrease. Effective ART

does not consistently normalize macrophage activation. ART initially reduces neuroinflammation, but reduced MCP-1 CSF levels often rebound (Letendre and Ellis 2012). Neuroimaging techniques demonstrate neuronal injury, inflammatory response, gliosis, brain atrophy, and decreased cerebral blood flow in the brains of HIV-infected patients using magnetic resonance spectroscopy (MRS), volumetric MRI, diffusion tensor imaging (DTI), and functional MRI (fMRI) (Ances and Hammoud 2014).

Conclusion

The presence of virus in the CNS early after infection in the periphery initiates Neuro-AIDS. Hallmarks of Neuro-AIDS include activated monocytes and increased numbers of these cells in the blood, the presence of replicating virus in the CNS, and then latent infection with reemergence with AIDS. Cuffs of perivascular macrophages that are CD14⁺ CD16⁺ CD163⁺ cells that can be virally infected are present. Additionally, MAC387⁺ macrophages that are not infected accumulate early in disease. Accumulation of macrophages, some of which are infected, is a histopathologic hallmark of Neuro-AIDS, as elevated myeloid shed sCD163 and CD14 in the plasma and proteins shed in the CSF. Blips of viral RNA are detected in the CSF of individuals on ART, which demonstrates that there is likely an ongoing low-level CNS infection in individuals on durable ART. Functional neuroimaging demonstrates changes in BBB permeability early with infection and white matter changes consistent with pathogenesis. CD8 T lymphocytes and CD4 T cells both regulate immune activation that can result in Neuro-AIDS.

Cross-References

- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [Medication Adherence and HIV-Associated Neurocognitive Disorders \(HAND\)](#)

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Neurocognitive Functioning in HIV-infected Substance Users

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Definition

This entry summarizes neurocognitive research on HIV+ individuals who have past or current substance use disorders. This entry focuses on findings since the introduction of combination antiretroviral therapy. We focus on neurocognitive correlates of specific drugs of abuse, placing special emphasis on the independent and additive effects of these drugs on the brain and cognition and on implications for clinical management. Finally, strategies for treating this vulnerable population are provided, with a specific focus on neurocognition.

Introduction

The frequency of substance use disorders (SUDs) among HIV+ individuals often makes it difficult to distinguish substance-related neuropathological and cognitive effects from those effects due to HIV-associated neurocognitive disorders (HAND). Detecting the presence of HAND in individuals with a concurrent SUD is further complicated by multiple SUD-associated comorbid conditions, including head injury, psychiatric illness, cerebrovascular risk, and attention deficit/hyperactivity disorder. Neurocognitive impairment, whether secondary to HIV, substance abuse, or both, complicates clinical management, increases high-risk sexual and injection practices with exacerbation in the risk of HIV transmission

and superinfection, and limits the capacity for successful treatment adherence and ability to function independently. Additionally, the clinical management of HIV+ substance-dependent individuals is further complicated by psychosocial consequences such as chaotic living environments, homelessness, and risk of incarceration. Therefore, understanding the complex relationship between SUD and HAND is critical in both the clinical and research milieu. Herein, we summarize relevant research regarding neurocognitive impairment among HIV+ individuals with past or current SUDs. We focus our review on studies conducted since the introduction of combination antiretroviral therapy (cART), with special emphasis on their independent and additive effects on the brain and cognition and their translational implications for clinical management.

Alcohol

Comorbid alcohol dependence in HIV+ persons can disrupt multiple cognitive functions, most evident among those with chronic heavy drinking. Heavy drinking (e.g., average consumption of at least 100 (80 for women) standard alcoholic drinks per month) and a positive HIV serostatus are individually associated with poorer neurocognitive test scores, and these together result in an additive or even synergistic effect on tasks requiring speeded information processing (Levine et al. 2012). Importantly, neurocognitive impairment can be attenuated with adequate antiretroviral treatment among alcohol users (Rothlind et al. 2005). Additional evidence of additive or synergistic effects of alcohol and HIV have been reported in recent years, including greater impairment on measures of immediate episodic memory, psychomotor speed, sustained attention, and associative learning (Levine et al. 2012).

Overall, studies have consistently demonstrated a greater risk of neurocognitive impairment among HIV+ individuals with heavy alcohol use, particularly among heavy drinkers who have consumed alcohol within the past 12 months and who have poorer viral suppression. It is also inferred that some degree of cognitive impairment could persist for up to one year following cessation of drinking, with most

prominent disruption in information processing. However, since psychomotor slowing is a prominent neurocognitive deficit among HIV+ individuals, it may be difficult to distinguish alcohol effects from those of HIV.

Cannabis

In recent years, there has been a growing trend to facilitate access to cannabis for individuals living with HIV/AIDS in order to help alleviate various symptoms associated with the disease. While recent research suggests promising therapeutic applications for smoked cannabis in the context of HIV/AIDS, including treating AIDS-associated anorexia and neuropathic pain, such studies have not addressed the long-term implications of cannabis use on neurocognitive functioning. It is important to note that dronabinol, an orally consumed synthetic tetrahydrocannabinol (the main psychoactive ingredient in cannabis), has been approved by the Food and Drug Administration for AIDS-associated anorexia since 1992. Nonetheless, FDA approval of dronabinol has not diminished the demand and research for botanical cannabis and pharmaceutical compounds that may mimic more closely the effects of smoked cannabis, as dronabinol is orally consumed and possesses different metabolism and drug action compared to smoked cannabis.

Studies examining the acute effects of dronabinol and smoked cannabis on the neurocognitive performance of HIV+ individuals are conflicting. One found that smoked cannabis had no acute impact on any of the neurocognitive tasks, but 30 mg of dronabinol adversely affected neurocognitive performance, and 20 mg of dronabinol adversely affected neurocognitive performance among a group with low body fat composition. However, another study concluded that both oral dronabinol and smoked cannabis use did not significantly change performance on cognitive measures compared to placebo (Levine et al. 2012).

The few published studies to date that have specifically examined the effects of longer-term cannabis use on the brain functioning of HIV+ individuals are also equivocal. One group found that symptomatic HIV+ individuals that used

cannabis frequently performed more poorly on a global measure of neuropsychological functioning compared to those with a history of minimal or no cannabis use. In contrast, a separate group found that the use of cannabis did not affect the neuropsychological performance among HIV+ subjects, as they reported no significant interactions between HIV serostatus and history of cannabis use. However, when they compared brain metabolite levels across groups using magnetic resonance spectroscopy, they found evidence of negative additive effects of cannabis use and HIV for some (but not all) metabolites in the basal ganglia and thalamus. In the third of existing studies, the researchers examined procedural learning (PL; i.e., acquisition of motor skills and habits) among HIV+ adults with a history of substance use disorders. Both positive HIV serostatus and a history of cannabis dependence were independently associated with poorer performance in complex motor skills with significant adverse additive effects of HIV and cannabis use history on performance.

Nicotine

Rates of cigarette smoking have been estimated at up to 88% among individuals in substance abuse treatment, and HIV+ individuals are three times as likely to smoke as the general population. However, there are minimal data regarding the neurocognitive consequences of nicotine use among HIV+ smokers. Compared with HIV-seronegative individuals, scans of non-smoking HIV+ persons with heavy alcohol use show significantly smaller volume in the prefrontal cortex; however, HIV+ persons with both chronic cigarette use and heavy drinking show significantly lower volumes in the frontal, temporal, and parietal cortex and also show poor performance on tests of memory and learning as compared to HIV+, nonsmoking, heavy drinkers. These findings illustrate the need to address the history of nicotine dependence as a source of additional neurocognitive and neurobiological burden among HIV+ subjects with alcohol dependence.

There may be seemingly paradoxical effects of nicotine use on the neurocognitive functioning in

HIV+ patients. A recent study of HIV-seronegative and HIV+ women found that current cigarette smoking was associated with higher plasma HIV viral burden and greater immunosuppression; however, no associations between neurocognitive functioning and either current or past history of smoking were found (Levine et al. 2012). Additionally, when analyses were restricted to the HIV+ women, individuals with a history of smoking performed significantly higher on measures of frontal/executive functioning. While this finding may seem counterintuitive, it is consistent with reports from animal studies that nicotine has protective effects. Currently, the mixed findings regarding nicotine use and neurocognitive functioning in HIV make it impossible to make conclusions regarding nicotine use in HIV. However, considering the multiple health hazards associated with cigarette smoking, cessation programs are likely to be beneficial for HIV+ persons.

Cocaine

The use of crack or powder cocaine is common among HIV+ drug users, but surprisingly few studies have evaluated their neurocognitive effects. Large studies have failed to find an increased risk for neurocognitive impairment in HIV+ cocaine users as compared to those with HIV or cocaine use alone (Durvasula et al. 2000). However, this may be due to lack of adequate measures or narrow demographic inclusion criteria, as others have found deficits specifically in sustained attention, verbal memory, and visuospatial functioning among active HIV+ cocaine users as compared to HIV+ nonusers (Levine et al. 2006). Neuroimaging studies have found neurophysiological differences, with HIV+ cocaine users having decreased dopamine transporter in the caudate as compared to HIV+ nonusers and HIV-negative controls; however, no clinical correlation was detected with neurocognitive tests (Chang et al. 2008).

Clearly, more detailed studies are needed to determine what factors are associated with neurocognitive deficits among HIV+ users of cocaine. Among these is sex; current evidence has raised the question of increased vulnerability

to neurocognitive impairment among HIV+ female crack users. The literature on gender differences in drug dependence has shown that compared with men, women are more highly responsive to cocaine/crack effects and that crack use is highly predictive of high-risk sexual behavior among women but not men. It has been reported that compared with women with no or non-crack drug abuse history, persistent crack users had significantly lower CD4 counts and higher HIV RNA levels; further, persistent users were three times as likely to die from AIDS-related causes (Levine et al. 2012). Recently, neuroimaging studies among HIV+ women have revealed aberrant functioning in the anterior cingulate and left dorsolateral prefrontal cortex and associated verbal memory deficits (Meyer et al. 2014).

Methamphetamine

Perhaps the most commonly studied drug in the context of neuroAIDS, studies suggest an additive and synergistic effect of HIV and methamphetamine use on neurocognition and biochemical, structural, and functional brain imaging. The risk of neurocognitive impairment increases with each additional risk factor (HIV and methamphetamine abuse) (Rippeth et al. 2004). Clinicopathological studies indicate that global neurocognitive impairment and memory scores are significant predictors of the severity of neuronal damage, which was significantly more extensive for HIV+ methamphetamine users with HIV encephalitis compared with methamphetamine users without HIV encephalitis and with HIV+ non-methamphetamine users without HIV encephalitis (Chana et al. 2006). While these findings suggest an additive effect, others found evidence for the synergistic impact on brain functioning affecting physiological parameters such as decreased baseline cerebral blood flow (CBF) (Ances et al. 2011). Still, the impact of methamphetamine on everyday functioning is less clear. For example, comorbid HIV and methamphetamine dependence did not increase the likelihood of complaints about problems with everyday functions, such as driving and managing finances, beyond

that found with either HIV or methamphetamine dependence alone (Sadek et al. 2007). The results suggest that the consistent neurocognitive deficits detected in the aforementioned studies may not necessarily translate to everyday functioning.

Club Drugs

“Club drugs” are those that have traditionally been used at dance clubs and raves and include 3,4-methylenedioxymethamphetamine (MDMA, or “ecstasy”), ketamine (“special K”), and gamma-hydroxybutyrate (GHB). Club-drug use is more prevalent among men who have sex with men (MSM) as compared to the general population. Despite the high rate of club-drug use among MSM, there are no published studies that examine the cognitive effects of these substances in the context of neuroAIDS. Still, based on their actions on the catecholamine system, as well as their high potential for abuse among HIV-prone populations, there are numerous reasons to suspect an interactive or additive effect of these drugs with HIV (Levine et al. 2012).

Neurobehavioral Treatment Issues in HIV+ Substance Users

HIV+ individuals with comorbid SUD present unique treatment challenges. Those with untreated SUD are less likely to receive cART therapy or achieve viral suppression even when cART is prescribed. These individuals often face a difficult road; the dual stigma of HIV and substance abuse makes access to treatment more difficult, and the lack of effective and consistent medical care may make abstinence more difficult. Further, both researchers and clinicians must contend with direct and indirect medical consequences of drug abuse, such as head injury, stroke, and malnutrition. Additionally, SUDs are frequently accompanied by a spectrum of neuropsychiatric conditions, including mood disorders, anxiety disorders such as PTSD and social phobia, ADHD and learning disabilities, and personality disorders, adding to the complexities and challenges of treatment. Clinicians should be mindful

of comorbid conditions and make appropriate referrals to substance use clinics and mental health professionals.

Adherence

Viral suppression requires optimal adherence to cART. While the definition of optimal adherence varies across studies, it generally can be said that at least 80% of scheduled doses must be taken (Malta et al. 2008). Adherence rates among active substance users have consistently been found to be suboptimal (Levine et al. 2012). Fortunately, abstinent drug users or individuals with access to substance use and mental health treatment have better adherence rates (Hinkin et al. 2007). While active substance use is associated with poorer adherence, various treatments improved this (Malta et al. 2008). For example, higher adherence rates were found among individuals receiving care in structured settings, as well as those in drug substitution therapy (e.g., methadone treatment). Additional factors resulting in poor adherence included poor self-esteem, recent incarceration, and negative outcome expectations.

Studies examining the interaction between neurocognitive impairment and adherence in substance users have shown that neurocognitive functioning is an intermediary between drug abuse and adherence (Levine et al. 2012). Findings from such studies have led to remediation and compensatory programs, as discussed in the following section.

Managing Neurocognitive Deficits in the Treatment of HIV+ Substance Using Individuals (SUI)

Fortunately, there has been considerable interest in the treatment for SUDs in HIV+ individuals, including those with neurocognitive deficits. There are several ways in which neurocognitive deficits common among HIV+ individuals may have a detrimental effect upon HIV-preventive behavior (Norman et al. 2009). For example, cognitive-behavioral techniques commonly used with substance abusers might be difficult to

remember and implement in the face of learning and executive deficits experienced by HIV+ individuals. To counter this, Norman et al. (Norman et al. 2009) suggested cognitive remediation strategies, including the frequent use of multimodal presentation of materials in order to facilitate learning and consistent training in a distraction-free environment to overcome impairments in retention and attention. Patients should be assessed frequently so that they have the opportunity to evaluate the efficacy of their treatment regimen. In order to facilitate the generalization of skill learning to day-to-day life, treatment should include real-world scenarios and situations. Assistance from the patients' social support network and other health-care providers may also improve the chance of success. In fact, group treatment may be the preferred treatment modality for neurocognitively impaired HIV+ SUIs, as this context encourages and strengthens prosocial behaviors. Group treatment could also reduce feelings of isolation and provide interpersonal support from peers with similar backgrounds.

From a medical standpoint, arguably the most important aspect of HIV treatment is maintaining strict adherence to cART. Thorough knowledge of potential obstacles could improve treatment outcomes. Wood et al. (2008) undertook a substantive review in order to summarize the evidence regarding barriers and facilitators to cART adherence among IDUs, which can also be applied to SUIs in general (Wood et al. 2008). They identified decreased access to treatment and poor adherence to treatment as existing challenges. Within each of these are sociopolitical, individual, and provider-based barriers. For example, adherence to medication among SUIs includes sociopolitical barriers such as incarceration and financial constraints, individual barriers such as fear of side effects and psychiatric illness, and provider-based barriers such as physicians' belief that SUIs will not be able to adhere to their regimen. In the context of this review, cognitive deficits would fall under individual barriers. A number of evidence-based interventions have been developed that are aimed at reducing these specific barriers to treatment. Examples of strategies

aimed at addressing sociopolitical barriers include outreach programs and meeting SUIs on their own terms (e.g., through needle exchange programs). Examples of strategies that address individual-based concerns include increased efforts to improve health insurance coverage and free access to medical care, utilizing HIV-experienced physicians in order to improve the treatment relationship and providing substance abuse treatment and housing support in order to reduce physicians' reluctance to prescribe cART. The recommendations put forth by Norman et al. (2009), described above, can also be considered individual-based strategies. Finally, provider-related strategies include modifications of clinics in order to improve uptake and adherence to cART by being highly flexible, comprehensive, and interdisciplinary. Key features of such programs include on-site pharmacists, HIV specialist nurses, drop-in services, geographic proximity, and case management services. A final important strategy mentioned by Woods et al. is the linking of addiction treatment (e.g., substitution therapy) with cART. Such "directly administered therapy programs," which also provide daily supervision of antiretroviral intake, are associated with improved adherence. Note, however, that there are potential difficulties with substitution therapies. For example, concurrent psychoactive medication and opioid substitution therapies such as methadone have been associated with impaired cognitive performance (Mintzer and Stitzer 2002). Further, pharmacokinetic studies of various ARTs and methadone or buprenorphine indicate that, when taken together, there are alterations in metabolic concentrations of both drug classes that may have adverse consequences on HIV disease status and opioid-therapy effectiveness. However, findings from a 5-year longitudinal study provide a more sanguine outlook, indicating that consistent opioid substitution therapy is predictive of virologic suppression (Bruce et al. 2010). Clinicians' awareness of such research will help facilitate its application and development.

Finally, integrated behavioral and medical interventions have been successfully applied to HIV+

substance users, although the optimal structure remains unclear. For example, variations in ease of communication between health-care providers, availability of office space, professional autonomy, and available services exist between integrated HIV clinics/substance use treatment centers and those that are simply collocated (Lombard et al. 2009). However, some essential features emerge from the literature, including the need for both conditions to be treated simultaneously by health care with proper experience/training, minimizing the stigma associated with either condition, installing valid methods for detecting the presence of either condition, and providing other key services for clients within a single location.

Conclusion

Despite the information provided in this entry regarding the putative additive risk of substance abuse on neurocognitive functioning in those with HIV, the interaction between substance abuse and HAND has not been well detailed among numerous commonly abused drugs. Despite their high prevalence among those with HIV, drugs such as marijuana, cocaine, and the class collectively known as “club drugs” have received relatively little attention. Further, research across different substance types among HIV+ individuals has not been balanced; most post-cART studies focus on stimulants such as methamphetamine. In addition, studies examining drug combinations with synergistic neurotoxic effects (e.g., alcohol and cocaine) are necessary. Finally, the available literature indicates the importance of additional studies of sex differences in neuroAIDS and substance use, which have potentially critical implications for clinical practice and treatment.

Continued research into evidence-based treatment strategies must occur in tandem with the investigation of mechanisms of HAND neuropathogenesis among SUIs. The treatment hurdles associated with each condition alone are difficult to overcome; individuals with comorbid SUD and HAND represent an especially difficult population. However, identifying the individual

barriers to treatment makes it possible to address each simultaneously and effectively.

Cross-References

- ▶ [Comorbidity: Opioids](#)
- ▶ [Harm Reduction for Injection Drug Users](#)
- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [HIV Prevention Efforts Within Substance Use Disorder Treatment Settings](#)
- ▶ [Neuropsychological Testing in HIV-Infected Individuals](#)

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Neurocognitive Outcomes in HIV-Infected Children and Adolescents

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Introduction

Perinatally acquired HIV (PAH) infection was first described in 1981. Within a short time, it became clear that, as with adults, PAH could lead to devastating central nervous system damage in infants and children. Through the early years of the epidemic it also became clear that many infected infants had more subtle static neurocognitive deficits and behavioral disorders.

With the advent of effective antiretroviral therapies and earlier initiation of therapy for infected children, the incidence of more serious HIV-related neurocognitive effects decreased dramatically, and they are rarely seen today. In regions with access to effective pediatric antiretroviral therapy (ART), the vast majority of youth with PAH have learning and behavioral profiles similar to age and sociodemographically matched uninfected youth. With the ability now to achieve lifelong control of the infection, the hope is to develop treatment strategies that best ensure optimal long-term neurocognitive and behavioral outcomes.

Pathophysiology

The exact mechanisms by which HIV enters and damages the central nervous system is still under study. As mentioned elsewhere (refer to entries on neuropathology), HIV-related neurologic changes are a direct consequence of HIV infection and subsequent immune activation.

Very early in the course of HIV infection, the virus spreads to the brain across the blood-brain

barrier, most likely carried by CD14+/CD16+ monocytes. Using a SIV infection model in primates, viral infection of the CNS was found within 1–3 days of an oral challenge with a high titer of SIV. In adults, HIV entrance into the CNS has been shown to occur as early as 2 weeks to 3 months after infection and most likely occurs even earlier. Among adults with acute HIV infection, 80% had measurable HIV RNA (>100 cpm) in the CSF at a median of 18 days postexposure (Peluso et al. 2015). In perinatally infected infants, systemic infection is estimated to occur within 2 months prior to delivery for a third of exposed infants, while the remainder of infections occur during the intrapartum period (in the absence of breastfeeding). CNS infection may also occur in utero or in the early perinatal period. Because of an immature blood-brain barrier in neonates and very young infants, they are at higher risk for early and severe CNS manifestations compared to older children and adults.

Infection of CNS microglial and macrophage cells leads to established infection. Infection of astrocytes, and possibly neurons, can occur as well, but their involvement in the neuropathogenesis of HIV encephalopathy is less clear. The interplay between characteristics of viral infection and replication, ongoing immune activation and host genetic factors and responses leads to the symptomatology seen among individuals.

Multiple markers of brain inflammation, immune activation and CNS neuronal damage have been shown to correlate with the level of neurologic impairment. These include serum and CNS markers of monocyte and macrophage activation (increased percentage of CD14+/CD16+ monocytes and levels of sCD14, sCD163, and quinolinic acid and TNF-alpha), as well as neuronal cell injury (CSF neurofilament light (NFL)). Several of these products, such as TNF-alpha and quinolinic acid, are neurotoxic. In addition, plasma levels of P-selectin and fibrinogen (markers of vascular dysfunction) have also been associated with pediatric cognitive outcome (CO) (Kapetanovic et al. 2010).

Most of these markers have been best studied in adults, and appear to correlate with ongoing CNS activation, viral replication and cellular

damage. The prospective use of these markers to identify children and adults most at risk for ongoing CNS infection/damage, and receive potential adjunct therapies to preserve CO, have not been clinically studied.

Neuroimaging of HIV-infected adults shows subtle changes within months of initial HIV seroconversion. The severity of changes seen on later imaging correlate with clinical severity of CNS disease in both children and adults. Similarly, employing magnetic resonance spectroscopy (MRS), findings in symptomatic PAH revealed changes associated with neuronal cell loss and inflammation. In addition, MRS studies in asymptomatic PAH were consistent with delayed brain development and neuro-inflammation. In infants with rapidly progressive CNS disease, cerebral atrophy and secondary compensatory enlargement of ventricles, with/without basal ganglion calcifications, can be seen in the first year of life and correlate with neurodevelopmental outcomes.

The issue of viral/host interactions in the pathogenesis of HIV-related CNS disease has been an area of intense interest. Differences exist in the reported incidence of HIV-related neurocognitive disease between HIV-1 and HIV-2, as well as between different HIV-1 viral clades and subtypes. So-called macrophage trophic viral strains appear to have an increased propensity for CNS infection, compared to T-cell trophic viral strains (though it appears that both viral strains can establish productive CNS infection). In addition, within subtypes, specific viral strains with increased neuro-tropism has been demonstrated. Finally, studies in adults and children have noted different rates of HIV-related CNS disease among infected individuals based on genetic-based differences in chemokine receptor density. For instance, among HIV-infected children and adults who are heterozygous or homozygous for a mutation (delta 32), that results in a decreased density of CCR5 receptors on cell surfaces, the incidence of HIV-related CNS disease appears decreased. Other specific genetic polymorphisms (such as polymorphisms in the gene coding for the CXCR2 receptor) have also been identified that appear to play a role in individual patient susceptibility to HIV-related CNS disease.

Among the early hypotheses of the pathogenesis of HIV-related CNS disease, was the possible contribution of other CNS infections acting synergistically with HIV infection. In the pre-ARV era, studies suggested that HIV-infected adults and infants co-infected with CMV were at increased risk of CNS disease and poor neurocognitive outcomes. However, data from the HAART era indicate that cognitive outcomes are the same for co-infected children compared to those who are only HIV-infected.

Clinical Presentation

The most catastrophic CNS aspect of pediatric HIV infection is a progressive encephalopathy (PE), characterized by Belman, Epstein and others (Belman et al. 1985; Epstein et al. 1985). The definition of PE was generally accepted to include at least two of three of the following: loss of previously acquired skills (or a confirmed >15 point loss in full-scale IQ testing), or a prolonged plateau period without any developmental advancement, with an acquired microcephaly and/or neuroimaging with evidence of cerebral atrophy with/without basal ganglion calcifications, and/ or corticospinal tract signs (paresis/spasticity/weakness). As noted, the severity of the changes on neuroimaging (atrophy and calcifications) were generally associated with a worse clinical status. Several patterns of the course (trajectory) of PE were eventually appreciated. In many infants, after a normal developmental trajectory at first, the disease ran a rapidly progressive and fatal course, with a rapid loss of skills; in pretreatment studies, average survival was 12 months following a diagnosis of PE. However, other children were noted to have a prolonged plateau phase of developmental advancement, followed by a slow increase in skills. Still others exhibited a pattern of rapid loss of skills interspersed with plateau or stabilization phases. Seizures were relatively rare, and generally seen late in the course of the disease.

The incidence rate of PE was greatest in the first 3 years of life (as high as 10% in the first year of life), falling to around 0.5–1%/year after age

3. In general, the onset of symptoms occur in the first years of life (with >50% diagnosed by age 3, and 75% by 4–5 years of age). Cumulative rates of PE in the USA, prior to HAART, were 20–35%, with a 4%–10% incidence rate by age 1 (Patel et al. 2009).

Infants infected in utero (as defined by a positive HIV PCR DNR, RNA or culture or day 1 of life) were at high risk for a rapid progressive disease trajectory, including peak viral loads (>5–5.5 log₁₀ cpm) and lower CD8 percentages in the first year of life, significant HIV-related symptoms within 24 months of life, and death by age 4. From the pre-ARV error, infants with PE were generally those with this more rapid progression pattern in the first 2 years of life. It should be noted however that not all of the infants with these high-risk markers developed HIV-related CNS disease.

Among infected children without the rapid progression pattern, PE was unusual, except as a very late event. The incidence of HIV-related encephalopathy among PAH, after infancy, is comparable to that among recently infected adults at about 1% per year. This led to the consideration that PE represented an in utero CNS infection, or infection with a more neurotrophic/pathogenic viral strain, as discussed above.

In addition to PE, in the pre-HAART era, an additional 20% of children were noted to have a static chronic encephalopathy (SE). In these children there was a stable delay in acquisition of cognitive and motor skills, but without loss of previously acquired milestones. Overall, prior to the advent of antiretroviral therapies, 40% of PAH developed either PE or SE CNS manifestations of HIV infection.

As noted, it was unusual to diagnose new-onset PE in children after age 4 or 5, except as a very late, preterminal event. One longitudinal study noted that among children who had neither PE nor SE at first testing (mean age 4 years), full-scale IQ results were not statistically different after a median follow-up of 4.75 years (Shanbhag et al. 2005). A more recent study from the same site determined that those PAH patients surviving to at least age 13 (mean age 15 years) had a mean full-scale IQ not

significantly different from those tested at a median age of 8 years (87 vs. 92) (Wood et al. 2009).

Treatment of Pediatric HIV-Related CNS Disease

Surprising results were seen in the original pediatric HIV treatment study using zidovudine, conducted at the National Cancer Institute. There, Pizzo and colleagues treated HIV-infected children with a 6-month course of a continuous infusion of zidovudine (Pizzo et al. 1988). Infants who entered the study with neurologic abnormalities improved over the 6-month treatment period; in addition, infants thought to be within the normal range also showed significant gains in developmental testing following the 6-month treatment period. There was also surprising improvement in the results of neuroimaging of these children. Among children with ventricular enlargement and cerebral atrophy noted on CT imaging prior to zidovudine treatment, several had a marked improvement in the degree of ventricular enlargement and cerebral atrophy while on therapy. Unfortunately, these gains dissipated over the ensuing months of treatment from what we now understand to be viral resistance developing in the setting of monotherapy.

In addition, there were scattered reports that prednisone could also temporarily reverse or stabilize CNS symptomatology, with improvement of cerebral atrophy and ventricular enlargement noted on neuroimaging. The fact that both therapies (zidovudine and prednisone) could lead to temporary improvement in function and neuroimaging led to the realization that pediatric HIV encephalopathy was a more plastic, dynamic process than originally thought and that even in children with evidence of brain volume loss, the process might be slowed and potentially partially reversed with treatment. As with prednisone or zidovudine therapy, however, treatment with other mono-, or dual-, ARV regimens only transiently affected the course of HIV-related CNS disease.

However with the advent of HAART in the late 1990s, it became clear that early aggressive

treatment, besides delaying or halting progression to AIDS in PAH, could prevent the onset of PE. The cumulative incidence of HIV encephalopathy by age 5 dropped to 2–10% in the early years of HAART compared with a pre-HAART rate of 20–35%. Since 2000, with the widespread use of HAART at the time of diagnosis in infants, the incidence rates of PE have further decreased, to less than 0.4/100 patient years in the USA (Nachman et al. 2009; Patel et al. 2009).

With substantial evidence that HAART initiated early in life can prevent PE, a major question still to be answered is whether HAART improves the neurocognitive outcome in those children without a diagnosis of progressive encephalopathy, and, if so, is there a threshold age by which HAART must be initiated to preserve cognitive outcome (CO). Recent studies, mostly in adults, have examined ARV's effectiveness in protecting and possibly improving CO by looking at the relationship between specific ARV's CNS penetration effectiveness (termed CPE) rating and changes in CO and neurologic status. Although data is conflicting, it seems those agents with higher CPE are more effective in sustaining or improving CO.

In pediatrics, less data is available on the utility of higher CPE regimens. One study found a decreased incidence of new cases of PE with HAART, versus non-HAART regimens, and that higher CPE regimens prolonged survival among those with preexisting PE (Patel et al. 2009). The few studies to date have not been able to find a convincing effect of CPE scores on CO for PAH; any HAART has been shown to be protective against progression to AIDS.

Two US studies retrospectively reviewed the age of initiation of HAART, CPE scores, age of virologic suppression and CO among PAH. In both studies, though there was no relationship found between treatment regimen CPE scores and CO, virologic suppression at an early age was associated with a trend toward improved CO (Crowell et al. 2015; Lazarus et al. 2015). These studies both suffered from a retrospective analysis, and compared to adult studies, were underpowered to detect smaller differences in outcomes. In addition, among HIV-infected

children, there are a limited number of treatment options, compared to those available for treatment of adults, so that CPE scores of the treated children may not have varied as widely as those in adult studies.

Abundant evidence exists to indicate that it is through suppression of CNS HIV viral replication that HAART preserves CO. Pretreatment CNS levels of viral replication have been associated with the presence of neurocognitive abnormalities, and the risk of progression of CNS symptoms is decreased in those with CNS viral suppression. Recent studies of adults find that up to 10% of those with suppressed plasma viral load have ongoing CNS viral replication. This ongoing CNS viral replication has been associated with continued CNS immune activation and risk of neurocognitive decline. In the pediatric literature, there are anecdotal reports of children with worsening neurocognitive function despite plasma viral suppression and without evidence of other causes of the deterioration. The supposition is that among these individuals there is compartmentalization between plasma and CNS viral populations, where the CNS viral strain replicates independently of the plasma compartment, and possibly due to inadequate CNS drug levels, develops resistance to the HAART regimen. In these situations, it might be critical to ensure adequate CNS penetration of the HAART regimen.

The age of initiation of HAART may be crucial. In a southern African population, infants randomized to very early therapy (commencing before 12 weeks of age) versus deferred therapy (CD4 guided) had significantly better performance on neurodevelopmental testing, at a mean age of 11 months, than those on the deferred arm, and the scores in the early treatment cohort mirrored that of HIV-uninfected infants (Laughton et al. 2012). Conversely, the PREDICT study found that when initiating therapy in Thai treatment-naïve children 1–12 years of age, starting therapy at a CD4 range of 15–25% versus initiating therapy for CD4 <15% did not improve the CO at week 144, and both groups scored worse than a control group of HEU and HU children (Puthanakit et al. 2013). From combined studies, it can be inferred that treatment before the

development of an AIDS diagnosis may be critical in preserving optimal CO. So it becomes an issue of starting therapy before immunologic decline, to prevent the development of AIDS; the age that this may occur is highly variable among a population of infants and children with PAH. The decision as to when to initiate therapy however is now less controversial, as most treatment guidelines recommend initiating therapy for all diagnosed infants and children less than 5 years of age, regardless of immunologic status, and strong consideration of treatment initiation at the time of diagnosis in older children and youth as well.

With increasing numbers of HIV-exposed neonates being exposed to multidrug ARV regimens in utero, it is important to note that a number of large cohort follow-up studies on HIV-exposed, but uninfected, infants and children (HEU) support the overall safety of in utero exposure to ARVs on the developing fetal brain. Early reports of very small numbers of exposed infants suggested mitochondria-related symptomology compared to infants without exposure to in utero ARVs; however, those concerns have not been found in other large cohorts. The increased risk of CNS-related mitochondrial disease or toxicity, related to in utero exposure to ARVs, has not been noted in the USA or elsewhere, when reviewing data from treatment networks.

Neuropsychological Profiles

There are multiple methodological issues with studies of neurocognitive outcome in PAH. Studies from the USA and international areas with access to HAART are generally retrospective in design and include heterogeneous populations, with youth identified at different ages and stages of illness, and during different periods of ARV availability and treatment initiation guidelines. In addition, the assessment tools employed differ over time, populations, and countries of origin.

PAH, particularly in young children and prior to advances in HIV treatments, was associated with global deficits, ranging from mild to severe. Domains of particular vulnerability included general cognition, receptive and particularly

expressive language, visual-spatial integration, and gross motor functioning (Smith and Wilkins 2015). As treatments advanced and youth survived into adolescence, the profile of impairments changed significantly, shifting to a profile of more subtle and specific deficits. Although there is increased risk for overall lower cognitive functioning, most studies agree that comparisons of mean performance show little to no differences when compared to PHEU, who are sociodemographically similar.

As noted above, when comparing PAH without PE to noninfected youth and when the groups were well matched in socioeconomic and family factors, the overall CO has approached that of same age-vulnerable youth (those that were HIV exposed in utero but uninfected). However, both groups on average perform lower than expected compared to normative samples, with standardized scores in the average to low-average range, suggesting that concomitant environmental risk factors contribute greater risk than HIV itself.

These groups' mean scores approach those reported of other cohorts of high-risk urban children. For example, in a longitudinal study of inner city youth with and without gestational cocaine exposure, but no HIV exposure, the mean FSIQ of both groups was about one standard deviation below national population mean, and in line with that reported from a longitudinal study of PAH adolescents followed in the same city over the same time period (Hurt et al. 1997; Wood et al. 2009).

Concomitant risk factors that youth with PAH may be exposed to include urban poverty, family instability, and disadvantaged minority status. Compared to the general population, PAH are more frequently exposed to maternal substance use, suboptimal prenatal care and nutrition, and are at higher risk for prematurity (protease inhibitor-based therapy may increase risk of prematurity), and lower birth weights. All of these birth-related factors have been associated with an increased risk of poorer CO among HIV-uninfected populations. Exposures after birth, including community- and family-related stressors (poor educational environments, maternal mental health and substance use problems, inadequate/unsafe housing, exposure to domestic and community violence, food and financial

instability), also impact on cognitive, behavioral, and mental health outcomes as well.

Over the last two decades, accumulating information describes the profound effect that these socioeconomic and emotional stressors can have on the developing brain. Lower cognitive score, achievement results, school success, and components of EF/WM have been well documented. With the advent of HAART, HIV-infected youth appear close to their SES matched peers in a number of neuropsychological domains.

While overall cognitive functioning appears to be fairly stable for older youth, more recent investigations have focused on specific neurocognitive functioning and its impact on daily living, including executive functioning, processing speed, memory, achievement, and adaptive functioning.

Executive functions are the cognitive processes involved in self-monitoring of emotions and behaviors and planning and execution of problem solving. It includes processes such as inhibition, working memory, and flexibility in shifting thoughts and behaviors. These processes are of particular concern as youth age into adolescence, when impairment of these skills may have serious implications for decision-making processes that could lead to high-risk behaviors, poor medication management, delayed vocational planning, and transmission of the virus. Findings have been mixed and interpretation has been difficult due to methodological differences among and within studies. Most studies support the finding that youth with PAH demonstrate impairments in EF abilities. However, studies with appropriate control groups and those that were able to control for psychosocial risk factors suggested that those impairments were not due to PAH infection.

An important area that has consistently been found to be of particular vulnerability in adults and youth is processing speed, and studies suggest that this impairment is highly associated with disease markers such as high viral load (VL) and having a previous Class C (AIDS-defining) diagnosis. This finding holds even after controlling for psychosocial influences. Impairments in this area have implications for a youth's ability to focus, process, and respond in an appropriate and timely manner in a broad range of circumstances.

Other domains of neuropsychological functioning are similar in nature to the neurocognitive findings; average to low-average functioning compared to normative samples, but comparable to youth who are not HIV-infected and have similar psychosocial demographics. Academic achievement and adaptive functioning, two domains that contribute to a youth's quality of life, are two of the domains with similar findings. Language is also a domain considered particularly vulnerable to PAH infection, but post-HAART studies again suggest that although youth exhibit high rates of impairment, they are not consistently attributable to PAH. Lastly, although motor impairment was highly prevalent in the pre-HAART era, it is not a common finding in youth who have access and are adherent to treatment.

One subgroup of youth with PAH are at greater risk for poor neuropsychological outcomes even after controlling for psychosocial contributions. Youth who experienced an early AIDS-defining illness (prior to 3–5 years), despite current virologic and immunologic recovery, continue to demonstrate deficits in several areas, even into adolescence, including cognition, EF, language, processing speed, and achievement (Smith and Wilkins 2015). Additionally, those who experienced HIV encephalopathy in early childhood are at even greater risk for moderate-to-severe neurocognitive impairments, indicating that although the process can be arrested, full return to normal function is generally not achieved. This suggests that the CNS may have less recovery potential and/or that recovery is different from the peripheral system possibly due to the evolution of viral replication in this independent reservoir. In addition, it may be that these youth experienced earlier infection (i.e., in utero) and were exposed to high viral loads and neurotoxic immune responses during critical periods of brain development.

Mental Health/Behavioral Health

Early studies, without control groups, suggested an increased incidence of MH disorders (among them depression, anxiety, and ADHD) and more importantly, psychiatric hospitalizations, among

PAH. Several studies have reported that children and adolescents with a past diagnosis of AIDS are more at risk for psychiatric illness and hospitalization, compared to national population incidence.

More recent work indicates that compared to control groups of PHEU and HIV-negative but at-risk youth, rates of mental health diagnoses are not significantly different for PAH youth, but generally higher than rates among the general population. Again, methodological issues make solid conclusions difficult, including differences in study design/confounding issues, different tools for assessing risk/diagnosis, and selection bias among those consenting for inclusion in a study.

As stated previously, environmental conditions under which one develops is one of the greatest influences to neurocognitive and MH outcomes. Youth with PAH face a number of concomitant risk factors including urban poverty which may bring increased exposure to stress, community and domestic violence, suboptimal education systems, and resource instability. In addition there are stressors unique to living with PAH that may increase the cumulative contributions of risk to overall mental health. Stigma is a complex societal process for people living with HIV. It can result in devaluation and discrimination and often influences a youth's decision to disclose their diagnosis to peers and intimate partners. Parental loss, either in the form of death, unstable family dynamic, or impaired functioning due to HIV, is another common stressor experienced by youth with PAH. Many of these stressful life experiences have been shown to be contributory factors in poor physical and mental health outcomes for children (Elliot-DeSorbo et al. 2009).

Mental health problems and high-risk behaviors often co-occur in adolescents, including those with PAH, and have been associated with poor adherence, substance use, and risky sexual behaviors (Mellins and Malee 2013; Mellins et al. 2011). Adherence can be a significant challenge for adolescents with any chronic illness, however for youth with PAH, adherence is often complicated with the increased risk of inadvertent disclosure to others. Additionally, many PAH adolescents are aging into adult care after more than a decade of personalized pediatric care and

often disengage from consistent medical care. The majority have been treated with ARVs for more than 15–20 years, frequently initially on what is now known to be suboptimal regimens. Therefore, many have resistance to multiple drugs, pill-taking fatigue, and poor family or community supports for medical adherence.

Many PAH voice a fear of sexual intimacy, related to need of disclosure, and fear of transmission of the virus. Others plunge into multiple relationships without disclosure of their status to anyone. In the few studies examining sexual activity among youth with PAH, rates of sexual intercourse were comparable to national averages and to PHEU youth, however the majority of youth with PAH had unprotected sex (with significantly higher rates of sexually transmitted infections) and did not disclose their status to their partners (Tassiopoulos et al. 2013).

Summary

With earlier initiation of more tolerable and effective treatment regimens, it is expected that infants with perinatally acquired HIV infection will have much improved CO, and much lower risk of PE, compared to those born in the pre-HAART era. However, for the aging population of PAH, further research into ameliorating the effect of ongoing CNS viral replication and immune activation continues to be a high priority. A major unanswered question is whether they will be affected by the progression of subtle HIV-related neurologic deficits and an accelerated process of CNS aging described in HIV-infected adults. Continued close monitoring of this unique population who have had early and chronic exposure to HIV, and multiple treatment regimens, as well as to significant environmental stressors is critical in hopes of offering targeted CNS therapies and necessary psychological, social, and educational support to best optimize outcomes.

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Neuroinflammation and HAND: Therapeutic Targeting

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Definition

Neuroinflammation associated with HIV infection is viewed as a state of glial cell activation and expression of inflammatory molecules and

mediators of oxidative stress within the brain, which can disrupt normal neuronal function and architecture. The severity and temporal expression of such inflammation and associated effects are less predictable in individuals on suppressive antiretroviral therapy (ART), but the severity of clinical manifestations of neurological dysfunction is dramatically and consistently reduced in such individuals. The syndrome of HIV-associated neurocognitive disorders (HAND) represents such a group of cognitive dysfunctional states of graded severity that persist in up to 30–50% of virally suppressed individuals, and possible roles for chronic or episodic neuroinflammation have been suggested as mediators of and therapeutic targets for HAND.

Identifying Neuroinflammation and Associated Pathways Induced by HIV-1 Infection of the CNS

- (a) **Central nervous system (CNS) HIV infection.** HIV infection of the brain occurs within days to weeks of systemic infection, and levels of HIV RNA in the cerebrospinal fluid (CSF) correlate with the severity of HAND in individuals who are ART naïve (Clifford and Ances 2013). Evidence suggests a “Trojan horse” mechanism of entry of HIV-infected CD4⁺ T lymphocytes (Sturdevant et al. 2015) and monocytes into the brain and the establishment of viral reservoirs that are at least partially inaccessible to ART drugs. Thus far, in brain autopsy studies, perivascular macrophages have been identified as a major CNS site of productive HIV replication, although endogenous brain microglia and astrocytes (up to ~15% infection rate) can also contain HIV genomes and possibly express viral proteins (Burdo et al. 2013). Although recent evidence for infiltration into the CNS and compartmentalized evolution of T lymphocyte-tropic, non-macrophage-tropic, HIV strains within the CNS has emerged, the anatomic site of this lymphocyte reservoir has not been identified (Sturdevant et al. 2015).

In ART-naïve subjects, brain HIV infection is associated with robust expression of pro-inflammatory molecules including cytokines and chemokines, production of potentially damaging reactive oxygen species and other markers of oxidative stress, expression of some HIV proteins, and increased production of glutamate and other non-proteinaceous neurotoxins, each of which has been linked to disrupted neuronal function and architecture. However, consistent expression of these inflammation factors and their associated linkage to neuronal injury and dysfunction in individuals on suppressive ART has not been as strongly established. Nonetheless, surprisingly high rates of HIV escape (“blipping”) from suppressive ART, as determined by sequential sampling of CSF and episodic detection of both HIV RNA and markers of immune activation in individuals felt to be in a state of HIV suppression, suggest a role for persistent CNS HIV infection and neuroinflammation, even if intermittent, in HAND pathogenesis in ART-experienced patients (reviewed in Price et al. 2014).

(b) **Oxidative stress within the CNS in HIV infection.** Oxidative stress is observed in both systemic and CNS compartments in HIV-infected individuals. Through effects of immune activation and associated signaling, HIV infection can induce pathological oxidative stress, as suggested by detection of diminished levels of reduced glutathione in plasma, lymphocytes, and PBMCs isolated from HIV-infected individuals, reduction of the thiol antioxidant thioredoxin in HIV-infected cells, and elevated serum levels of the lipid peroxidation products in HIV-infected individuals (Kamat et al. 2012). Oxidative stress can in turn drive immune activation through enhancing NF- κ B-driven HIV replication and release of pro-inflammatory cytokines. Furthermore, glutathione deficiency in CD4+ T lymphocytes in HIV-infected individuals correlates with systemic disease progression and mortality.

HIV infection can induce CNS oxidative stress through chronic immune activation and

production of free radicals and lipid peroxidation products, including pathways generating superoxide anions, peroxynitrite, nitric oxide (NO), ceramide, sphingomyelin, and 4-hydroxynonenal (Reynolds et al. 2007; Steiner et al. 2006). Furthermore, several of these in vivo markers of oxidative stress correlate with neurocognitive impairment as well as systemic disease progression in HIV-infected individuals (Reynolds et al. 2007; Steiner et al. 2006).

Although suppression of HIV replication with ART significantly reduces immune activation and oxidative stress, this suppression is incomplete. Neuroinflammation and neuropathological damage can likely persist to some degree in virally suppressed ART-treated individuals, and persistent systemic and CNS markers of monocyte/macrophage activation predict neurocognitive impairment, even in individuals with virological suppression (Kamat et al. 2012). These findings highlight the potential therapeutic benefit of targeting neuroinflammation and oxidative stress to ameliorate disease progression in ART-treated patients.

(c) **Immune activation and neuroinflammation pathways in CNS HIV infection.** In addition to CNS oxidative stress induced by HIV infection within the CNS compartment, systemic effects of HIV infection likely also contribute to neuroinflammation and associated dysfunction. Persistent chronic immune activation has been linked to early pathological injury to the gut in HIV-infected individuals (Burgener et al. 2015). Such immune activation is thought to result from translocation of microbial products across a damaged gastrointestinal tract, which is rapidly depleted of gut-associated lymphoid tissue after HIV infection. Among the identified translocated microbial products in HIV infection is LPS, which can induce systemic monocyte activation, as indicated by elevated blood levels of soluble CD14 (sCD14) and sCD16, and elevated expression of monocyte-associated CD14 and CD16 (Burgener et al. 2015). Based on accumulating evidence for

associations between the gut microbiome and neuroinflammation-associated CNS disorders such as multiple sclerosis (Wang and Kasper 2014), it is interesting to speculate that some gut microbiomes could associate with CNS disease progression in HIV infection.

Elevated plasma markers of immune activation correlate with systemic morbid events during ART, and some evidence exists for a link between systemic immune activation, CNS immune activation, and HAND. Elevated blood levels of CD16⁺ monocytes, sCD14, sCD163, and total CD14⁺ monocyte HIV DNA content associated with microbial translocation have been shown to correlate with an increased risk for HAND (Valcour et al. 2013). Thus, ART-treated individuals with HIV suppression are at risk for CNS complications of persistent immune activation, particularly monocyte/macrophage activation in the systemic and CNS compartments, which likely puts them at an increased risk for HAND. The pathophysiological factors associated with establishing and maintaining a state of immune activation are important considerations for HAND prevention strategies.

HIV-induced neuronal injury *in vitro* is associated with the release of various immune activators and toxins from HIV-infected macrophages, and immune-activated astrocytes, and glutamate receptor-mediated excitotoxicity may be one common injury pathway, among others. Monocyte and macrophage activation, either due to viral infection or inflammation, results in the production and release of known neurotoxins including glutamate, quinolinic acid, and other glutamate receptor agonists, nitric oxide, platelet-activating factor, reactive oxygen species, TNF- α , and other pro-inflammatory cytokines and chemokines. Subjects with HAND have elevated levels of several such pro-inflammatory cytokines, including IL-1 β , TNF- α , and IL-6 in the brain and/or CSF (Chen et al. 2014). Immune activation of astrocytes could similarly increase expression of these potentially neurotoxic factors, including glutamate, even in the absence of HIV infection.

(d) **Blood-brain barrier breakdown.** Additional mechanisms of HIV-induced CNS inflammation might involve increased permeability of the blood-brain barrier (BBB) and increased entry of infected monocytes and lymphocytes into the CNS mediated by systemic and/or CNS inflammation. Several viral and host factors alter the stability and function of the blood-brain barrier (BBB), and this is believed to contribute to HIV neuroinflammation and neuropathogenesis. Drugs of abuse, including cocaine and methamphetamine, and TNF- α have been proposed to exacerbate neurocognitive impairment in HIV infection through further disruption of the BBB, resulting in enhanced immune cell infiltration, elevated CNS inflammation, and alteration in cellular homeostasis. Cocaine, methamphetamine, and TNF- α also alter tight junction and cell adhesion molecule expression, permeability, and immune cell migration across brain microvascular endothelia (Hauser and Knapp 2014). The host response to HIV replication and drug-mediated BBB dysfunction can promote immune cell (HIV-infected and noninfected) infiltration into the CNS and promote neuroinflammation, which in turn may drive further loss of BBB integrity. Therefore, reducing inflammation in the periphery as well as within the CNS could be expected to improve neurocognitive outcomes in HIV-infected individuals.

Potential therapeutic targeting of neuroinflammation and oxidative stress in HAND.

Considering therapeutic targeting of neuroinflammation and oxidative stress in HIV infection requires identification of pathological activation mechanisms and pathways of immune activation and oxidative stress in the CNS. As discussed, a number of such pathways and mechanisms have been tentatively identified, and some of these have been therapeutically targeted (Table 1), but effective strategies for altering neurological outcomes by such targeting have remained elusive. Whether past therapeutic failures represent misidentification of suitable targets, ineffective drugs, or inadequate clinical trial

Neuroinflammation and HAND: Therapeutic Targeting, Table 1 Proposed therapeutics for HAND with direct and indirect anti-inflammatory, antioxidative effects

Generic (brand) name	Mechanism	Proposed effects	References
Memantine (Namenda)	NMDA receptor antagonist	Prevent NMDA receptor-mediated excitotoxicity	Zhao et al. (2010)
Selegiline (Deprenyl)	MAO-B inhibitor	Reduce oxidative burden of cell	Schifitto et al. (2009a)
Minocycline	Inhibitor of 5-lipoxygenase and others	Anti-inflammatory and antioxidative effects	Zink et al. (2005), Nakasujja et al. (2013)
Lithium, sodium valproate (Depakote)	GSK-3 β inhibitor	Anti-inflammatory and other effects	Schifitto et al. (2009b)
SSRIs: citalopram (Celexa), paroxetine (Paxil)	Serotonin transporter inhibitor	Anti-inflammatory and other effects	Steiner et al. (2015)

designs for clinical outcomes remains to be determined.

- (a) **CNS HIV replication and ART treatment strategies.** Although ART has significantly decreased the severity of HAND, presumably by lowering systemic and CNS viral loads, the relatively poor penetration of ART drugs into the CNS limits their ability to completely suppress HIV replication in this compartment. Improved CNS penetration of ART drugs into the CNS might reduce HAND prevalence further by more effective suppression of CNS HIV replication, neuroinflammation and oxidative stress pathways associated with infected and activated macrophages/microglia and immune-activated astrocytes. However, clinical trials in patients treated with ART regimens with increased CNS penetration have not demonstrated reduced incidence or progression of HAND. Additionally, increasing ART penetration into the CNS could expose the brain to neurotoxic effects of these drugs. Effective therapies for HAND will likely require combination therapy that not only safely suppresses HIV replication but also addresses the associated neuroinflammation and oxidative stress that contribute to HIV-associated neurodegeneration in ART-treated individuals.
- (b) **Oxidative stress and associated pathways.** Targeting oxidative stress and associated pathways within the CNS can be rationally considered through the use of antioxidants

that might directly correct the cellular glutathione deficiency associated with HIV infection and/or through drugs that induce the endogenous host cellular antioxidant response pathway. The former has already been investigated, and the latter is receiving much current attention. The cellular antioxidant response is initiated through rapid induction of a family of genes driven by the Nrf2 transcriptional factor, which activates an element (the antioxidant response element) within the antioxidant gene promoter. Translocation of Nrf2 from the cytoplasm to the nucleus occurs rapidly after cellular injury and stress and results in antioxidant gene induction and a subsequent cytoprotective response. This cytoprotective response involves the detoxification of the cell's pro-oxidative state that is associated with injury-induced cellular stress. Several previous neuroprotection trials have focused on several therapies that do not directly activate the Nrf2 antioxidant pathway (selegiline, minocycline) but which nonetheless produce antioxidant effect through either glutathione restoration or anti-inflammatory properties. Newer therapies under development target the induction of Nrf2 antioxidant pathways.

Selegiline (Deprenyl), a monoamine oxidase B (MAO-B) inhibitor used in the treatment of early-stage Parkinson's disease, is proposed to act as a neuroprotectant by reducing the oxidative burden within the

cell. However, recent studies of HIV-infected individuals have shown no evidence of improved neurological outcomes with short-term (24 weeks) treatment with selegiline; selegiline also failed to reduce biomarkers of oxidative stress in further analysis of this study, which suggests ineffective drug targeting of oxidative stress pathways *in vivo* (Schifitto et al. 2009a).

Minocycline is a broad-spectrum tetracycline antimicrobial that can cross the blood-brain barrier and that can suppress HIV replication in cultured microglia, macrophages, and lymphocytes (Zink et al. 2005). In addition, minocycline has anti-inflammatory and antioxidative properties as shown by its inhibition of TNF- α , IFN- γ , and IL-2 production by lymphocytes. In SIV-infected macaques, minocycline administration resulted in decreased CSF expression of CCL2, a biomarker of inflammation, and decreased the severity of SIV encephalitis (Zink et al. 2005). However, a short (24 weeks) minocycline neuroprotection trial with ART-naïve, HIV-infected individuals demonstrated no improvement in cognitive functioning (Nakasujja et al. 2013). Notably, however, analysis of CSF from a subset of treated subjects demonstrated a significant reduction in lipid markers of oxidative stress (ceramides), suggesting effective *in vivo* reduction of at least some pathways of oxidative stress. This suggests that a longer treatment period with minocycline should be considered to more effectively evaluate its ultimate neuroprotection potential in HIV-infected individuals.

- (c) **Immune activation and neuroinflammation pathways in CNS HIV infection.** Considerable attention has been paid to the potential neuroprotective effects of statins, which can modulate several neuroinflammation pathways associated with the pathogenesis of HAND. Statins have been prescribed for the treatment of hyperlipidemia, which might be associated with an increased risk of dementia in both HIV-infected and noninfected individuals. Statins also promote cardiovascular and, presumably, cerebrovascular health through

antioxidant and anti-inflammatory effects and improved endothelial function. A recently published systematic review of the role of statins in preventing cognitive decline and dementia reviewed randomized controlled trials and observational studies in HIV-negative cohorts. The conclusion was that initiation of statins in later life does not prevent cognitive decline over the ensuing 3–5 years (Power et al. 2015).

Nonetheless, the potential for statins to provide neuroprotection against HAND requires specific investigation of their effects against associated pathological processes in HIV-infected individuals. These processes include chronic inflammation and the production of excitatory neurotoxins from activated monocytes/macrophages, which are recruited to the CNS during HIV infection. Atorvastatin has been demonstrated to have anti-inflammatory effects in cultured monocytes and in ART-treated HIV-infected individuals, and a double-blind, placebo-controlled crossover pilot clinical trial designed to determine effects of atorvastatin on monocyte activation, gene expression patterns, and neurocognitive outcomes in HIV-infected individuals receiving ART is currently under way (ClinicalTrials.gov Identifier: NCT01600170). Other ongoing research is also examining the role of other statins on monocyte-mediated inflammation and activation in association with neurovascular risk factors in HIV-infected individuals.

Glycogen synthase kinase-3 β inhibitors such as sodium valproate (VPA) and lithium are approved for treatment of bipolar disorder and related mood disorders, and both provide neuroprotection against HIV-induced toxicity *in vitro* and in mouse models of HIV encephalitis. Several small pilot studies have demonstrated improved neuropsychological performance in HAND patients following short-term VPA or lithium therapy (Schifitto et al. 2009b).

Selective serotonin reuptake inhibitors (SSRIs), including paroxetine, citalopram, and fluoxetine, also express some anti-

inflammatory effects and are also under consideration as adjunctive therapies for HAND (Steiner et al. 2015). SSRIs were predicted to directly decrease levels of HIV replication, although clinical trials have not yet found a significant improvement when an SSRI is used as an adjunctive therapy.

- (d) **Glutamate receptor (N-methyl-D-aspartate/NMDA) subtype antagonists.** Noted clinical similarities between the neurodegeneration seen in HIV infection and other neurodegenerative diseases as well as depression have prompted investigations into broadly neuroprotective therapies for the treatment of HAND (Table 1). Because neurodegeneration is often associated with neuroinflammation, oxidative stress, and excitotoxic injury (through glutamate and other NMDA receptor agonists), agents that block the NMDA receptor have been investigated for their neuroprotective properties in several neurodegenerative diseases. Memantine (Namenda) is approved in the treatment for Alzheimer's disease and acts as a noncompetitive NMDA receptor antagonist. *In vitro* and *in vivo* animal studies have shown that memantine can attenuate some features of HIV- and SIV-linked neurotoxicity and neuronal dysfunction, and short-term (20 weeks) and long-term (~1 year) clinical trials in HAND patients demonstrated that memantine improved neuronal metabolism, indicative of neuroprotection, but did not lead to significantly improved neurocognitive outcomes (Zhao et al. 2010). These studies suggest that NMDA receptor targeting, in addition to targeting pathways leading to production of toxic levels of NMDA receptor agonists (glutamate, quinolinic acid) produced in the HIV-infected brain, might provide clinically significant neuroprotection.

Conclusions

Because HIV infection is associated with chronic immune activation and associated inflammation

and oxidative stress, even in individuals with virological suppression due to ART, adjunctive therapies to ART are necessary for improved long-term clinical outcomes. The persistence of HAND in such individuals supports a need for adjunctive therapies capable of penetrating the CNS. Several classes of drugs that might ultimately meet these needs include GSK-3 β inhibitors and drugs that directly or indirectly suppress oxidative stress within the CNS. Some drugs that have antioxidant effects (selegiline, minocycline) have not proved effective, for reasons that might include ineffective CNS modulation of oxidative stress or even because of therapeutic clinical trials of limited duration. Newer approaches to modulating oxidative stress include drugs that can directly activate the host's endogenous antioxidant response. This response, which is mediated Nrf2 transcriptional factor, drives expression of a family of antioxidant genes after stress-associated injury. One major mediator of this host antioxidant response is the inducible enzyme heme oxygenase-1 (HO-1), which is a detoxifying enzyme that is critical for limiting oxidative stress, inflammation, and damage within the CNS and other tissues in response to acute cellular injury. Recent studies demonstrated a unique deficiency of HO-1 within the brains of individuals with HAND and a mechanistic link to HO-1 deficiency and dysregulation of glutamate metabolism, suggesting a potential role for HO-1 deficiency in the pathogenesis of HAND and its potential therapeutic targeting (Gill et al. 2014). Among the drugs known to induce HO-1 expression are statins and a recently-approved drug, dimethyl fumarate (Tecfidera) that has recently been approved for the treatment of multiple sclerosis (Gill et al. 2014). Additional drugs that activate Nrf2-driven gene expression are currently in development as neuroprotectants for various neurodegenerative diseases, which could facilitate their application to HAND neuroprotection. Finally, modified ART regimens with less potential neurotoxicity and with improved CNS penetration might still be feasible for improving neuroprotection against HAND (Clifford and Ances 2013).

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Neuropsychological Testing in HIV-Infected Individuals

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Definition

Recent estimates from the US Centers for Disease Control and Prevention were that 1.2 million individuals were living with HIV/AIDS in the United States and over 35 million worldwide (CDC 2013). The last few decades have witnessed dramatic improvements in the treatment and management of HIV disease, including the advent of combination antiretroviral therapy (cART) in 1996. These advances remarkably improved HIV-related health outcomes and transformed HIV from what was once a condition with very poor prognosis and high mortality rates into a more

chronic, long-term manageable condition. Despite these significant advances, HIV-associated neuropsychological disorders (HAND), particularly in milder forms, remain highly prevalent in the cART era and can have significant adverse effects on everyday functioning and health-related outcomes.

This chapter will provide an overview of neurocognitive (NC) assessment as it pertains to the diagnosis of HAND in clinical and research settings. We will begin with a brief developmental history of the current HAND diagnostic criteria, followed by an overview of the epidemiology and nature of NC disturbances in HIV. We will then review the approaches used to evaluate NC impairment in HIV, which will include methodological considerations (e.g., the selection of NC tests and interpretation of test findings) and will provide brief summaries of methods used to evaluate NC change over time and screen for impairment in clinical or other resource-limited settings.

History of HAND and Current Diagnostic Criteria

Some of the earliest published reports of central nervous system (CNS) consequences due to HIV appeared in the mid-1980s to early 1990s. One of the most common was a neurological condition characterized by moderate to severe cognitive, behavioral, and motor disturbances. Originally termed the AIDS dementia complex (ADC; Navia and Price 1987), and now referred to as HIV-associated dementia (HAD), this constellation of disabling symptoms was observed most typically in the later stages of AIDS and was associated with significant mortality risk. Prevalence estimates of NC impairment in the earlier stages of disease (e.g., medically asymptomatic stage) were more controversial, although a preliminary study by Grant and colleagues (Grant et al. 1987) suggested that, with use of a comprehensive NC test battery with adequate normative standards, increased rates of NC impairment could be documented at each successive stage of infection (including the medically asymptomatic stage). This finding was supported by a

subsequent review (White et al. 1995), which further emphasized the importance of comprehensive assessments covering multiple NC ability areas that could be affected by HAND.

During the early years of the epidemic, several useful classification systems were proposed for the diagnosis of HAND. One of the more widely used early classification systems was developed by an AIDS Task Force of the American Academy of Neurology (AAN; AAN 1991). This system identified two diagnostic categories of HAND: HIV-associated dementia (HAD) and minor cognitive motor disorder (MCMD). In order to meet criteria for HAD, a patient must demonstrate (1) an acquired deficit in two or more non-motor NC domains that cause impairment in work or other activities of daily living (ADLs) and (2) an acquired abnormality in motor function and/or a decline in specific neurobehavioral or psychosocial areas (e.g., motivation, emotional control). If the diagnostic criteria for HAD were not met, a patient could meet criteria for MCMD if they demonstrated acquired impairment in at least two cognitive, motor, or behavioral domains that caused mild impairment in ADLs or at work. These AAN categories were later expanded to include the “sub-syndromic neurocognitive impairment” diagnostic category in response to research suggesting a subgroup of patients with NC disturbances that did not appear to interfere with everyday functioning (Grant and Atkinson 1995).

While the AAN classification system provided a valuable first step toward developing guidelines for classifying the neurological complications of HIV, there were several significant limitations to their applicability in research and clinical settings. One major concern was that the criteria were loosely defined and not operationalized, which can lead to inconsistencies across studies and/or clinics and unreliable estimates of prevalence and nature of HAND across studies. Furthermore, the AAN criteria for HAD with mild functional decline appeared to overlap with the criteria for MCMD, which could also lead to diagnostic inconsistencies.

Another limitation was that the AAN criteria did not emphasize NC (non-motor) impairment as

the essential component of HAND. In fact, given the AAN criteria's emphasis on motor and other noncognitive (e.g., mood, personality) changes, a patient could meet criteria for MCMD without demonstrating any cognitive changes (e.g., motor symptoms and mood/personality changes might meet criteria). This was problematic as motor and/or mood and personality abnormalities frequently are difficult to attribute to HIV.

Significant changes in the HIV epidemic over the years also limited the applicability of the AAN criteria. With the introduction of cART, the nature and course of the HIV epidemic improved dramatically. Effective cART not only improved systemic disease but also decreased the prevalence of severe neurological consequences that often occurred in the pre-cART era, including HAD. The stable, and if anything, increasing prevalence of milder forms of NC impairment (Heaton et al. 2011) warranted more distinct and operationalizable criteria. The changing epidemic also witnessed a growing population of older adults as well as an increase in non-HIV-related comorbid risk factors for NC impairment in HIV, including, but not limited to, traumatic brain injury, metabolic syndrome, coinfection with hepatitis C, serious psychiatric disorders, and substance use disorders (e.g., alcohol and methamphetamine abuse/dependence). As such, there was a need for detailed guidelines regarding how to assess aging and aging-related comorbidities (e.g., vascular disease), as well as other common comorbidities listed above. Careful assessment of the potential NC and functional impact of these comorbid conditions, in addition to the effects of premorbid confounds (e.g., learning disabilities), is required and must be distinguished from the effects of HIV prior to assigning HAND diagnoses.

In response to the abovementioned concerns, in 2005 the NIH supported an international meeting of interdisciplinary neuroAIDS experts in Frascati, Italy, to develop a classification system with more clear and objective guidelines and criteria for diagnosing HAND in research settings. Consensus discussions resulted in the Frascati classification system for the diagnosis of HAND

(Antinori et al. 2007), which defined NC impairment as at least mild impairment in two or more NC ability domains, and described three categories of HAND: (1) asymptomatic neurocognitive impairment (ANI), (2) mild neurocognitive disorder (MND), and (3) HIV-associated dementia (HAD). Formal criteria may be found in Antinori et al. (2007). In brief, to be diagnosed with ANI, a patient must demonstrate at least mild, acquired impairment in at least two NC ability areas, but without any clear evidence that the impairment adversely affects their daily functioning. A diagnosis of MND also requires at least mild impairment in at least two NC ability domains, though unlike ANI, a diagnosis of MND requires evidence that the NC impairment is impacting everyday functioning. A diagnosis of HAD, which in the cART era is rare (i.e., <5%), requires at least moderate impairment in at least two ability areas associated with serious problems with everyday functioning (e.g., incapable of working competitively and/or living independently). For all three HAND classifications, the impairment and/or disability must be attributed at least partly to HIV, rather than solely to other common pre- and/or comorbid conditions (including opportunistic CNS disease and other comorbid conditions mentioned above).

These "Frascati criteria" improved the identification and classification of HAND diagnoses in several ways. First, these criteria included specific operational definitions as well as standardized procedures and algorithms to evaluate the prevalence and severity of both NC impairment and functional decline. For example, for "mild NC impairment" (necessary for ANI and MND classification), a patient's NC performance must be >1 SD below the mean of demographically corrected scores on at least two NC domains; "moderate NC impairment" (necessary for HAD) requires scores >2 SD below the mean on at least two NC domains. Second, the Frascati system emphasized NC impairment as the principal diagnostic criterion rather than other criteria such as motor disorders and/or emotional/personality changes, given consistent findings throughout the literature demonstrating strong associations

between NC abnormalities and underlying pathologic changes in the brain (see Grant and Sacktor 2011). Third, the Frascati classification system was the first to provide detailed operational guidelines for evaluating and interpreting the potential adverse impact of common pre- and comorbid conditions on the expression of HAND and functional declines (see Antinori et al. 2007, Table E-4). This is particularly important, as the Frascati criteria (like prior classification systems) specify that, prior to assigning a HAND diagnosis, the observed NC impairment and functional disability must be attributed at least partly to HIV as opposed to comorbid conditions.

Neuropsychological Assessment in HIV

History of Neuropsychological Assessment

Clinical neuropsychology has long focused on assessment methods to detect and characterize NC impairments in persons with known or suspected brain disorder. In earlier years of the field, NC tests were developed primarily for diagnostic purposes, such as the identification and localization of brain lesions. As the field evolved, the focus of neuropsychology shifted its diagnostic focus to include methods for evaluating the impact of brain damage or dysfunction on NC and behavioral outcomes themselves, which today represents one of the most important and valuable contributions of neuropsychology. Considerable progress has been made in this regard and as a result has expanded the role of NC assessment to include differential diagnosis, assessing change in cognitive functioning over time and predicting everyday functioning outcomes.

Role of Neuropsychological Assessment in HIV

Comprehensive NC assessments have played a major role in detecting and characterizing CNS complications of HIV disease. As mentioned above, HAND remains common in the cART era, even in treated and virally suppressed individuals, with current prevalence estimates of

35–50% (Heaton et al. 2010, 2011). It is most likely to emerge in persons with histories of severe immunosuppression though can even be seen in cases of primary infection. In the cART era, HIV-associated NC deficits are typically mild to moderate in severity and most prominently observed in the domains of executive functions, episodic memory (learning and delayed recall), attention/working memory, speed of information processing, verbal fluency, and fine perceptual-motor skills. HAND is generally nonprogressive, though fluctuations may occur due to disease- or treatment-related factors (e.g., changes in antiretroviral treatment). Importantly, even in its mild forms, HAND has been consistently linked to an array of adverse functional and health-related outcomes, including problems with ADLs (e.g., medication and financial management), vocational difficulties, and reduced overall quality of life (for a review, see Blackstone et al. 2017).

A thorough characterization of NC functioning in HIV requires a detailed (2+ hours) NC assessment covering the seven ability domains mentioned above. This is because of the varied and somewhat “spotty” nature of HIV-associated NC deficits (consistent with neuroimaging findings in this disease; Jernigan et al. 2011), resulting in variable patterns of deficits across patients. Moreover, a comprehensive evaluation of NC functioning not only identifies NC weaknesses, but it also provides information regarding strengths, which together can be highly informative for differential diagnosis and when developing rehabilitation strategies to improve impairment (or at least reduce the impact of impairment in daily life). Relatedly, unlike other measures of CNS integrity (e.g., neuroimaging) NC evaluations are able to detect brain dysfunction that impacts everyday functioning. As mentioned above, NC assessments are also useful tools for detecting and monitoring changes in brain function over time, which may occur with disease progression and/or as a result of treatment (e.g., changes in ART regimens) or comorbidity-related changes (e.g., aging). Lastly, NC evaluations are helpful in determining the impact of incident comorbidities on the expression of HIV-associated NC impairment.

Approaches to Neuropsychological Evaluation

Neuropsychological Test Selection

When considering tests for the assessment and diagnosis of HAND, standardized and well-validated instruments should be selected based on their sensitivity to HIV-associated NC impairment and underlying frontostriatal dysfunction. Selecting multiple and diverse measures sensitive to HAND is necessary for detecting the often mild and “spotty” HIV-associated deficits, even at relatively early stages of disease. Detecting impairment early can have significant implications for treatment, as this may allow for the initiation of directed therapies prior to significant HIV-associated neurological damage. Examples of recommended tests for each of the domains likely to be affected in HAND are provided in Table 1 (for a more comprehensive list with references, see Table E-3 of Antinori et al. 2007).

Another important requirement should be the availability of demographically corrected normative standards appropriate for the individual or population being assessed. Demographically corrected normative data provide guidance with regard to how a particular individual would be expected to perform on a test in the absence of a brain disorder. Demographic factors such as age, education, sex, and ethnicity, can significantly influence NC test performance, so failure to use norms that are corrected for these factors will increase the risk of misclassifying NC impairment (Heaton and Marcotte 2000).

Importantly, although reliable HIV effect sizes can be obtained from group mean comparisons in studies that include demographically comparable HIV-uninfected controls, in the absence of adequate norms, it is not possible to determine the prevalence of HAND in the HIV+ group or determine which HIV+ individuals have HAND. The latter determinations obviously are essential within

Neuropsychological Testing in HIV-Infected Individuals, Table 1 Standardized Neuropsychological Tests Commonly Utilized in HNRP-Associated NC Test Batteries to Document Impairment in NC Ability Domains

Standardized Neuropsychological Assessment (HNRP)	
7 NC Ability Domains	Examples of Neuropsychological Tests
Verbal Fluency	Controlled Oral Word Association Test – Letter Fluency (F-A-S)
	Category Fluency (Animals)
	Action/Verb Fluency (“Things people do”)
Abstraction/ Executive Functioning	Wisconsin Card Sorting Test-64 card version (WCST-64)
	Trail Making Test – Part B
	Stroop Color and Word Test – Interference score
	Halstead Category Test
	Color Trails II
Attention/Working Memory	Wechsler Adult Intelligence Scale (WAIS)-III Digit Span Subtest
	Wechsler Adult Intelligence Scale (WAIS)-III Letter-Number Sequencing
	Wechsler Memory Scale (WMS)-III Spatial Span Subtest
	Paced Auditory Serial Addition Task (PASAT)
Learning (Verbal/Visual)	Hopkins Verbal Learning Test-Revised (HVLT-R) – Total Learning
	Brief Visuospatial Memory Test-Revised (BVM-T-R) – Total Learning
Memory (Verbal/Visual)	Hopkins Verbal Learning Test-Revised (HVLT-R) – Delayed Recall
	Brief Visuospatial Memory Test-Revised (BVM-T-R) – Delayed Recall
Speed of Information Processing	Wechsler Adult Intelligence Scale (WAIS)-III Digit Symbol Subtest
	Wechsler Adult Intelligence Scale (WAIS)-III Symbol Search Subtest
	Trail Making Test – Part A
	Color Trails I
	Stroop Color Word Test – Color Naming
Motor Speed & Dexterity	Grooved Pegboard Test (dominant and non-dominant hands)

the clinical context, but also are necessary in research aimed at assessing associations of HAND with disease characteristics, treatment effects, biomarkers related to pathogenesis, and other indications of brain changes (e.g., neuroimaging).

Definitions and Cutpoints for NC Impairment

One of the most important roles of NC assessment is the ability of tests or a battery of tests to detect the presence or absence of a brain disorder. Ideally, a cutpoint that defines “abnormality” on NC tests would correctly identify all patients with a brain disorder (100% sensitivity) and at the same time correctly identify as normal all those who do not (100% specificity). Unfortunately this degree of accuracy is almost never achievable except in conditions with very severe and generalized cognitive effects (e.g., advanced Alzheimer’s disease). In such cases, there is virtually no overlap between test scores of patients with these severe conditions and those of neurologically normal people with similar demographics. For clinical conditions such as HIV, however, in which deficits are more mild and variable, there tends to be an overlap of NC test score distributions for those eventually diagnosed with a brain disorder relative to those who are not diagnosed. In general neuropsychological practice, therefore, it is not possible to achieve 100% specificity and 100% sensitivity; there is also an inverse relationship between the two, such that a gain in specificity would inevitably result in a loss of sensitivity and vice versa. Therefore, when choosing cutpoints to define impairment, it is important to strike a balance between specificity and sensitivity with consideration of the relative implications of a false positive error (i.e., misclassifying a normal individual as impaired) versus a false negative error (i.e., misclassifying an impaired individual as normal).

While there is no universal consensus regarding optimal cutpoints for these classification decisions, it is recommended that specificity be maintained at a reasonably high level and balanced as much as possible with sensitivity. Prior research with other conditions with different NC tests and test batteries suggests that optimal balance between sensitivity and specificity is

typically achieved using a 1 SD cutpoint (Heaton et al. 2004; Taylor and Heaton 2001). This is consistent with the Frascati definition of mild NC impairment being >1 SD below the mean on demographically corrected scores; although it will result in a false positive error rate of approximately 16%, more conservative cutpoints generally provide more loss in sensitivity than gain in specificity. Importantly, the Frascati definition of moderate impairment (>2 SD below the mean), a criterion for HAD, will detect dementia but not mild impairment and results in close to 100% specificity (2% expected false positive errors).

Methods of Data Interpretation

Clinical Ratings

Clinical ratings by neuropsychologists were recommended by the National Institute of Mental Health (NIMH)-sponsored workgroup as the best method for detecting NC impairment in HIV/AIDS (Butters et al. 1990). Such ratings are considered the “gold standard” for assessing HAND (Antinori et al. 2007). Clinical ratings are derived using demographically corrected standard scores from individual NC tests that are categorized by domain of NC functioning (e.g., executive functions, learning). Trained neuropsychologists assign a rating for each NC domain according to a standardized clinical rating algorithm (see Heaton et al. 1994; Woods et al. 2004). A global clinical rating is then assigned by considering ratings across domains, with greater weight afforded to impaired test performances. The global rating does not simply average the domain ratings, but considers impaired domains more importantly, such that the global rating equals the rating of the two worst NC domain scores (if these scores have the same levels of impairment) or the worse NC domain score minus one (if these scores are different). Of note, in accordance with the Frascati guidelines for classifying HAND (Antinori et al. 2007), in order for an individual to be classified as having global NC impairment (i.e., global rating ≥ 5), he/she must show definite impairment (\geq mild) in at least two ability domains; this

ensures that no patient can be classified as globally impaired if they have an isolated deficit in one domain, even if it is severe.

A significant advantage of clinical ratings is that they allow trained neuropsychologists to consider individual patterns of strengths and even mild deficits across the NC test battery when making HAND classifications. Ratings also meet the published international guidelines for NC impairment in HAND classifications (Antinori et al. 2007), have excellent inter-rater reliability (Woods et al. 2004), and have been associated with a host of everyday functioning and health-related outcomes (e.g., ADLs, medication adherence, and health-related quality of life; Blackstone et al. 2017). Clinical ratings have also been associated with underlying HIV-associated CNS changes (e.g., synaptodendritic injury; Moore et al. 2006) and HIV disease-related outcomes (e.g., immunosuppression or failure to achieve virologic suppression on treatment; Heaton et al. 2011). A major limitation of this approach is that assigning clinical ratings is time consuming and requires that raters have training and experience in NC test interpretation. In addition, it could be argued that clinical ratings are at least somewhat subjective and less convincing relative to more objective, actuarial approaches, such as the global deficit score (see below).

Global Deficit Score (GDS)

The GDS is an alternative approach used to summarize and evaluate NC impairment on an entire test battery. The GDS is a summary score calculated by an actuarial approach, which weighs the NC test data in a similar manner to clinical ratings, by considering both the number and severity of deficits in an individual's performance across the test battery and assigning less weight to performances within and above the average range (Heaton et al. 1995; Carey et al. 2004a). The GDS is calculated by converting demographically corrected T-scores on individual NC tests to deficit scores ranging from 0 (no impairment; $T \geq 40$) to 5 (severe impairment; $T < 20$), which are then averaged to derive individual domain deficit scores and the GDS. A GDS cutpoint of ≥ 0.5 is a standard cutoff that yields the most optimal balance between sensitivity and

specificity (Heaton et al. 1995). This cutpoint on individual tests approximates the specificity of a 1 SD cutoff and is roughly equivalent to requiring an average of mild impairment on at least half of the administered measures for a diagnosis of neurocognitive impairment.

The GDS is entirely objective, well validated for use in HIV, and relative to clinical ratings is much less time- and resource-intensive. Similar to clinical ratings, the GDS is significantly associated with a number of functional and health-related outcomes, including ADLs (Scott et al. 2011), medication adherence (Andrade et al. 2013), and biological markers of HIV disease (e.g., CD4 count, cerebrospinal fluid viral load; Gonzalez et al. 2003).

Comparing Diagnostic Classification Systems: Clinical Ratings Versus GDS

Both the clinical ratings and the GDS approaches are sensitive to the mild and variable deficits observed in HIV. They also improve upon the group mean differences approach in identifying NC impairment in research (Heaton et al. 1995) and are valid indicators of functional and health-related outcomes. While the two approaches provide comparable classification rates, their overlap is imperfect. Evidence from a large multisite study conducted by the CHARTER group (Blackstone et al. 2012) compared the diagnostic classifications of both approaches in 1,574 HIV+ individuals and found an 83% ($n = 1,305/1,574$) concordance rate between clinical ratings and GDS impairment classifications. Of those discordant, the majority (16%) were classified as impaired by clinical ratings (i.e., meeting criteria for a HAND diagnosis) but not according to the GDS. Less than 1% were classified as impaired according to the GDS only. Therefore, while the GDS is slightly more conservative (slightly greater specificity), an impaired GDS virtually guarantees that an individual will be impaired by clinical ratings and meet Frascati NC criteria for HAND.

Measuring NC Change

Assessing NC change can be helpful in tracking the progression of disease, monitoring any cognitive effects of treatment (e.g., changes in ART

regimens), and detecting NC impairment associated with incident comorbidities (e.g., a new substance use diagnosis or a medical condition coinciding with aging). There are several challenges with regard to measuring NC change, including practice effects (improved performance due to prior exposure to the same tests), variable test-retest reliabilities across instruments, and perhaps most important, determining how to evaluate whether any individual change is real (versus chance variation) and clinically meaningful (e.g., what degree of change might indicate altered brain function or cause a significant increase in everyday functioning problems?). As with use of NC normative standards to classify HAND at a single point in time, reliable detection of cognitive change requires referencing test-retest results against appropriate normative standards. In this case, the comparison samples would be neuromedically stable individuals (e.g., HIV-uninfected controls), who ideally have been retested with all or most of the same tests in the NC battery being used. The latter would allow comparable norms for change on the entire NC battery, rather than on isolated tests.

A variety of statistical approaches have been used to evaluate NC change, including standard deviation change scores, the reliable change index (RCI), and regression-based change (RBC) formulas (Temkin et al. 1999). However, most studies provide normative change formulas for individual tests rather than summary change scores for more comprehensive batteries like those used to evaluate HAND. More recently, studies have proposed modifications of these approaches for use in deriving summary change scores based on multiple cognitive domains (Woods et al. 2006; Cysique et al. 2011). Woods et al. (2006) proposed a summary change score using the reliable change index (RCI) plus practice effect approach (i.e., RCI-PE), whereas Cysique et al. (2011) employed a multiple RBC score normative approach.

An advantage that the multiple RBC score approach has over the RCI + PE and others is that it predicts a follow-up score on each test while considering multiple other factors that could influence retest performance, including the

baseline score on the test in question and general NC “competence” at baseline (mean standard scores on all other tests in the battery). While demographic characteristics and test-retest interval may also be considered in the prediction models, they usually do not contribute much to predicted scores at follow-up. Importantly, this approach takes into consideration not only levels of baseline NC functioning but also normal test-retest variability (including practice effects) and regression to the mean and provides a basis for summarizing all changes across the NC test battery.

Using published, regression-based norms for NC change from Cysique et al. (2011), a recent study (Heaton et al. 2015) examined the incidence and time-dependent predictors of NC change within a longitudinal cohort of 436 HIV+ individuals who had at least four semiannual visits over a mean of 35 months. Of the entire sample, 22.7% declined, 60.8% remained stable, and 16.5% improved. At baseline, there were no differences in NC performance between those who declined, remained stable, or improved, but the three groups differed very substantially in NC outcomes at the last visit. Significant time-dependent predictors of NC change (i.e., decline or improvement) included markers of disease severity (e.g., albumin, total protein), ART status (on/off), some baseline demographics and estimated premorbid IQ, and comorbidities such as methamphetamine use, mood disorders, and current depression symptoms. While preliminary, this method of quantifying global NC change provides a significant step forward in our ability to detect and monitor HIV disease- and/or treatment-related NC changes and identifies potentially modifiable risk factors of decline that may be amenable to treatment as well as protective factors that may prevent decline.

The Use of Brief Screening Measures

Given the variable patterns of NC deficits in HAND, comprehensive NC testing is the optimal way of detecting these disorders, as well as determining the nature and severity of strengths as well as deficits (Butters et al. 1990). However, comprehensive test batteries can be burdensome and

costly, and as such, are not always practical or feasible, particularly in clinical settings and other settings in which time and/or other resources are limited. Below we will provide a brief overview of screening methods that have been used to detect HAND, with validity estimates based upon comparisons with more comprehensive testing. For a more detailed review of cognitive screeners (with associated references) and recommended guidelines, see Valcour et al. (2011) and Kamminga et al. (2013).

Traditional screening measures such as Folstein's Mini-Mental State Examination and the Mattis Dementia Rating Scale (DRS), which were developed to detect dementia in neurological populations (e.g., Alzheimer's disease), are generally ineffective at detecting HAND, except in its most severe forms. The HIV Dementia Scale (HDS), which was developed specifically for use in HIV, and its international counterpart, the International HDS (Sacktor et al. 2005), are more sensitive to HAND relative to traditional screeners, though less effective in detecting mild forms of HAND. As with other NC tests, performance of screeners may be improved with the application of demographically corrected normative standards.

Several fairly brief computerized screening measures have been used in HIV (for a detailed review with references, see Kamminga et al. 2013) including the California Computerized Assessment Battery (CalCAP), the CogState battery (www.cogstate.com), and the computer assessment of mild cognitive impairment (CAMCI). Similar to other screeners, these computerized methods demonstrate utility in detecting HAND, though their effectiveness in detecting milder forms of impairment is inconclusive. There is preliminary evidence to suggest that the CAMCI may hold some promise (Becker et al. 2011). The recently released National Institutes of Health (NIH) Toolbox Cognition Module is a computerized cognitive screener designed to assess five of the NC domains commonly affected by HIV (attention, executive functions, working memory, processing speed, and episodic memory) and has excellent demographically corrected norms based upon a large and diverse US sample (Casaletto et al. 2015; see also Weintraub et al.

2013). The five tests of the above cognitive abilities take only 20 min to administer and may have promise in screening for HAND, though validating studies have not yet been done within the HIV population.

Brief NC screening batteries comprised of small combinations of standardized NC tests (e.g., 2, 3, or 4) have also been developed to detect HIV-associated NC impairment (e.g., Carey et al. 2004b), even in the earlier stages of disease (Moore et al. 2012). Carey et al. (2004b) developed two brief screening batteries (each using two-test combinations), which demonstrated fairly good accuracy in detecting even mild NC impairment: (1) the Hopkins Verbal Learning Test-Revised (HVLT-R) and the Grooved Pegboard Test (nondominant hand) and (2) the HVLT-R and the Wechsler Adult Intelligence Scale (WAIS-III) Digit Symbol subtest. Moore et al. (2012) applied this approach to a cohort of patients with early-stage infection and found that tests of verbal learning (HVLT-R) and processing speed (Stroop color test) provided the most sensitive two-test screener for detecting impairment in this population; also the addition of a measure of attention/working memory (Paced Auditory Serial Addition Test [PASAT]) provided the best three-test combination. Importantly, these brief combinations of more traditional NC measures include well-standardized, reliable, and validated NC tests with available demographically corrected normative data, which is essential in achieving the best classification accuracy statistics. A drawback of these brief cognitive screens is that they assess a limited number of cognitive domains and therefore run the risk of missing some individuals whose "spotty" deficits primarily affect other domains.

Conclusions

HAND remains common in the cART era, affecting up to 50% of individuals with HIV/AIDS. By far the most prevalent forms of HAND (i.e., ANI and MND) are relatively mild and involve somewhat variable patterns of specific cognitive deficits across patients. As such, they are difficult to

reliably detect using existing NC screening methods. More comprehensive NC test batteries that tap multiple NC domains are needed to sensitively detect these mild forms of HAND. All tests included in NC batteries should have appropriate normative standards for classifying impairment, as well as for detecting meaningful change over time. When interpreting NC test results, it is important to use demographically corrected standard scores and carefully consider cutpoints for classifying impairment. Current Frascati criteria for classifying HAND provide the most clearly operationalized criteria for determining NC and functional impairment in addition to detailed guidelines for assessing the potential impact of comorbid conditions in the context of HAND. Data summary and interpretation methods (e.g., clinical rating algorithms, global deficit scores, summary regression-based change scores) should consider performance across all tests and domains in the battery in order to reliably detect HAND and disease- or treatment-related changes over time.

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Neurotoxic Consequences of Antiretroviral Therapies

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Antiretroviral Therapy (ART) and the Central Nervous System (CNS)

With the introduction of combination antiretroviral therapy (CART), also known as highly active antiretroviral therapy (HAART), for the control of human immunodeficiency virus (HIV) infection, it has been possible to suppress viral quantity in patients' blood, often to undetectable levels. This intervention has decreased the overall HIV/AIDS disease burden and increased the life span of HIV-infected individuals. However, HIV reservoirs persist in tissue compartments where there is limited access of antiretroviral drugs and/or the presence of long-lived latent HIV reservoirs. Given these circumstances, the complete control of HIV replication and the eradication of HIV have been difficult and infected individuals must continue on treatment regimens indefinitely. Antiretroviral therapies (ARTs) are essential for the

effective treatment of HIV infection, but these compounds also have intrinsic toxicity and with long-term treatment can damage organs over time. Thus, the therapeutic antiretroviral effects must be weighed against the toxic side effects in deciding on treatment options.

One of the more challenging HIV reservoirs is the central nervous system (CNS). HIV rapidly enters the CNS after primary infection where it establishes a persistent viral reservoir. CNS HIV infection frequently results in a spectrum neurological disorder manifested as cognitive, motor, and behavioral symptoms, known as HIV-associated neurocognitive disorders (HAND). The phenotypes in HAND range in severity from asymptomatic neurocognitive impairment (ANI) to mild neurocognitive disorder (MND) to HIV-associated dementia (HAD); it is difficult to predict what signs and symptoms will be apparent in any given individual. CART improves cognition and has greatly reduced the prevalence of HAD (<5%) among patients with HAND over the past decade. However, asymptomatic and mild neurocognitive impairments persist in ~25–40% of patients on CART with a trend toward increasing prevalence as patients live longer (Cysique and Brew 2009; Heaton et al. 2011). Although the magnitude of the reservoir and the extent of viral replication within brain tissue are not well understood, there is general consensus that control of HIV replication within the CNS would be advantageous in terms of preventing CNS disease and limiting the transfer of HIV from the CNS to systemic reservoirs capable of accelerated virus production. Consequently, strategies are under development to increase the penetration of ARTs across the blood-brain barrier through selection of antiretroviral drugs with high CNS penetration effectiveness (CPE, <https://hnrp.hivresearch.ucsd.edu>), together with other approaches that enhance drug delivery into the CNS parenchyma.

Increased delivery of ARTs to the CNS, however, comes with an increased risk of adverse effects due to drug interactions with neural tissues. Although these compounds have well-described toxic actions in many organs including the liver, kidney, fat, bone, muscle, and peripheral

nervous system (Thompson et al. 2012), little is known about the toxicity of ARTs in the CNS. Improvements in drugs and wider treatment options have reduced serious systemic side effects and have also improved tolerability and drug adherence. CNS side effects with current CART regimens have typically been minor relative to systemic toxic effects, but this may be due to the limited penetration of ARTs. As these efforts to suppress or eradicate HIV from the CNS advance, the potential risk(s) of adverse CNS events will likely increase.

Clinical Evidence for Neurotoxic Effects of Antiretroviral Therapies

A number of adverse effects have been reported in patients on ARTs as summarized in current treatment guidelines and reviews (Reust 2011; Thompson et al. 2012). The earliest and clearest clinical evidence of antiretroviral neurotoxicity was observed in the peripheral nervous system. The nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), didanosine (ddI), dideoxycytidine (ddC), and stavudine (D4T) in particular, are known to cause peripheral neuropathy, while zidovudine (AZT) was recognized early on for its ability to cause a distinctive myopathy. HIV-infected patients taking the so-called d-drugs (ddC, ddI, D4T) developed distal symmetric sensory polyneuropathy, primarily in the feet. Pathologically, there is a “dying back” axonal neuropathy, which initially affects the distal branches of the longest nerves and then progresses proximally in a height-dependent manner. The clinical presentation is defined by numbness, aching, and/or burning in the toes progressing to the feet over weeks to months resulting in severe neuropathic pain. This drug-induced neuropathy can be difficult to distinguish from distal sensory polyneuropathy caused directly by the viruses, with only a history of d-drug exposure at the time of neuropathy onset as a discriminating feature. More recent studies of patients on CART regimens that avoid the neurotoxic ARTs and

maintain effective HIV RNA suppression (<400 copies/ml) and CD4+ cell counts >350 report ~30% of subjects with clinically detectable peripheral neuropathy (Evans et al. 2011), of which ~10% are symptomatic. In contrast very little information is available on the actions of ARTs in the CNS.

CART regimens with high penetration of the CNS are currently under investigation. While some studies have found beneficial cognitive effects with higher CNS penetration, results have been mixed (Cysique and Brew 2009). These equivocal findings may be due in part to combined beneficial and adverse effects of treatment. Additionally, the CPE score is largely predicated on ART concentrations in cerebrospinal fluid, which may not reflect tissue ART concentrations. Although the effects of ARTs in the CNS have not been adequately explored, a number of studies have indicated that ARTs can have adverse effects. Documented adverse effects that may reflect ART interactions with the CNS include sleep disturbances, abnormal dreams, depressed mood, nausea, fatigue, vomiting, fever, headache, and neuropsychiatric symptoms (Reust 2011). The strongest evidence of CNS toxicity is evident in the neuropsychiatric side effects of efavirenz, which include short-term effects of suicidal ideation, dizziness, clouded thinking, and lucid dreaming, as well as long-term impairments in neuropsychological performance (Ciccarelli et al. 2011).

One group showed that cognition improved for up to 96 weeks in a group of immunologically and virologically stable subjects who elected to interrupt ART (Robertson et al. 2010). Patients in these studies were not optimized for antiretroviral penetration suggesting that perhaps low ART concentrations can also penetrate the brain sufficiently to exert deleterious effects. Other studies have reported that abacavir may be associated with adverse psychiatric symptoms (Foster et al. 2003). Using proton magnetic resonance spectroscopy, a group directly assessed metabolic profiles in brains of HIV-infected patients treated with ARTs with known mitochondrial toxicity

(Schweinsburg et al. 2005). *N*-Acetylaspartate (NAA) levels were measured as an index of neuronal mitochondrial metabolism revealing that in white matter, NAA levels were significantly decreased in patients receiving didanosine and/or stavudine. Decreases in NAA levels were correlated with combinations of drugs and longer treatment duration. These studies provided the first in vivo indication that ARTs might be associated with neural cell damage.

Collectively, the above studies contribute to a growing body of indirect evidence in support of CNS ART neurotoxicity. However, the causal relationship between ART exposure and CNS effects is unknown, and it is often difficult to determine if the effects were due to direct toxic actions of the ARTs or secondary effects due to changes in systemic pathology, interactions with other drugs, or other confounding variables (Cysique and Brew 2009). For example, cardiovascular disease, lipodystrophy, and diabetes seen in HIV-infected patients are all correlated with changes in neurocognitive performance. Common symptoms such as nausea, vomiting, fatigue, and headache reported for NRTIs may be due to direct CNS interactions or systemic effects. Changes in sleep and cognitive and mood disorders associated with non-nucleoside reverse transcriptase inhibitors (NNRTIs) are more likely to be due to direct CNS effects. However, development of serious systemic effects such as hyperlipidemia or liver toxicity may confound the analyses. Protease inhibitors (PIs) carry increased risks of hypertriglyceridemia, hypercholesterolemia, and hyperglycemia, all of which could have wide-ranging effects on the CNS. Thus, there is considerable uncertainty regarding the direct impact of ARTs on the brain and its functions. As newer therapeutic strategies aim to deliver higher concentrations of ARTs to the CNS, the potential for toxic interactions will increase and may include novel effects not seen with current treatments. Support for this possibility comes from emerging in vitro studies, summarized below, in which the direct effects of antiretroviral compounds on neural tissue are beginning to be investigated.

Experimental Evidence for Antiretroviral Therapy-Mediated Neurotoxicity

Most information on the mechanisms that underlie potential toxic effects of ARTs has been generated for the NRTIs based on the early recognition of inhibitory effects on mitochondrial polymerase gamma (MtPol γ). These nucleosides, modified to terminate HIV reverse transcription, also inhibit MtPol γ activity leading to mitochondrial DNA depletion, increased oxidative stress, and a loss of mitochondrial function (Koczor and Lewis 2010). Organ systems with high dependence on mitochondrial protein synthesis are particularly vulnerable to these effects. Lactic acidosis, liver steatosis, and myopathy were prominent in patients treated with first-generation NRTIs. An increased risk for chronic kidney disease and development of peripheral neuropathies (particularly d4T and ddC) and dyslipidemia were also associated with chronic NRTI treatment. Newer NRTIs have been screened for these adverse effects and have a lower affinity for MtPol γ with fewer side effects. However, side-effect profiles of the NRTIs often cannot be explained solely by their ability to inhibit MtPol γ as the appearance of effects often does not correlate with the level of MtPol γ inhibition (Koczor and Lewis 2010). In addition, distal sensory polyneuropathy, often attributed to effects on MtPol γ in the past, continues to be recognized among well-controlled patients receiving PIs which are pharmacologically distinct from the NRTIs (Evans et al. 2011); this finding raises the possibility that PIs might also contribute to the development of polyneuropathy. Many factors may contribute to observed effects of ARTs including the confounding influences of HIV infection itself, pharmacokinetics, tissue distribution, cellular metabolism of each drug (e.g., NRTIs are prodrugs which require phosphorylation to triphosphate forms by cellular kinases), and unique drug actions. Little direct evidence is available to indicate how these compounds will interact with CNS cells at concentrations required to control HIV replication. Several studies have begun to

Neurotoxic Consequences of Antiretroviral Therapies, Table 1 In vitro neurotoxic activity of antiretroviral compounds

Class	Drug	TC ₅₀ (μM)	Log plasma conc/TC ₁₀
NRTI	Abacavir (ABC)	0.0077	2.80
NRTI	2',3'-Dideoxycytidine	5.0426	-0.63
NRTI	2',3'-Dideoxyinosine	0.0780	2.66
NRTI	Emtricitabine [(-) FTC]	21.3875	-0.31
NRTI	Tenofovir (TDF)	0.2651	1.07
NRTI	Lamivudine (3TC)	0.8434	1.79
NRTI	Zidovudine (AZT)	6.1348	0.59
NNRTI	Efavirenz (EFV)	0.6319	2.03
NNRTI	Etravirine (ETR)	0.0156	3.11
NNRTI	Nevirapine (NVB)	0.5678	2.61
PI	Amprenavir (APV)	0.0969	2.64
PI	Atazanavir sulfate (ATV)	0.0197	2.91
PI	Darunavir (DRV)	17.6049	0.58
PI	Ritonavir (RTV)	3.2940	0.77
EI	Maraviroc (MVC)	5.7972	-0.50

In vitro toxic activity was assayed on cultured primary rat neurons. NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; EI, entry inhibitor; TC₅₀, median toxic concentration in primary neuronal cultures; TC₁₀, the drug concentration that is expected to cause minimal (10%) toxic activity based on the concentration-effect curve.

examine ART effects on neuronal function in vitro and ex vivo to assess potential interactions.

In vitro studies of ART neurotoxicity. The ability to examine effects of compounds on primary neuronal cultures allows sensitive direct assessments of potential toxic mechanisms under controlled conditions. Initial in vitro studies of the effect of various ARTs on cultured rat neurons demonstrated neurotoxic activity as assessed by alterations in calcium homeostasis and the development of dendritic pathology (Robertson et al. 2012). These pathological endpoints were chosen to provide assays of neuronal function likely to be sensitive to mild toxic effects expected in vivo. In this study, concentration-effect curves were generated for 15 antiretroviral drugs including 7 NRTIs, 3 NNRTIs, and 4 PIs and one entry inhibitor to determine the relative sensitivity of neurons to each compound. The median toxic concentration (TC₅₀) calculated for each drug varied widely as summarized in Table 1. Lower TC₅₀ values represent higher potencies. Potency does not take into consideration in vivo factors such as CNS penetration that contribute to the likelihood of neurotoxicity. Toxicity was not restricted to a

particular class of compounds. Of the NRTIs, abacavir, dideoxycytidine, tenofovir, and lamivudine showed the highest toxic potency. All NNRTIs tested had relatively high toxic potency. The toxicity of the PIs was generally lower than the NRTIs and NNRTIs but spanned a wide range with a toxicity rank order of atazanavir > amprenavir ≫ ritonavir > darunavir. To gain insight into the potential neurotoxicity at concentrations that would effectively suppress viral replication in the brain, a toxicity index was calculated as the log of the therapeutically effective plasma concentration divided by the TC₁₀ (representing a minimal 10% deflection of the concentration-effect curve). A toxicity index of 1 would then indicate that a therapeutic concentration was at the threshold of toxic activity with higher values indicating greater risk of toxicity. Overall, approximately half the compounds tested had an index ≥2, suggesting a clear risk of neurotoxicity. No correlation was seen with changes in the mitochondrial membrane potential and the rank order of CNS toxicity; abacavir > dideoxyinosine > tenofovir, lamivudine > dideoxycytidine, zidovudine > emtricitabine

(Robertson et al. 2012) did not parallel the reported inhibitor efficacy on MtPoly: zalcitabine \geq didanosine (dideoxyinosine) \geq stavudine \gg lamivudine $>$ tenofovir $>$ emtricitabine \geq zidovudine $>$ abacavir (Koczor and Lewis 2010). No significant cell death was seen for most compounds at relevant concentrations indicating that even though the compounds disrupt normal neural function, they are not highly toxic to nervous tissues. This increases the likelihood that neurotoxicity in vivo may be managed by careful drug selection and adjunctive neuroprotective therapies.

When clinically relevant combinations of the antiretroviral compounds were applied to cultured neurons at therapeutic doses, the results were unpredictable. In some cases, the expected additive effects were seen, whereas in other cases potential protective effects were observed (Robertson et al. 2012). Although more data are required to understand the nature of these interactions, the latter observation indicates that specific combinations of drugs might minimize neurotoxicity. Individual drug effects and drug-drug interactions in the CNS will undoubtedly be complex and mechanisms are only beginning to be explored. In some cases, toxic metabolites may also play a role. Enhanced neuronal calcium dysregulation and pathology has been reported in response to the efavirenz metabolite, 8-OH-efavirenz, which was an order of magnitude more toxic than the parent compound (Tovar-y-Romo et al. 2012). CNS cells other than neurons may also be targets. The NRTI, abacavir, and the PI, indinavir, at 10 μ M have been reported to inhibit the ability of microglia to phagocytose amyloid beta (Giunta et al. 2011), a process thought to be important for suppression of Alzheimer-like pathology that has been reported in HIV infection. NRTIs have also been linked to inhibition of telomerase activity in human peripheral blood mononuclear cells suggesting that they contribute to accelerated aging (Leeansyah et al. 2013). Each of the in vitro studies has limitations that must be taken into account when attempting to interpret in vitro results in the in vivo setting. In vitro assays cannot easily assess the effects of

long-term treatment, may depend on specific conditions of the assay, and cannot recapitulate the complex interactions that occur in vivo. Thus, the impact of particular ART interactions on CNS function must be validated in appropriate in vivo studies.

In vivo studies of antiretroviral therapy neurotoxicity. An important recent study has provided an in vivo assessment of antiretroviral toxicity in both macaques and rats treated with various antiretroviral drug combinations (Akay et al. 2014). Decreases in synaptic density in the hippocampus, assessed by synaptophysin immunoreactivity, were specifically linked to ART exposure in SIV-infected macaques and in rats treated for 7 days with the ARTs, zidovudine, ritonavir, and saquinavir. These same studies further demonstrated that the generation of reactive oxygen species contributed to cellular injury and that adjunctive antioxidant treatments may have therapeutic utility.

The above studies show that ARTs can be toxic to neurons and provide preliminary evidence highlighting how these drugs might cause cellular damage. More studies are needed to better understand the potential risks and the underlying pathogenic mechanisms. Future studies should include evaluation of interactions between antiretroviral drugs as well as interactions with other medications likely to be used to treat comorbid conditions in an aging HIV population.

Conclusions

Treatment of replicating HIV in the CNS carries a risk of off-target neurotoxic effects. Under current treatment regimens with limited penetration of ARTs into the CNS, there are few reports of serious CNS adverse events, perhaps because the virus also exerts direct and substantial adverse effects on the CNS. However, a growing body of data has demonstrated neurotoxic effects of several ARTs, which must be taken into consideration as new strategies are developed to suppress HIV replication in the CNS. Although too few studies are available at present to recommend particular

treatment strategies, preliminary evidence suggests that adverse effects of ARTs in the CNS may be avoided at least partially by the careful selection of specific ART combinations that minimize the risk of neurotoxicity. While effects on MtPoly are the most well-documented toxic mechanisms, other actions are likely to contribute to ART toxicity in the CNS. Indeed, the mechanisms of ARV toxicity in the CNS are poorly understood, necessitating more studies to understand the full range of neurotoxic effects and the mechanisms mediated by ARTs. CART selection strategies are currently guided by efficacy, resistance, toxicity, drug interactions, and penetration of the CNS. As improved strategies are developed to target the CNS viral reservoir, greater knowledge of the effects of ARTs on neural tissues will be needed to direct their use which both maximize their antiretroviral actions and minimize the neurotoxic effects and the associated clinical complications.

Cross-References

- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [HAND Adjunctive Therapies: Reversing Neuronal Injury](#)
- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [Medication Adherence and HIV-Associated Neurocognitive Disorders \(HAND\)](#)
- ▶ [HIV-2 Neurological Manifestations](#)
- ▶ [HIV Neurocognitive Diagnosis, Natural History, and Treatment](#)
- ▶ [Neuro-AIDS, Immunopathogenesis of](#)
- ▶ [Neurocognitive Outcomes in HIV-Infected Children and Adolescents](#)
- ▶ [Neuroinflammation and HAND: Therapeutic Targeting](#)
- ▶ [Overview of HIV CNS Infection](#)

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NKT Cells: Bridging Innate and Adaptive Immunity

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Definition

Invariant natural killer T-cells (iNKTs) are an innate T-cell subset unique in their possession of an invariant T-cell Receptor (TCR) and non-classical antigen presentation mechanism through the CD1d molecule. iNKTs recognize glycolipids presented on the nonpolymorphic major histocompatibility complex (MHC) class I-like CD1d molecule, unlike conventional T-cells which recognize peptides presented on MHC class I/II molecules. iNKTs can rapidly produce multiple cytokines in response to activation. This rapid cytokine production allows iNKTs to be powerful modulators of the immune response. iNKTs can produce T-helper type 1 (Th1), Th2, and Th17 cytokines depending on stimulus strength, type of stimulation, iNKT subset, and presenting cell type. As a result, iNKTs are able to affect and modulate immune responses to a variety of diseases, both chronic and infectious – including a potentially important role in controlling human immunodeficiency virus (HIV) infection. The ability of iNKTs to produce and direct a variety of immune responses shows the promising potential for development of immune therapies and adjuvants by harnessing and directing iNKT functionality. A better understanding of iNKT function would provide a powerful tool to direct both chronic and infectious diseases through modulation of the immune response.

Introduction

Invariant natural killer T-cells (iNKTs) are an innate, multifunctional T-cell subset. iNKTs are

able to produce T-helper type 1 (Th1), Th2, Th17 cytokines and influence the function of other immune cell subsets (Tupin et al. 2007; Godfrey et al. 2015; Van Kaer et al. 2015). This ability to produce cytokines covering a range of immune functions highlights the important role of iNKTs in immune modulation.

iNKTs were first discovered in nonobese diabetic (NOD) mice in the context of diabetes. In this murine model, mice spontaneously developed diabetes which was ultimately attributed to the absence of a subset of T-cells expressing NK1.1, a marker previously thought to be exclusively expressed on natural killer cells (NK cells). Natural killer T-cells were thus named for their mix of NK and T-lymphocyte markers. Further investigation led to the understanding that this newly identified population of cells was a heterogeneous T-cell subset. This in turn led to the classification of NKTs into at least two distinct groups: type I NKTs – which this chapter will focus on – and type II NKTs.

Common to both types of NKTs is their recognition of antigen presented in the context of CD1d molecules. CD1d molecules are major histocompatibility complex (MHC) class I-like molecules that are nonpolymorphic and comprised of a heavy chain with three alpha subunits associated with β 2-microglobulin. The CD1d molecule is highly conserved across species ranging from mice to humans and has a hydrophobic binding pocket that allows binding and presentation of lipid ligands. Type I NKTs, also known as classical or invariant NKT cells, are activated by a class of lipid ligands known as sphingolipids. iNKTs use an invariant TCR α chain – V α 24-J α 18 in primates and V α 14-J α 18 in mice – that preferentially binds to V β 11 and V β 8 in primates and mice, respectively. Both human and mouse iNKT cells are enriched in CD4⁺ and CD4/CD8 double-negative populations. Humans and non-human primates (NHPs) have a CD8⁺ population of iNKT cells that is absent in the mouse model.

Type II NKT cells, or nonclassical NKTs, are also CD1d restricted but possess more TCR variability than iNKTs. Like iNKTs, type II NKT cells are also activated by lipid ligands; however, the specific lipids able to activate type II NKTs differ from the lipid ligands of iNKTs. One subset of type

II NKT cells are activated by sulfatides instead of the sphingolipids employed in iNKT activation. Studies of NKT cells have been hampered by lack of tools for clear differentiation and identification of NKT subsets. The initial definition of NKT cells as T-cells expressing NK1.1 in mice and CD161 in primates is neither specific nor does it cover all NKT cells. Flow cytometry based methods using tetramers have greatly facilitated accurate identification of NKT cells, particularly iNKTs.

Currently iNKTs are most readily detected by the use of CD1d tetramers as well as a monoclonal antibody (clone 6B11) which recognizes the invariant region of the iNKT TCR. iNKT identification involves co-staining with T-cell receptor (TCR) V α -chain specific antibodies, such as V α 24 (in primates, V α 18 in mice), and 6B11 or CD1d tetramers (CD1dTM) loaded with the lipid antigen alpha-galactosylceramide (α -GalCer). Both the 6B11 antibody and α -GalCer loaded CD1dTM bind to the glycolipid binding site on the TCR of iNKTs. These Va24+ CD1dTM+ or Va24+ 6B11+ cells are defined as iNKTs. A subset of type II NKT cells can be identified with CD1dTM loaded with sulfatide lipid ligands.

The remainder of this entry will focus on iNKTs and their unique multifunctionality. Characteristics of iNKTs as well as their role in both chronic and infectious disease – with a particular interest to HIV – will be discussed.

Characteristics of iNKTs

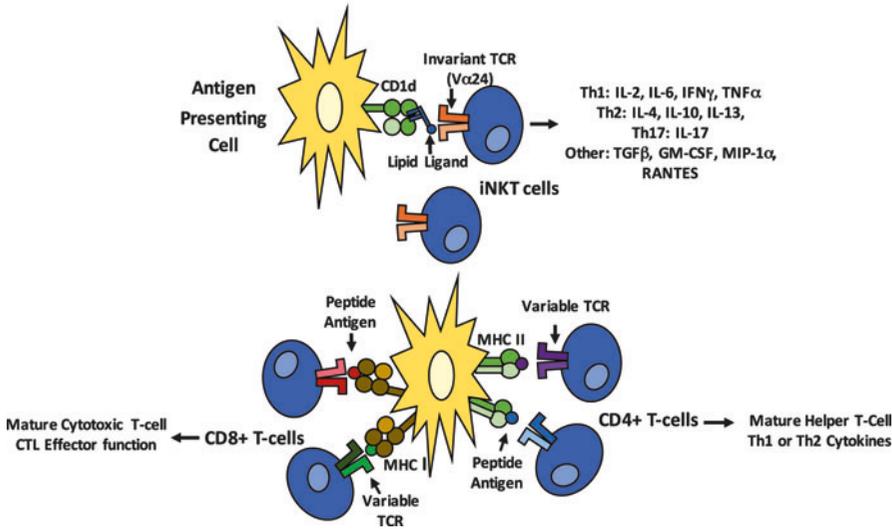
iNKTs are a nonclassical T-cell subset for multiple reasons (Table 1). While all T-cells have an alpha

(α) and beta (β) TCR chain, in the case of classical T-cells, the antigen recognition domain of the α and β chains are infinitely variable. This property allows classical T-cells to specifically recognize and respond to a broad range of peptides presented on MHC class I or II molecules (Table 1). While classical T-cells are a part of the adaptive immune system, iNKTs function as innate T-cells by virtue of having an invariant complementary determining region three (CDR3) region of the α chain and a restricted (but not invariant) β chain that recognizes glycolipid ligands presented on nonpolymorphic CD1d molecules (Godfrey et al. 2015). Due to their innate nature, iNKTs can rapidly recognize lipid antigens after encountering them for the first time.

The best studied lipid antigen capable of activating iNKTs is alpha-galactosylceramide, or α -GalCer, a derivative of the marine sponge, *Agelas mauritanus*. α -GalCer was first identified as a natural killer T-cell agonist in studies investigating novel antitumor agents. α -GalCer has a strong avidity for the iNKT TCR when presented by CD1d molecules on antigen presenting cells (APCs) and rapidly activates iNKTs leading to proliferation and cytokine production. While α -GalCer is not necessarily a biologically relevant lipid antigen for iNKTs in vivo, analogous glycolipids (both endogenous and exogenous or pathogen derived) have been identified. It has been suggested that endogenous lipids play a role in iNKT development and differentiation in the thymus. Endogenous lipids may also play a role in iNKT activation during infection with some pathogens where concurrent pathogen and “self” lipid recognition is necessary to elicit a response.

NKT Cells: Bridging Innate and Adaptive Immunity, Table 1 iNKTs versus classical T-cells

	Invariant natural killer T-cells	Classical T-cells
TCR	Invariant	Infinitely variable/diverse
Binding specificity	Limited to few lipid ligands	Multiple and diverse peptide-MHC combinations
Antigen presentation	CD1d (nonpolymorphic)	MHC class I/II (polymorphic)
Antigen	Lipids (glycosphingolipids)	Peptides
Effector function	Diverse Th0/Th1/Th2/Th17/ Cytotoxicity	CD4+ Th1 or Th2 helper T-cells or CD8+ Cytotoxic T-cells
Time to effector function in vivo	Rapid (hours to days)	Slower (days to weeks)
Type of immunity	Innate	Adaptive



NKT Cells: Bridging Innate and Adaptive Immunity, Fig. 1 Antigen presentation in iNKTs and Classical T-cells. Antigen presenting cells present antigens to

T-cell subsets – lipid antigens on CD1d in iNKTs and peptide antigens on MHC class I/II in classical T-cells – resulting in activation/maturation

α -GalCer is composed of a glucose head connected to a ceramide, which consists of a sphingosine backbone with a fatty acid tail. Sphingolipids with a similar sugar-ceramide structure make up the family of lipid ligands that can activate iNKT cells.

Modes of antigen presentation differ between iNKTs and classical T-cells (Fig. 1). In the case of iNKTs, APCs expressing the CD1d molecule – such as dendritic cells, macrophages and B-cells – present lipid ligands to iNKT cells that are recognized by the iNKT invariant T-cell receptor. Activated iNKTs produce a wide range of cytokines depending on multiple factors including the type of APC, ligand binding affinity, and duration of binding. In contrast, classical CD4 and CD8 positive T-cells recognize peptide antigens presented on APCs expressing either MHC class I or II molecules. Upon activation, classical CD4+ T-cells mature into helper T-cells with a Th1 or Th2 cytokine profile while CD8+ T-cells mature into cytotoxic T-cells with cytolytic activity. iNKTs are able to produce multiple effector functions from a single cell subset, whereas conventional T-cells generally have a single function specific to each T-cell subset. For example, a single subset of iNKTs can produce Th1 and

Th2 (Th0) cytokines as well as having cytolytic activity. In contrast, in conventional T-cell subsets there are CD4+ Th1 producing subsets and Th2 producing subsets that are distinct (Tupin et al. 2007).

iNKTs Across Species

As a component of the innate immune system iNKTs are evolutionarily conserved across multiple species. iNKTs can be found in a wide range of vertebrates including mammals such as mice, humans, NHPs, pigs, and horses. Functional CD1d is notably lacking from ruminants showing that while present in many mammals, iNKTs are not universal (Looringh van Beeck et al. 2009). CD1d/NKTs are not restricted to mammals and have been identified in birds, reptiles, and amphibians; although functionality of CD1d in these species has not been determined (Ohta and Flajnik 2015). The wide range of species possessing iNKTs suggests that these cells developed relatively early in evolutionary history. iNKTs have been most widely studied in mice and humans; more recently studies in NHPs have been performed.

NKT Cells: Bridging Innate and Adaptive Immunity, Table 2 iNKT frequency and phenotype varies between species

Species		Peripheral blood iNKT frequency (mean or range, % T lymphocytes)	CD4/CD8 phenotype				Tissue iNKTs	References
			CD4+	CD8+	CD4+ CD8+	CD4- CD8-		
Mouse	<i>Mus musculus</i>	0.5–2%	+	–	–	+	Highly enriched in liver; significant populations in bone marrow, thymus, spleen; lower in lymph nodes	Berzins et al. 2011
Primates								
Humans	<i>Homo sapiens</i>	0.1% (highly variable between individuals)	+	+	–	+	Enriched in omentum and liver; significant populations in spleen, bone marrow, and lymph nodes; lower in the thymus	Godfrey et al. 2015
Sooty mangabey	<i>Cercocebus atys atys</i>	0–0.13%	–	+	–	+	ND	Rout et al. 2010
Rhesus macaque	<i>Macaca mulatta</i>	0–0.04%	+	+	–	+	ND	Rout et al. 2012
Pigtailed macaque	<i>Macaca nemestrina</i>	0–2.2% ^a	+	+	+	+	ND	Fernandez et al. 2009
Cynomolgus macaque	<i>Macaca fascicularis</i>	0.008–0.6%	+	+	+	+	ND	Rout et al. 2012

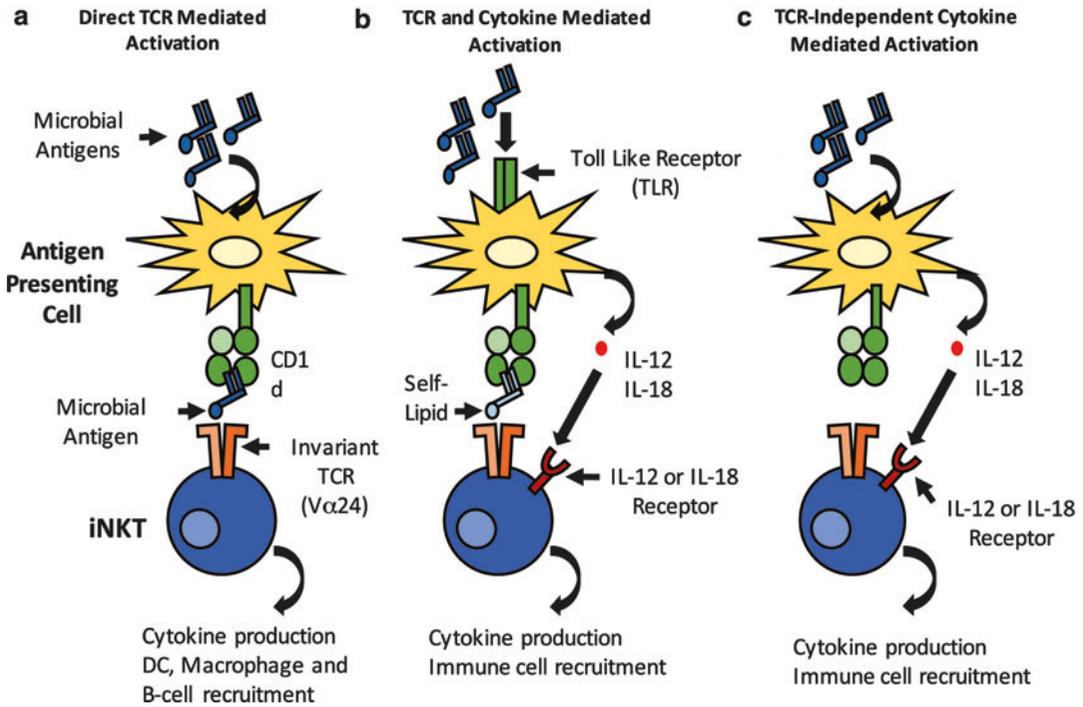
^aPercent total lymphocyte population
 ND not described

iNKT frequency, distribution within the host, phenotype, and function vary based on tissue and host species (Table 2). In the murine model, iNKTs are enriched in liver and bone marrow. Significant iNKT populations are also seen in the thymus, spleen, and peripheral blood but to a lesser degree. iNKT frequencies between mice are relatively stable. Humans, however, have lower NKT frequencies than mice, and frequencies vary greatly between individuals. Not surprisingly, NHP iNKTs are more reflective of what is seen in humans than in mice. The NHP species that have been studied – sooty mangabeys and macaques (rhesus, cynomolgus, and pigtailed) –

consistently have lower iNKT frequencies than mice, and iNKT frequencies vary widely between animals (Fernandez et al. [2009](#); Rout et al. [2010](#), [2012](#)). Cynomolgus macaques have more similar iNKT frequencies to humans and therefore may represent the best NHP model to understand iNKT frequency and functionality in humans.

How Do iNKTs Act?

iNKTs act either directly or through downstream effects on other immune cell subsets. iNKTs activate and are able to cross-talk with other immune



NKT Cells: Bridging Innate and Adaptive Immunity, Fig. 2 iNKT pathogen response mechanisms. There are three proposed routes of iNKT activation: direct TCR

mediated activation (a), TCR and cytokine mediated activation (b), and TCR-independent cytokine directed activation (c)

regulatory and effector cells. Pathogen recognition causes iNKTs to produce a wide range of cytokines including IL-2, IL-4, IL-6, IL-10, IL-13, IL-17, transforming growth factor beta (TGF- β), interferon gamma (IFN- γ), and tumor necrosis factor alpha (TNF α). Through the rapidity of their response and their prolific cytokine production repertoire, iNKTs are able to directly affect a variety of downstream immune responses and recruit other effector cells. iNKTs can enhance immune responses through interaction with dendritic cells (DCs). iNKTs can induce activation and maturation of DCs through CD40 ligand on its surface interacting with CD40 on DCs and this in turn enhances DC-mediated antigen presentation to T lymphocytes and improves immune responses mediated by CD8+ cytotoxic T lymphocytes (CTLs) and CD4+ T-helper cells (Juno et al. 2012). NK cells, B-cells, and regulatory T-cells can also be directly influenced by iNKTs. iNKTs can also be directly cytolytic through granzyme B,

perforin, FasL, and TNF-related apoptosis-inducing ligand.

While iNKTs can directly recognize and react to pathogens possessing lipid antigens that are presented on CD1d molecules, they can also react to pathogens without these hallmark lipids. How are iNKTs activated by pathogens lacking lipid antigens? As shown schematically in Fig. 2, there are at least three proposed models for iNKT activation in response to foreign antigens (Tupin et al. 2007; Van Kaer et al. 2015).

The first proposed model of iNKT activation is through direct recognition. iNKTs can directly recognize and respond to pathogens with glycolipids analogous to α -GalCer. These are primarily bacterial or parasitic organisms which have glycolipids on their outer cell surface such as the glycosylceramides in the cell wall of *Novosphingobium* species (*Sphingomonas*, gram negative bacteria) as well as diacylglycerols expressed by *Borrelia* species, the spirochete etiologic agent of Lyme disease (Tupin et al. 2007).

The second proposed model is iNKT activation through simultaneous TCR and cytokine engagement. In this scenario, byproducts of invading micro-organisms are recognized by Toll-Like-Receptors (TLRs) on APCs initiating a stress response which results in upregulation of self-lipids presented on CD1d molecules and production of cytokines – namely IL-12 and IL-18 that can bind to their respective receptors expressed on the surface of iNKT cells.

Finally, iNKTs can be activated entirely in a TCR-independent cytokine directed manner through the receptors for IL-18 and IL-12 on their cell surface binding to cytokines produced in response to pathogen detection by a number of innate immune cells such as DCs and macrophages. While a given pathogen may primarily use one route of activation, it is likely that iNKT activation involves multiple activation routes.

The type of effector response of iNKTs is directly related to the type of stimulus as well as the route and strength of stimulation. Depending on the pathogen and type of immune response required, iNKTs can be protective or deleterious. Questions remain regarding the determinants of effector function in iNKTs: are there distinct NKT populations with specific effector function or is it the milieu or environment of NKTs that determine their ultimate effector function?

iNKTs and Disease

iNKTs have been shown to play an important role in several bacterial, parasitic, and viral infections (summarized in Table 3), and in cancer and diseases of chronic inflammation and immune activation such as autoimmune diseases, asthma, and metabolic syndromes (Mallevaey et al. 2006; Tupin et al. 2007; Berzins et al. 2011; Van Kaer et al. 2015). Murine studies have provided evidence for a protective role of iNKTs against tumor proliferation as well as against autoimmune conditions such as diabetes, multiple sclerosis, and systemic lupus erythematosus, whereas iNKTs can exacerbate certain diseases such as asthma and atherosclerosis. Protective and deleterious effects of iNKTs have also been described for several pathogens (Table 3).

Clinical studies in humans have also suggested that deficiencies in iNKTs, in frequency and/or function, contribute to a variety of chronic and infectious diseases. iNKT defects, either low frequency or incompetent cytokine production, have been suggested to play a causal role in the pathogenesis of autoimmune diseases (i.e., diabetes, lupus, arthritis, and multiple sclerosis), cancer, and infectious diseases including influenza and HIV. A pathologic role of iNKTs in human disease has been suggested for atherosclerosis, graft-versus-host disease, and asthma (Berzins et al. 2011). It should be noted that due to conflicting results in similarly designed studies as well as lack of a stringent, consistent definition for iNKTs in human studies, the link between iNKT dysfunction and disease is less clear than in the mouse model. A major question that remains across even the most convincing data linking iNKT defects to disease in humans is whether the observed defect is causal or reactive to disease progression. Further longitudinal investigation of the role of iNKTs in disease, especially in the NHP model, promises to fill gaps in understanding between human and mouse studies. Investigating the role of iNKTs in both chronic and infectious diseases involving inflammation will lead to a better comprehension of the role of iNKTs in HIV infection and progression to AIDS, a hallmark of which is chronic inflammation and immune activation.

iNKTs and HIV

In vitro studies, as well as in vivo studies in animal models and humans have begun to complete the picture of the role of iNKTs in HIV infection. While the mechanism and details of iNKT involvement in HIV/AIDS are unknown, multiple factors suggest that iNKTs play an important role in HIV progression to AIDS. First, the ability of HIV proteins to downregulate CD1d expression suggests the evolution of iNKT-related immune evasion mechanisms in HIV infection. Second, iNKTs are highly susceptible targets of HIV infection. Finally, massive dysregulation of iNKTs in AIDS susceptible hosts (i.e., humans and macaques) with maintenance of iNKT function

NKT Cells: Bridging Innate and Adaptive Immunity, Table 3 Examples of pathogens where iNKTs play a role in protection from or exacerbation of disease

Pathogen type	Pathogen	Disease	Route of activation			iNKT role in disease	References
			Direct	TCR with self-lipid	Cytokine mediated		
Parasites	<i>Schistosoma mansoni</i> , Blood Flukes	Schistosomiasis	–	–	+	Important in acquired immune response	Mallevaey et al. 2006
	<i>Leishmania major</i>	Cutaneous Leishmania	+	–	?	Protective-parasite clearance	Tupin et al. 2007
Bacteria	<i>Pseudomonas aeruginosa</i>	Mild disease in healthy persons (rash and ear infections), severe disease in immunocompromised patients (pneumonia)	+	–	?	Unclear-protective in some cases and pathologic in other	Tupin et al. 2007
	<i>Borrelia burgdorferi</i>	Lyme disease	+	–	?	Protective against Lyme arthritis	Tupin et al. 2007
	<i>Salmonella spp.</i>	Salmonellosis, Salmonella poisoning	–	+	+	Pathologic-contributes to liver injury	Van Kaer et al. 2015
	<i>Chlamydia spp.</i>	Chlamydia	+	–	+	Protective (<i>C. pneumonia</i>) or pathologic (<i>C. muridarum</i>) depending on <i>Chlamydia</i> species	Tupin et al. 2007
Viruses	Influenza	Flu	–	–	+	Protective-improve virus specific immune responses	Juno et al. 2012
	Herpes Simplex Virus	Genital or oral herpes, cold sores	–	–	+	Protective-early viral control	Tupin et al. 2007
	HIV	AIDS	–	+ ^a	+ ^a	Unknown	Juno et al. 2012

^aRole proposed

in natural hosts (sooty mangabeys) that do not progress to AIDS is another added factor implicating iNKTs in having a protective role in AIDS. Despite the strong evidence supporting iNKT involvement in AIDS, longitudinal and prospective studies are needed to show causality between iNKT dysfunction and AIDS progression. The sections below summarize our current knowledge on the interactions between iNKTs and HIV infection.

HIV Downregulates CD1d

HIV has evolved mechanisms to interfere with CD1d trafficking impeding antigen presentation through this route. HIV, for example, employs viral proteins Vpu and Nef to prevent CD1d expression on dendritic cells (DCs), which play an important role of antigen presentation to iNKTs (Juno et al. 2012). Vpu sequesters CD1d in early endosomes, impeding the ability of DCs to activate iNKTs. Furthermore, Nef promotes the



internalization of CD1d molecules from the cell surface to the trans-golgi network ultimately impairing the ability of the cell to present CD1d and activate iNKT cells. The evolution of mechanisms to overcome iNKT activity suggests an iNKT based role in HIV control. These mechanisms could effectively block the ability of iNKTs to respond to pathogens, thereby dysregulating normal immune response mechanisms.

iNKTs Are Susceptible to HIV Infection

Infection of iNKTs by HIV or simian immunodeficiency virus (SIV), the simian analogue to HIV and the primary model for HIV research, have been studied *in vitro* as well as *in vivo* in both humans and NHP models. *In vitro* studies have shown that iNKTs are highly susceptible targets of HIV/SIV infection (Fleuridor et al. 2003). Many iNKTs are CD4 positive and the majority express CCR5 and to a lesser degree CXCR4, the co-receptors of HIV, making them preferred targets for HIV/SIV infection (Sandberg et al. 2002). *In vivo*, in both NHP models and humans, CD4+ iNKTs are depleted early in SIV/HIV infection indicating they are primary target cells of these lentiviruses (Sandberg et al. 2002; Ibarrondo et al. 2013). This depletion is most likely due to direct infection of CD4-positive iNKTs as well as Fas-mediated apoptosis of the remaining CD4 negative iNKT subsets. Activation induced cell death is probably the main way that the CD4-negative iNKT subsets are depleted in HIV infection.

Studies in HIV-infected subjects on highly active antiretroviral treatment (HAART) have investigated the repopulation dynamics of iNKTs depleted during acute HIV infection. While several studies report iNKT recovery following treatment with HAART, there are conflicting results concerning the extent and rate of repopulation of CD4-positive and CD4-negative iNKT subsets. Due to the conflicting data on iNKT recovery, the effect of HAART on iNKT reconstitution remains inconclusive.

iNKT Dysfunction Is Associated with Chronic Immune Activation

Consistent with iNKTs being a primary target of HIV/SIV infection, the early depletion of gut

iNKTs in HIV infection is thought to contribute to iNKT dysregulation, a skewing toward pro-inflammatory cytokine production and ultimately chronic immune activation and progression to AIDS (Ibarrondo et al. 2013). NHP studies in natural and nonnatural hosts of SIV infection have highlighted the importance of preserved iNKT frequency and function in protection against AIDS (Rout et al. 2012). Natural SIV hosts such as the sooty mangabey monkey (*Cercocebus atys atys*) show marked differences in response to SIV infection when compared to nonnatural hosts, such as macaques (cynomolgus and rhesus) and humans. Natural hosts typically maintain high levels of viremia with no chronic immune activation and no progression to AIDS. In contrast, nonnatural hosts have high virus loads paired with chronic immune activation and inevitable progression to AIDS.

In the natural host, iNKT populations remain intact and functional, helping to maintain and find balance in the immune response. In chronically SIV infected macaques, the loss of CD4+ iNKTs correlates with an increase in SIV viral load as well as activation of CD4+ memory T-cells, contributors to increased inflammation. This maintenance of iNKT function and positive SIV outcome in sooty mangabeys contrasted with dysregulation of iNKTs and poor outcome in macaques highlights the importance of maintained iNKT function in protection from chronic immune activation and progression to AIDS (Rout et al. 2012).

Cytokine profiles of iNKTs in natural hosts compared to nonnatural hosts differ in distinct and possibly meaningful ways. For example, when comparing SIV-negative mangabeys and macaques, iNKTs from members of both species produce a variety of Th1 (IFN- γ , IL-2) and Th2 (IL-10, IL-6, TGF- β) cytokines. However, there are quantitative and qualitative differences between species with sooty mangabey iNKT activation resulting in higher levels of IFN-g, IL-13, and IL-17 production relative to the macaques (Rout et al. 2012). These differences in cytokine production in mangabeys versus macaques most likely represent functional differences between the species that may have a bearing on differential AIDS pathogenesis. Additionally, in the context

of SIV, macaque iNKTs lose much of their ability to produce cytokines following stimulation with α -GalCer and the residual effector activity is skewed towards a dominant proinflammatory IL-6 response, a factor that may contribute to increased immune activation (Rout et al. 2012).

Harnessing the immunomodulatory action of iNKTs could provide a promising tool to control immune responses and thus disease outcomes in the HIV setting as well as other diseases of chronic inflammation where iNKTs play a prominent role. It remains unclear whether the changes in iNKT frequency, phenotype, and functionality in the presence of HIV/SIV infection are causal with respect to negative disease outcomes. Longitudinal studies are needed to show direct causality.

Manipulation of iNKTs: Potential for a Therapeutic Role

In recent years much interest has been placed on the potential role of iNKTs in therapeutic measures. The ability to manipulate iNKTs would allow modulation of T-helper immune responses: the host immune response could be skewed towards an environment beneficial to pathogen control. Immune modulation through iNKT manipulation is a promising field that will lead to therapeutic interventions – both as direct therapies or as adjuvants to improve vaccine induced immunity – for a range of diseases in the future.

Orchestration of the immune response by iNKTs could be applied as an important therapy in disease settings where iNKTs play a role. Antitumor therapies involving iNKT activation are currently being studied. Researchers have looked into methods involving soluble α -GalCer (and other synthetic lipid) administration as well as lipid ligand loaded onto autologous antigen presenting cells in various formulations. These studies together show some positive clinical outcomes in patients including tumor reduction, reduction of tumor markers in the serum (Singh et al. 2014). While the current focus on iNKT therapies is to target various cancers, such therapies could be applicable to other iNKT-mediated diseases. Harnessing the immunomodulatory capacity of iNKTs as adjuvants to improve

immune responses, especially mucosal immunity, is also a promising field.

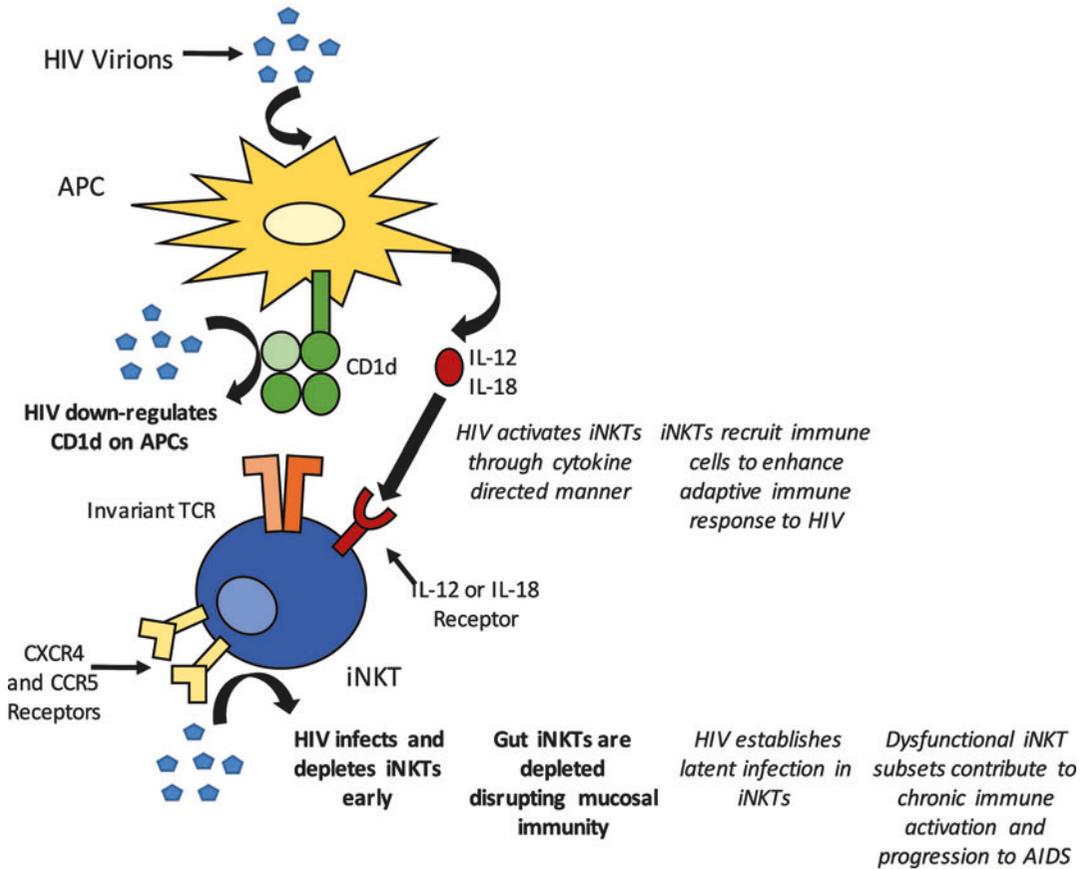
Conclusions and Remaining Questions

iNKTs are a heterogeneous nonclassical T-cell population. These innate T-cells are able to rapidly produce a range of cytokines and direct the immune response following activation by lipid ligands presented on the nonpolymorphic MHC class one like molecule CD1d. iNKTs recruit other immune cells such as DCs and macrophages and additionally shape the adaptive immune response through recruitment of B and T-cells. iNKTs can also have direct cytolytic activity. iNKTs play an important immunomodulatory role in multiple diseases related to chronic inflammation, most likely including HIV.

During HIV infection, iNKTs are rapidly depleted, showing they are a preferred target for HIV infection. Through this depletion, iNKT populations are disrupted in terms of frequency, phenotype, and function. In NHP models of HIV infection, dysregulation of iNKT function following SIV infection correlates with increased immune activation, inflammation, and progression to AIDS. HIV has evolved mechanisms to downregulate CD1d expression on antigen presenting cells, further impairing iNKT functionality. Although mounting evidence in human and NHP studies suggests that iNKTs are important in HIV infection and pathogenesis, further study is needed to conclusively show causality and illuminate mechanisms of iNKT activity during HIV infection.

Many questions remain in understanding iNKT biology, effector function, and potential therapeutic role in the context of HIV infection and mucosal immunity (Fig. 3 and Table 4).

The mechanism of iNKT activation in HIV infection and the consequence of HIV infection-mediated CD1d downregulation on *in vivo* iNKT activation are not known. It is proposed that iNKTs are activated by HIV through a TCR independent, endogenous lipid independent, cytokine mediated mechanism; however, the actual mechanism has yet to be definitively shown. It is known that iNKTs are able to improve adaptive immune responses by



NKT Cells: Bridging Innate and Adaptive Immunity, Fig. 3 iNKTs in the context of HIV. Schematic diagram showing known and proposed interactions between iNKT and HIV and AIDS pathogenesis

NKT Cells: Bridging Innate and Adaptive Immunity, Table 4 iNKTs and HIV: knowns and unknowns

	Known	Questions
Target cells	iNKTs are CD4+ and express HIV co-receptors CCR5 and CXCR4 iNKTs have enhanced susceptibility to HIV infection in vitro Both systemic and gut mucosal CD4+ iNKTs are infected and depleted early in HIV infection	Are iNKTs a latent reservoir for HIV? Is iNKT depletion irreversible? Does iNKT depletion lead to chronic immune activation?
iNKT dysfunction	CD4-negative iNKT are not depleted but become dysfunctional Increased expression of exhaustion markers PD-1 and LAG-3 on iNKTs in HIV-infected individuals	What is the consequence of iNKT dysfunction? 1. Impaired HIV/SIV-specific immunity? 2. Impaired immune response to opportunistic pathogens? 3. Enhanced proinflammatory environment? 4. Are iNKTs necessary to maintain gut mucosal immunity?
Immune evasion	HIV downregulates CD1d on APCs and as a result may evade NKT cells	How do iNKTs get activated in vivo in HIV infection? Does CD1d downregulation impair iNKT activation in vivo resulting in iNKT dysfunction?

recruiting immune cells and enhancing T-cell development in response to invading pathogens. While it seems likely that fully functioning iNKTs would play this role in HIV infection, it is unclear how iNKT loss and dysfunction impacts pathogen-specific immune responses against HIV and other pathogens. There are also open questions about the role of iNKTs in mucosal immunity and AIDS pathogenesis. Mucosal immunity is known to be important in HIV infection and pathogenesis. Gut iNKTs are depleted early on in infection, disrupting the protective gut mucosal barrier. Dysfunctional iNKT populations in the gut may contribute to further immune activation and progression to AIDS. Further study of gut mucosal iNKTs is needed to definitively determine the role of iNKTs in mucosal immunity during HIV infection. A major question concerning iNKTs in AIDS pathogenesis is whether the iNKT dysfunction and subsequent immune activation during chronic SIV infection is causal or reactive to viral infection and disease progression. Although iNKT depletion and dysregulation correlate with chronic immune activation and progression to AIDS, causality remains to be established. Many of these elusive questions can be best answered in NHP models where experimental conditions can be tightly controlled and there are opportunities for *in vivo* iNKT manipulation. Continuing to build the breadth of knowledge on this subject through longitudinal studies in humans and NHPs will lead to a full understanding of the capacity of iNKTs to regulate or contribute to disease in the context of HIV.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [Mucosal Immunity to HIV-1](#)
- ▶ [Nonpathogenic SIV Infection of Sooty Mangabeys](#)
- ▶ [Overview: Immunopathogenesis](#)

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Nonhuman Primate Models of HIV Transmission

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Definition

A nonhuman primate (NHP) model of HIV transmission is an experimental infection of monkeys with simian immunodeficiency virus (SIV), a virus that is closely related to HIV, or a chimera of SIV engineered to express select HIV genes (SHIV). NHP models are used to study the biology of HIV transmission and pathogenesis or to test new strategies for treatment and prevention.

Introduction

Because the NHP and human immune systems closely resemble each other, the search for an animal model to study HIV-1 infection in the early years of the epidemic focused on experimental infection of chimpanzees (*Pan troglodytes*). Although these studies provided initial key information about transmission, infection of chimpanzees rarely resulted in disease and did so only after more than 10 years of infection. During the early 1980s, outbreaks of immunodeficiency-associated diseases occurred in Asian macaques at multiple primate centers. The animals succumbed to neoplasms and opportunistic infections (OIs), paralleling the newly described human disease now known as AIDS. Thus, macaques became the NHP model of choice for the vast majority of HIV studies.

Nowadays there are several NHP models of HIV infection, which are characterized by the infection of different monkey species with different SIVs. These SIVs and the disease profiles they manifest in the different species exhibit specific traits that enable the study of particular aspects of HIV transmission and pathogenesis. The most

common species used for these models are Asian macaques (*Macaca* species) – *M. mulatta* (rhesus; RM), *M. nemestrina* (pigtailed; MNE), and *M. fascicularis* (cynomolgus). Disease in these animals reproduces the disease course in humans, including acute and then progressive loss of CD4⁺ T cells followed by clinical immunodeficiency, OIs, and neoplasms. Sooty mangabeys (SMs) and African green monkeys (AGMs) are two other species that exhibit a nonpathogenic infection and have been studied to better understand disease progression.

During the more than 20 years of their use, NHP models have dramatically increased our knowledge of (i) the transmission of immunodeficiency viruses across mucosal barriers, (ii) the definition of relevant challenge viruses via different routes, (iii) the timing of viral dissemination, (iv) the establishment in the newly infected host of quiescent sources of infectious virus (reservoirs), (v) the quality and magnitude of innate (00151) and adaptive immune responses (00152-3), and (vi) the protective effect of candidate vaccines and topical microbicides. Herein we will describe (i) the similarities and differences between HIVs, SIVs, and SHIVs, (ii) the importance of these models in the study of the early events after transmission, (iii) the continuous striving to create and utilize a model that recapitulates the modalities of transmission and pathogenesis in humans, (iv) the models of coinfection with other sexually transmitted infections (STIs), (v) the pathogenic versus nonpathogenic models that provide insight into natural mechanisms of protection, and (vi) the use of these models in vaccine and microbicide testing.

SIV and SHIV Versus HIV

SIVs exhibit similar genomic organization and genetic sequence homology (53% nucleotide identity) to HIV (Courgnaud et al. 2002). The SIV is composed of the same three major genes *gag*, *pol*, and *env*, which are responsible for the structural (capsid, nucleocapsid, matrix), enzymatic (reverse transcriptase [RT], protease, integrase), and virus entry (envelope [Env])

proteins, respectively. Several accessory genes are also common to both viral lineages; HIV and SIV express the *tat*, *rev*, *vif*, *vpr*, and *nef* genes. However, only SIVcpz and HIV-1 express *vpu*, while SIVsm, SIVagm, and HIV-2 express *vpx*. Disparate *vpu/vpx* expression has been implicated in varied pathogenesis.

Like HIV, SIV replicates predominantly in CD4⁺ T lymphocytes but also infects dendritic cells (DCs) and macrophages. DCs can capture infectious HIV/SIV and transfer it to CD4⁺ T cells and also can become infected, synthesizing new virions, which are then transferred to CD4⁺ T cells. Thus, DCs play an important role in amplifying HIV and SIV infection and macaque DC biology largely mimics that of human DCs (Teleshova et al. 2013).

Both HIV and SIV are transmissible through mucosal (vaginal, rectal, oral) and intravenous routes. Mimicking the HIV early stages of infection, within 2–4 weeks after mucosal transmission (acute phase), SIV viremia peaks in the plasma (10^8 – 10^9 RNA copies/ml) and then typically resolves to a set point (10^4 – 10^6 RNA copies/ml). This coincides with the detection of virus-specific antibodies (Ab), CD4⁺ T cell, and CD8⁺ cytotoxic T lymphocyte (CTL) responses in peripheral blood and is maintained throughout most of chronic infection. Therefore, both the replication rate of SIV and the immune response to the virus are similar to HIV in particular in the pathogenic models of macaques.

Although the course of SIV infection (00191) closely resembles that of HIV infection in humans, the testing of prevention methods and therapies in the SIV model is hampered by the still large genetic diversity between HIV and SIV. Genetic diversity can translate into important differences in the structures of the proteins identified as potential targets of vaccines and antiviral compounds between SIV and HIV, and thus vaccine-elicited immune responses to HIV and other strategies to prevent HIV transmission are specific to the HIV molecules they target. To overcome these difficulties, chimeric viruses were generated that contain relevant pieces of the HIV genome in an SIV background. These SHIVs exhibit similar biology (host cell types,

transmission, viremia) to SIV and HIV, and they have become crucial models for research into novel HIV prevention strategies. The SHIV-RTs encode the RT of HIV in an SIV genetic background and are particularly useful to test the efficacy of prevention strategies based on anti-retrovirals (ARVs) that target the RT of HIV.

SIVs encoding the HIV Env protein gp160 (or its subunits gp120 and gp41) are used to test compounds that aim to block the interaction of the surface subunit gp120 with receptors on the target cell surface as this is a necessary step for infection. Such SHIVs are also important for the development and testing of vaccines because most vaccine strategies aim to generate a neutralizing Ab (nAb) response, which is directed solely against Env.

Notably, SHIVs bearing recombinant gp120s with specific characteristics are potentially powerful tools to study characteristics of the Envs of transmitted viruses. A striking feature of HIV sexual transmission, which is a particularly rare event, is that while a genetically diverse population of viruses (called a quasispecies) exists in an HIV-infected person, only one (the founder/transmitted virus, T/F) or a select few of those variants infect their partner during sexual transmission. F/T viruses appear to share common characteristics in their Envs such as shorter length of the gp120 variable loops and fewer sites of sugar moiety addition (potential N-linked glycosylation sites, PNGs) than chronically replicating isolates (Parrish et al. 2013). Importantly, as for HIV, mucosal SIV infection proceeds through a similar “genetic bottleneck” involving a T/F virus expansion. Generating SHIVs based on T/F Envs may allow scientists to study the contribution of these characteristics to viral fitness during transmission.

Early Events During Transmission

Sexual mucosal transmission (rectal or vaginal) is the most common route of HIV transmission. Although it has been the focus of many studies, there is still a lot to learn about how the virus penetrates mucosal barriers and establishes a systemic infection. This knowledge is key to the

design of new ways to block infection before the virus spreads and the immune system starts to fail. Although it would be unfeasible and unethical to study the early events following sexual exposure to HIV in humans (as this would require sampling the mucosal tissue a few minutes or hours after sexual intercourse), it is possible to expose NHPs rectally or vaginally to a determined amount of virus, mimicking as much as possible the events occurring in humans. In doing so, we can study how the virus interacts with the mucosal microenvironment and what are the characteristics of the host and/or the virus that facilitate infection. With this experimental model, it is possible to collect samples of tissues and blood at time points (hours or days) much closer to the time of exposure than is possible in humans. Blood, tissues, and lymph nodes (LNs) collected during this early phase have informed on the timing required for the virus to replicate in the mucosa, travel to the draining LNs where it finds an abundance of target CD4⁺ T cells to expand, and disseminate systemically. Moreover, it is possible to identify the cells that are preferentially targeted in the early phase and study their phenotype and the differential role of specific cell types in spreading infection. This information guides the design of targeted approaches to block viral replication immediately after exposure (Haase 2010).

Using NHPs, it was possible to determine that sub-activated “resting” CD4⁺ T cells are likely the first cells that the virus infects following sexual exposure. However, these cells do not allow the rapid viral replication achieved in activated memory CD4⁺ T cells. Therefore, the latter cell type, although less abundant, may be the major source of virus during the early stages (Haase 2005). Moreover, it was possible to determine the important role of DCs in the mucosa and draining LNs. In fact DCs capture the virus and transfer it to neighboring T cells and in this way facilitate infection. Moreover, DCs are permissive to viral replication and produced virions can also be transferred to T cells, a process that can fuel infection especially in the draining LNs. The NHP models also helped to identify the gut tissue and the gut-associated lymphoid tissue (GALT) as the first and primary reservoir of viral infection.

In fact the gut and the gut-draining LNs contain the highest concentration of HIV and SIV target cells and favor exponential virus growth. Another advantage of using NHP to study early events after transmission is the ability to culture tissue biopsies (rectal or vaginal) and examine viral replication in the tissues *ex vivo*. Such studies allow the investigation of virus replication in differentially conditioned mucosal microenvironments.

Studying the early events following SIV infection allows the study of viral and host-related characteristics that increase the chances of this event to occur. Identifying what renders some animals more susceptible than others can help to identify those characteristics of the human rectal and vaginal tissue that increase the risk of acquiring HIV (00386).

Low-Dose Challenge Models

In the initial transmission studies using NHPs (up until the early 2000s), the animals were exposed to large doses of pathogenic SIV to ensure that all macaques in a study became infected. However, it became evident that this type of challenge did not recapitulate the early events after mucosal transmission in humans, among them the fact that sexual HIV transmission is a relatively rare event and there are specific (mostly unknown) factors or combinations of factors that allow this event to occur. Challenging with a high dose of virus masks these factors and does not mimic repeated viral exposure, which is more physiologically relevant. Exposure in humans is, in most cases, a recurrent event and a NHP model that includes a single exposure is far from mimicking these more realistic situations, but this approach did provide some first critical insights into the earliest events of HIV infection across the mucosae. To cope with these differences, most laboratories that use NHP models of HIV infection are starting to infect the animals using “low-dose repeated challenge models.” These models have several advantages, which go beyond the fact that they mimic a real-life situation. They are commonly used to assess the efficacy of HIV vaccine candidates or preventive

treatments and consist of challenging monkeys repeatedly with low doses of SIV or SHIV. Such challenge data provide a unique opportunity to assess the importance of exposure history for the acquisition of infection.

Reaching statistical power is one of the major issues in NHP models. The animals are costly and their use has ethical considerations; thus, there is a need to limit the number of animals to a minimum, which can make it difficult to interpret the data. Repeated low-dose challenge models have the benefit of increasing the statistical power of a study several folds since every challenge that does not result in infection adds one animal to the study. This is possible since repeated challenges do not immunize the animals (Regoes et al. 2005).

Although repeated low-dose challenge models have many important advantages, they also have several caveats. Infecting all control animals requires a longer period of time than the single high-dose studies since fewer animals become infected at each challenge. In a typical setup of the repeated low-dose model, the animals are challenged once or twice per week with a low viral dose. However, the dose, or viral titer, used for the challenge can vary even of 10–20-folds, depending on the animal species, the site of inoculation (rectal or vaginal), and the virus used. Generally, depending on the question that is asked, the studies are stopped when all the control animals get infected. A different way of setting up the repeated low-dose model is to set the total number of challenges at the beginning of the study. This can vary from 10 to more than 20 and therefore the studies can last 5–6 months.

In performing statistical analyses on the efficacy of a preventive strategy to reduce susceptibility, whether using a high- or a low-dose model, it is important to take into account the animal-to-animal variation in susceptibility. This seems to be higher in some species such as RMs than in others such as pigtailed macaques and could be explained by the differential susceptibility of the two species (with the pigtail macaques being more susceptible). Finally these studies are also relevant for understanding the role of exposure history on HIV acquisition and forecasting the epidemiological spread of HIV (Regoes 2012).

Interaction with Other STIs

The risk of HIV sexual transmission is higher if one of the partners has another STI (also called a coinfection or co-pathogen). In particular, HSV-2-infected individuals are at least three times more at risk of acquiring HIV than HSV-2-uninfected individuals (Wald and Link 2002). Although part of this increased risk can be explained by the presence of active HSV-2 replication (which can result in ulcers and inflammation), the increase in risk is present even after the infection abates and in the absence of detectable inflammation. Therefore, there is a lot that remains to be explained on what renders HSV-2-infected individuals more at risk of acquiring HIV and what are the characteristics of the mucosal microenvironment of such individuals that facilitate HIV infection. Knowing these factors could help not only to find ways to block the HSV-2-driven increase in susceptibility to transmission but also to understand HIV transmission in the absence of other STIs.

The first model of vaginal HSV-2 infection and HSV-2/SIV coinfection in NHPs was established in Melissa Robbiani's laboratory in 2009 (Crostarosa et al. 2009). Since then, a model of HSV-2 rectal infection of RMs was also developed in Dr. Robbiani's laboratory (Martinelli et al. 2011) and several other laboratories are adopting models of HIV/STI coinfection. The Center for Disease Control is using pigtailed macaques to study *Chlamydia trachomatis* and *Trichomonas vaginalis* (Henning et al. 2011). These models are useful to test the effect of prevention strategies in circumstances of heightened susceptibility to HIV. They also allow exploration of the interaction of HIV and SIV with other pathogens in the mucosa, how the immune response to a pathogen affects the response to HIV and SIV, and how other pathogens modify the mucosal microenvironment in a way that increases its susceptibility to HIV and SIV. These models may consist of (i) co-challenging the animals with the two pathogens simultaneously (in a single or repeated exposure setting) or (ii) infecting the animals first with the co-pathogen and then with SIV/SHIV. Both of these experimental conditions are valuable as they mimic real-life circumstances of transmission in

discordant couples in the presence of other STIs, which is a very common scenario in people at high risk of HIV acquisition. These models also allow the testing of preventative strategies for their ability to (i) prevent SIV/SHIV in the face of another STI and/or (ii) prevent the STI itself.

Pathogenic versus Nonpathogenic Models

SIVs have coevolved with their natural host species for thousands of years. Unlike HIV infection of humans, when these African monkeys are infected with their species-specific SIV, either in the wild or in captivity, the virus does not cause disease. In contrast, SIV infection of Asian macaques causes a simian acquired immunodeficiency syndrome (SAIDS) that resembles AIDS in humans though it progresses faster (~ 2 years vs. ~ 10 years). SAIDS is characterized by $CD4^+$ T cell depletion, [► Chronic Immune Activation in HIV](#), the acquisition of OIs, and premature death.

SMs and AGMs are two species of African monkeys used as models of natural infection because when infected with SIV, they do not develop SAIDS and remain healthy. The first pathogenic model of infection in macaques was generated by the inoculation of RMs with a virus isolated from SMs (SIVsm). The SIV isolated from RMs infected with SIVsm (termed SIVmac) was found to be highly pathogenic in different species of macaques. SIVmac infections of RMs, MNEs, and cynomolgus macaques are the principal pathogenic models of HIV transmission and disease. Since the isolation of the first SIVmac, several other isolates (quasispecies) of SIVmac have been generated by inoculating macaques with different strains of SIVsm and isolating the virus at different times following infection.

The infection profile during acute SIV infection is strikingly similar between natural and experimental hosts (and HIV-infected humans): the major initial targets of infection are $CD4^+$ $CCR5^+$ T cells, and the virus replicates to high titer, resolving to a set point concomitant with the induction of T cell responses (00152-3). Mucosal $CD4^+$ T cells are extensively depleted during

acute infection, in particular in the gut tissue and LNs. Adaptive immune responses consisting of virus-specific nAbs and CTLs (00151-4) are elicited but do not effectively control infection in either case. On the other hand, a higher innate antiviral response and in particular a higher, more prolonged production of the antiviral cytokine and interferon- α (IFN- α) by a subset of DCs seem to be more a characteristic of the pathogenic models. This has been implicated in determining the different course of infection during the chronic phase.

More extensive studies of natural SIVsm and SIVagm (virus isolated from AGMs) infection have revealed other key differences between natural and experimental SIV (and HIV) infection that provide clues to the determinants of pathogenesis (Chahroudi et al. 2012). In natural hosts, the innate immune response resolves rapidly during the transition from the acute to the chronic phase (approximately 4–8 weeks postinfection), and [► Chronic Immune Activation in HIV](#) is absent. Mucosal $CD4^+$ T cells recover from their early depletion and their presence is responsible for the balancing act of mounting inflammatory responses against extracellular bacteria and fungi while protecting the tissue from inflammation in response to commensal bacteria and food antigens. The integrity of the gut mucosa is maintained and [► microbial translocation](#) (the migration of bacterial products from the gut into the blood across a compromised epithelium) is noticeably absent. LN architecture is preserved. The fraction of $CD4^+$ T cells responsible for long-lived memory responses (central memory), which is the preferential target of pathogenic SIVs, is not a major target of nonpathogenic SIV infection. Instead in natural hosts effector memory $CD4^+$ T cells (that are short lived even in the absence of infection) are preferentially infected (Chahroudi et al. 2012).

Mother to infant transmission (MTIT) is also different in natural and experimental infection. Whereas MTIT of HIV is quite efficient (35–40% for MTIT in absence of antiretroviral therapy vs. $<1\%$ per sex act), it is rare in natural hosts ($<7\%$ for SMs, even less for AGMs), and the availability of $CD4^+$ $CCR5^+$ target cells has been implicated.

Putting together these pieces of the puzzle, scientists are defining a model of pathogenicity linked to the events and the immune responses (00151-4) during the earliest stages after transmission (00383). The factors identified through these models that may play a role in determining the disease progression relate to the integrity of the gut mucosal tissue, the lymphocyte subsets that populate it along with restricted infection to short-lived effector cells, and the absence of vertical transmission.

Vaccines and Attenuated SIVs

The success of early vaccines for epidemic viral diseases such as smallpox, rabies, measles, and polio was aided by the fact that virus eradication was linked to the elicitation of vigorous specific immune responses, which helped to identify correlates of protection. The nature of naturally occurring protective immunity to HIV is still mostly unclear. Nonetheless, the development of an effective vaccine is a central goal of HIV research.

To this end, NHP models of HIV infection have been critical for dissecting the initial immune response after transmission and why it fails, and for developing strategies to boost this response. SIV and SHIV challenge models have been exceptionally useful to test an array of vaccine strategies – live attenuated (LAVs), inactivated, protein subunit, DNA, and viral vector vaccines, as well as combinations (e.g., DNA prime protein boost). Because LAVs are actually replicating (but weakened) strains of virus, they have the best chance of recapitulating all the classical desired immune elements – nAbs to block infection, CTLs to kill residual infected cells at transmission, CD4⁺ helper T cells to boost nAb and CTL production, and immune responses specifically localized to the sites of transmission and replication (genital and gastrointestinal mucosa).

One particularly important model for HIV vaccine design is the inoculation of RMs with *nef*-deleted SIVmac (SIVΔ*nef*). SIVΔ*nef* results in a highly attenuated infection that protects against future challenge with wild-type (WT) SIV although the protection is lower or

absent against different genetically distant strains (Freissmuth et al. 2010). The immune correlates of this protection have been difficult to elucidate. The degree of attenuation of the vaccine strain is inversely correlated with the level of protection from WT (e.g., a triple deletion mutant is more attenuated and less protective than SIVΔ*nef*). Neonatal macaques with lower immune capabilities are not protected from SIVΔ*nef*. However, the bulk of the data show no discrete link between immune responses and protection. Protection is achieved in the absence of a strong nAb response to the challenge virus. Antibody transfer from SIVΔ*nef* inoculated macaques into naïve macaques does not protect them from WT. Infected animals generally exhibit intact T cell function, making SIV-specific CTL responses that are superior to those in WT-infected macaques, but the data are inconclusive as to whether SIV-specific cellular responses are sufficient for protection from pathogenesis (Freissmuth et al. 2010).

To evaluate protection by vaccine candidates that elicit nAbs against the HIV Env, researchers challenge macaques with SHIVs expressing different HIV Env proteins. Which SHIV to use has been the subject of much debate over the years, but scientists generally agree that the most relevant virus should (i) contain Env from a primary rather than a lab-adapted isolate, (ii) use the CCR5 coreceptor, and (iii) be mucosally transmissible. There is enormous diversity in the genetic sequence of the HIV Env among different subtypes (clades) of virus. Early SHIVs were generated based on Env sequences from clade B Envs, those common to North America and Europe. New SHIVs are being developed that express Envs from clades A and C, which are the most prevalent clades in Africa. Ultimately, designing vaccines that target conserved regions of Env requires careful iterative engineering, and after 30 years of research, a strong candidate remains elusive.

Microbicide Testing

Microbicides (00378) are topical agents, which are applied intravaginally or intrarectally and are designed to prevent the ► [HIV-1 sexual](#)

transmission of HIV. They contain diverse active pharmaceutical ingredients (APIs) – anti-retrovirals (ARVs) and nonspecific inhibitors/immune modulators – formulated in easy-to-use products such as gels or intravaginal rings (IVRs). Most microbicide studies in macaques utilize SHIV models because the viral protein targets of microbicides (thus far predominantly RT and Env) differ enough in genetic sequence between HIV and SIV making HIV-specific inhibitors less effective against SIV. To study RT inhibitors, SHIV-RT is usually used (HIV RT in SIV background). To study entry inhibitors (those interfering with Env function), SHIVs expressing different HIV Envs are used with SHIV_{SF162P3} being the most common.

Macaque studies of a gel comprising 1% of the RT inhibitor tenofovir (TFV) demonstrated that this product prevented both vaginal and rectal SHIV infection, providing the data necessary to move 1% TFV into clinical trials where it was partially effective against vaginal acquisition of HIV in studies demonstrating good adherence to the regimen (Abdool Karim and Baxter 2013).

Moreover, studies with NHP models have enabled scientists to determine the strength and duration of protection of products. These characteristics may help generating a product that is somewhat independent from how it is used by individuals (adherence), a key factor in the success of microbicide strategies. By challenging with high doses of virus and with viruses of varying virulence, it is possible to gauge the limit of protection. To establish the window of protection, animals are challenged at different times following vaginal or rectal application – i.e., does a product have to be applied just prior to exposure or could it be applied up to a day (or days) before, and can it be used repeatedly and still remain safe and effective? The ultimate goal is to develop products that can be applied under diverse scenarios that appeal to the women and men using them and offer the same level of protection (e.g., sporadic use, long-lasting protection, lack of toxicity if used frequently, etc.). For example, these types of studies were used to demonstrate that a promising microbicide gel, MZC (containing the anti-retroviral drug MIV-150, zinc acetate, and the

seaweed derivative carrageenan developed by Population Council), protects against vaginal SHIV-RT challenge providing up to 8 h of full protection when used once or daily for 2 weeks (Kenney et al. 2012). They also showed that MZC prevents rectal SHIV-RT infection but only when it is applied within an hour of challenge as protection against rectal infection wanes quickly with increasing time between application and challenge.

ARV-based microbicide approaches are dependent on the presence of therapeutic levels of functional drug in the mucosal epithelia. Pharmacokinetics (PK) and pharmacodynamics (PD) studies are crucial for identifying the presence of drug in vivo (PK studies) and its activity against virus (PD studies) in order to draw correlations between drug level/activity and protection. Although PK studies testing for API in the blood can be carried out easily in humans, tissue PK and PD studies are more invasive, and the macaque model enables extensive testing. Moreover, tissues can be collected for ex vivo PD analysis. However, while informative, there are some limitations to the ex vivo approaches since perfect mimics of in vivo efficacy are hard to establish (i.e., there is no dynamic environment; continued drug delivery postexposure cannot be mimicked).

By targeting specific steps in the viral life cycle, microbicides can cause the virus to develop or select for drug resistance mutations. This is of particular concern if an infected person unaware of her infection status uses the product. In these individuals the virus would be replicating under constant drug pressure. Drug resistance studies are carried out in macaques treated with different microbicides under a variety of conditions (e.g., infected animals vs. uninfected animals) to determine the likelihood that resistance will develop.

More Opportunities, Problems, and Pitfalls

In light of the complexity of the circumstances in which transmission can occur in humans and the consequent differences in susceptibility to HIV infection, new NHP models of transmission are

being developed. These include models of coinfection with other STIs and, more recently, models that try to mimic the hormonal environment of women at different stages of the menstrual cycle or women who use different contraceptive strategies. Although it has been known for several years that sex hormones influence the susceptibility to vaginal (and possibly also rectal) transmission, our understanding of the mechanism and degree of the hormones-linked increased risk of infection is lacking. This is particularly relevant for the widespread use of hormonal contraceptives by women in developing countries and especially those in groups already at risk of HIV acquisition. To evaluate the role of hormones in NHPs, progestins, estrogens, or combinations of these two hormones that mimic specific contraceptive strategies or different stages of the menstrual cycle are injected into NHPs. Ovariectomized animals can be used to isolate the effect of the single hormones and mimic the postmenopausal condition.

However, although particularly useful, NHP models of HIV transmission have several limitations especially in testing the safety and the efficacy of prevention strategies. On the one hand, macaques are a better species in which to test vaccines than other mammals. The structure of their Abs is very close to that of humans, enabling the animals to develop the types of Abs that have been shown to have the best neutralizing activity. Nevertheless, the immune system of macaques is not identical to the human immune system, and several vaccines that elicited protective immune responses in macaques did not demonstrate the same efficacy in humans. Subtle differences may prove to be important in developing a vaccine that confers complete protection.

Importantly, in the case of microbicide testing, macaque studies cannot predict adherence to a product and a regimen. Therefore, NHP studies need to be complemented with behavioral studies to identify the vehicle and administration strategy that promises to be most widely used.

Finally, the substantial differences in the menstrual cycle of macaques and humans render particularly challenging the studies that aim to understand the effect of hormones on susceptibility to sexual transmission. They impair studies of microbicides

and vaccine efficacy since monkeys breed seasonally, their susceptibility to infection can be heavily influenced by the season in which the study is performed. Moreover, the differences between humans and macaques in their menstrual cycle substantially limit the ability to test products with dual function as contraceptives and microbicides.

The difficulty in interpreting the results of macaque studies is evident when considering that they have already failed to predict the results of several clinical trials.

Conclusion

Although not perfect, NHP models are the best tool scientists have to study how the virus penetrates mucosal barriers, how it establishes infection, and what are the correlates of susceptibility and protection. Ultimately NHP models constitute the best system to test prevention strategies. Moreover, the data generated with these models have helped our understanding of the immune response to pathogens other than HIV and ultimately will guide more innovative strategies to tackle the virus at its portal of entry.

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(IDU) involves piercing through the skin into the body with a needle and a syringe. IDU may be intravenous, intramuscular, or subcutaneous. NIDU may involve prescription medications such as opioids or illicit drugs like alkyl nitrite (“poppers,” most often amyl nitrites), marijuana, crack or powdered cocaine, methamphetamines, and multiple drugs (Qian et al. 2011). Excessive alcohol consumption is often referred to separately from NIDU but is clearly in the same category. Also associated with disinhibition and high-risk sexual behavior is the frequent use of erectile dysfunction (ED) drugs, popular among men who have sex with men and other men who may seek multiple partners and commercial sex workers.

NIDUs may be defined as individuals who have a history of non-injection use of any of the abovementioned substances but no history of IDU (the latter would hierarchically preempt NIDU since its HIV risk is higher and since IDU almost invariably are also NIDU). Hence, persons who do not inject drugs and are recognized to use substances with or without dependence, and/or have entered any drug or alcohol rehabilitation program, and/or have any medical, legal, or social problems due to drug or heavy alcohol use, can be classified as NIDU (Qian et al. 2011).

Epidemiologic Overview

According to *World Drug Report 2013* (United Nation Office on Drugs and Crime 2013), the prevalence of illicit drug use in 2010 was between 3.6% and 6.9%, representing 167–315 million people aged 15–64 who used illicit substances at least once in 2010. Data suggested that the estimated number of drug-related deaths was 211,000, occurring mostly amid the younger population. Illicit drug use continues to jeopardize the health and welfare of populations all over the world, and HIV/AIDS is typically overrepresented in this population. Although having apparently declined a bit compared to 2008, an estimated 1.6 million people between 15 and 64 years of age are still using illicit drugs while living with HIV/AIDS. Evidence suggests that

Non-injecting Drug Users, Epidemiology of HIV/AIDS

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Definition

Non-injection drug use (NIDU) is a term used to describe substance use through oral ingestion or nasal inhalation. In contrast, injection drug use

IDU accounts for an increasing proportion of new HIV cases in Eastern Europe, South America, and East and Southeast Asia (Mathers et al. 2008). NIDU is common among HIV-infected individuals and may contribute to a substantial proportion of new HIV cases worldwide through sexual disinhibition (Kipp et al. 2011). Whether the drugs contribute to HIV risk behavior or whether drug use is simply a component of a high-risk lifestyle that would be extant even in the absence of drug availability has been debated.

HIV and Drug Use Pattern by Continent

Africa

Over a third of the infected persons globally live in just ten nations of southern Africa, by far the worst afflicted region. South Africa, for example, was the world's 25th most populous nation with 49 million persons in mid-2009 (U.S. Census Bureau 2014). Yet it ranked first for the number of persons living with HIV/AIDS with an estimated 5.6 million persons (range 5.4–5.9 million) in 2009. The sub-Saharan African epidemic is mainly driven by heterosexual contact (Vermund et al. 2009; Eyawo et al. 2010). Injection drug use (IDU) and male-to-male sexual contact may be increasing and represent future risk (Vlahov et al. 2010; McCurdy et al. 2010; Smith et al. 2009; Baral et al. 2011; Rispel et al. 2011; Merrigan et al. 2011; Johnston et al. 2010; UNAIDS 2014).

In West Africa, HIV prevalence is lowest in Senegal and highest in Nigeria. Compared to South Africa's adult HIV prevalence (17.3%), Nigeria's is much lower (3.7%). Yet as Africa's most populous nation, Nigeria has Africa's second largest number of people living with HIV. A principal driver of infection in the region may be commercial sex. Non-injection drug use, such as cocaine, is increasing with traffickers who traverse West Africa also marketing their drugs locally in the transit cities. For example, reports from Dakar, Senegal, indicate that consumption of cocaine and crack has increased, even as heroin use had declined since 2000.

In East Africa, HIV infection prevalence is moderate to high. Heroin is the most commonly

used substance with an especially severe problem in Zanzibar. Other commonly used substances include cannabis and alcohol (United Nation Office on Drugs and Crime 2013). The East Africa epidemic is similar to southern Africa, largely driven by heterosexual transmission (with accompanying maternal-to-child transmission). The epidemic in Central Africa is not as robust, also driven by heterosexual transmission. The role of unsafe needle/syringe or razor reuse by allopathic or traditional health personnel is also potentially contributing to transmission in sub-Saharan Africa. Alcohol use is prevalent and associated with increased risk taking.

In North Africa and the Middle East, sexual transmission among MSM accounts for a majority of HIV infections (Mumtaz et al. 2010). Information on drug use is available from Algeria and Morocco; prevalence of drug use is low in Algeria, but a documented increase in cocaine use has been noted in Morocco (United Nation Office on Drugs and Crime 2013). Iran has the region's highest injection heroin problem, and there are many non-injection heroin users as well (Khajehkazemi et al. 2013; Navadeh et al. 2013; Malekinejad and Vazirian 2012; Zamani et al. 2010; Farnia et al. 2010).

Americas

The HIV epidemic in North America has been driven by MSM, illicit drug use, and, to a lesser extent, heterosexual transmission (Vermund and Leigh-Brown 2012). Sexual risk taking is documented to be higher in persons using alcohol and/or illicit drugs proximate to sexual activity, including receptive anal intercourse (Shoptaw and Reback 2007; Colfax and Shoptaw 2005; Miller 2003). Party drugs such as nitrates, amphetamines, and cocaine have been associated with higher-risk sexual activity, an observation dating from very early in the HIV/AIDS epidemic (Goedert 1984; Drumright et al. 2006; Wooff and Maisto 2009). Persons addicted to crack-cocaine and methamphetamine may practice high-risk sex, and both women and men may sell sex for drugs or money to support drug habits (Wilson and DeHovitz 1997; Corsi and Booth 2008; Zierler and Krieger 1997; Norris et al.

2004). Adolescents are vulnerable to peer pressures to have sex and use drugs (Senf and Price 1994; Fortenberry 1998).

There is diversity in the south and Central American epidemic, but the dominant mode of transmission seems to be anal sex among MSM (McGowan et al. 2007; Caceres 2002; Coplan et al. 1996; McCarthy et al. 1996; Bastos et al. 2008). PWID contributes substantially in some regions such as urban Brazil but is a less common risk factor than in North America, Europe, or Asia (Lima et al. 1994; Hacker et al. 2005; Rodriguez et al. 2002; Caiaffa et al. 2003; Khalsa et al. 2003; Mesquita et al. 2003). Alcohol and non-injecting drug use fuel unsafe sexual behaviors (Bastos et al. 2007; Damacena et al. 2011; Cortez et al. 2011; Anastario et al. 2011; Bassols et al. 2010; von Diemen et al. 2010). Heterosexual transmission occurs and bisexual men are thought to be an important bridge population (Ramirez et al. 1994; Konda et al. 2011). Perinatal transmission is less common with most antenatal programs in endemic areas offering HIV testing and ART, albeit imperfectly (D'Ippolito et al. 2007; Read et al. 2007).

The Caribbean is the region with the second highest prevalence rates in the world, after sub-Saharan Africa (Figueroa 2003, 2004, 2008; Calleja et al. 2002; Inciardi et al. 2005). Heterosexual transmission, homosexual transmission, and illicit drug use (particularly IDU in Puerto Rico) are all dominant factors for the HIV epidemic in this region (Castro and Farmer 2005; Pape and Johnson 1993; Dorjgochoo et al. 2009). It is not known the extent to which non-injected drugs that are used with high prevalence may contribute to sexual risk, such as marijuana, but alcohol use in the context of "sun-and-fun" tourism cultures are very likely to increase sexual risk taking.

Europe

Non-injection drug use is prevalent in Europe, and its contribution to HIV spread is, as elsewhere, thought to be mediated by sexual disinhibition. People who inject drugs (PWID) may use non-injection drugs as well, such that sexual risk may persist even as IDU-related risk reduction

progresses. Eastern European countries, notably Russia, that have taken a punitive approach to IDU without concomitant public health (needle exchange) and medical (opiate substitution therapy) approaches continue to have growth in their epidemics, even as prevalence rates stabilize or even decline elsewhere in the world (Kelly and Amirkhanian 2003). Except for the prevalence of heroin use which is estimated to be 1.2% of the adult population, other patterns and types of illicit drug use in Eastern Europe are lower compared with global levels (United Nation Office on Drugs and Crime 2013).

Male homosexual transmission has been a principal driver of the epidemic in Western Europe, but IDU has also been a major contributor, particularly in Southern and Eastern Europe (Mathers et al. 2010). The HIV epidemic in Western Europe is similar to that in North America in many ways, though it is less intense, possibly due to more aggressive public health responses to HIV early in the epidemic (Vermund and Leigh-Brown 2012). The use of all illicit substances in Western Europe is higher than the average for the rest of the world, particularly with use of methamphetamine and cannabis (7.6% of the adult population). The proportion of persons using cocaine in a year was 1.2%, nearly threefold the global average (United Nation Office on Drugs and Crime 2013).

Asia

The HIV epidemic in East and Southeast Asia, and other parts of the region, is a complex one. While less intense than in Africa, the Asian epidemic remains intractable with persistent transmission in vulnerable populations. In Southeast Asia and Indonesia, heterosexual, MSM, and PWID transmission are all prevalent (Vlahov et al. 2010; van Griensven and de Lind van Wijngaarden 2010; Sharma et al. 2009; Ruxrungtham et al. 2004; Couture et al. 2011). In countries like Thailand and Cambodia, successes are notable in reducing transmission among PWID and heterosexual transmission with needle exchange and universal condom advocacy, respectively (Park et al. 2010; Ainsworth et al. 2003; Celentano et al. 1998). China has had some progress in addressing its

serious problem of HIV among PWID, but the epidemic among MSM is rising; fortunately, heterosexual spread is not common in the world's most populous nation, even among sex workers (Wu et al. 2007; Xiao et al. 2007; Tucker and Cohen 2011). In South Asia, India has experienced a substantial heterosexual epidemic concentrated in its southern states, with PWID-related transmission in northeastern states; the northern states have much lower incidence and prevalence (Chandrasekaran et al. 2006). Despite prevalence rates far lower than in sub-Saharan Africa, India, the second most populous nation on the globe, ranks just behind South Africa and Nigeria for the highest number of HIV-infected persons (about 2.5 million in 2009) living in the nation (Dandona et al. 2006). MSM are at risk throughout South Asia, including *hijras*, who are men who dress as women and have a long-standing cultural niche in such nations as India, Pakistan, and Bangladesh (Siddiqui et al. 2011; Sahastrabuddhe et al. 2012; Shaw et al. 2011; Phillips et al. 2010; Solomon et al. 2010; Verma et al. 2010). In Central Asia, the pattern of HIV transmission is similar to Eastern Europe, which is mostly through IDU, though homosexual and heterosexual also account for HIV epidemic in this region (Mathers et al. 2010).

Little is known about the role of non-injection drug use in Asia in driving the HIV epidemic. Countries like Thailand that have had tremendous reductions in HIV spread among both PWID, and high-risk heterosexuals still have large epidemics among MSM. It is likely that non-injection drug use helps fuel high-risk behaviors, including alcohol use.

Australia and Oceania

Australia has had a persistent and aggressive risk reduction program from the early days of their epidemic (Mao et al. 2011; Kang et al. 2010; Jones et al. 2010; Minichiello et al. 2001). Their widespread and "user-friendly" approaches to clean needle exchange and availability of opiate substitutions therapy through primary care practitioners are credited for keeping HIV rates exceedingly low (Miller et al. 2009; Falster et al. 2009; Maher et al. 2007; Dolan et al. 2003). MSM is a

principal challenge in parts of the region, though Oceania and New Zealand confront pockets of heterosexual transmission (Vallely et al. 2010; Washington et al. 2008; Vanualailai et al. 2003; Corner et al. 2005). The prevalence of the use of most illicit drugs remains relatively high in the Oceania region, especially in Australia and New Zealand. High prevalence of some non-injecting drugs was reported in this region, for example, cannabis (10.9%), "ecstasy" (2.9%), and cocaine (1.5%) (United Nation Office on Drugs and Crime 2013).

Drug Use, HIV Risk, and HIV Disease Progression

PWIDs are at substantial risk of contracting HIV from the transmission of blood-borne HIV via needle sharing (Mathers et al. 2008). Non-injecting users increase their HIV infection risk via several mechanisms: (1) Impairing decision-making may result in high-risk sexual behaviors (Kipp et al. 2011; Cook and Clark 2005); (2) trading sex for drugs or for money to buy drugs may result in unprotected sex; (3) the immune-modulating effects of drugs may increase HIV viral load among infected persons and decrease host defenses to viral exposure among uninfected persons, which would upregulate HIV-specific cellular receptors (Kipp et al. 2011; Astemborski et al. 1994; Brewer et al. 2007; Cabral 2006; de Souza et al. 2002; Edlin et al. 1994; Ramirez-Valles et al. 2008). Extant literature has indicated that, specifically among crack-cocaine users, non-IDUs may experience elevated risk of STI and HIV compared with other drug use group, and IDUs, explicitly due to increase in numbers of sexual partner and sex trade, engagement in complex sexual networks, decrease in condom use, and having multiple concurrent partnerships (Khan et al. 2013; Celentano et al. 2008; Ross and Williams 2001). In addition, global evidence suggests alcohol use among MSM is particularly high in many venues, though not all (Deiss et al. 2013; Balan et al. 2013; Wirtz et al. 2013; Bruce et al. 2013; Newcomb 2013; Grov 2012; Liu et al. 2014). Like heterosexuals, MSM may use alcohol

for sexual arousal in anticipation of subsequent sex (Woolf-King and Maisto 2011). MSM may also experience stigma, psychological, and social-legal problems (Yu et al. 2013; King et al. 2008), for which alcohol may be used for relief or coping (Dyer et al. 2012, 2013; Jeffries et al. 2013; Gorbach et al. 2009).

Interesting to note is the literature on marijuana use. Cannabinoid agonists actually have anti-HIV properties and have been proposed as salutary immune modulators for infected persons, though the quality of evidence from human studies is inadequate to judge its merit (Hu et al. 2013; Lutge et al. 2013; Rom and Persidsky 2013; Kaplan 2013). At the same time, marijuana may be a sexual disinhibitor associated with higher risk.

Illicit drug use among people living with HIV/AIDS (PLWHA) is a medical and public health concern. HIV-infected drug users may not fully benefit from combination antiretroviral therapy (cART) (Kipp et al. 2011). Drug abuse comorbidities are associated with delayed access to, or reduced uptake of, treatment (Spire et al. 2007); worse adherence compared to HIV-infected subgroups (Hinkin et al. 2007); and inferior virologic and clinical outcomes (Cook et al. 2008). Most published studies included PWIDs and clearly identify a positive association between drug use and adverse HIV disease progression outcomes (Spire et al. 2007; Grigoryan et al. 2009; Lert and Kazatchkine 2007). Non-IDU drug abusers may share similar biologic, social, and behavioral consequences (Kipp et al. 2011). However, data on non-IDU and HIV disease progression remain limited, and the findings are mixed. Non-IDU drug use has been associated with higher AIDS and all-cause mortality (Baum et al. 2009). Use of crack-cocaine independently predicts AIDS-related mortality, upregulation of immunologic and virologic markers of HIV disease progression, and more AIDS-defining illnesses among women (Cook et al. 2008). Crack-cocaine users have also been demonstrated to have more aggressive HIV disease progression and lower rates of cART adherence with postulated cocaine-related up-modulation of viral activity (Arnsten et al. 2002). However, an MSM study did not find any association between marijuana

and other recreational non-IDU drugs with HIV disease progression (Di Franco et al. 1996).

Conclusion

Non-injecting drug use is associated with an increased number of sexual partners. It is presumed that disinhibition contributes to this, but an alternative explanation is that persons seeking higher-risk sex are more likely to use drugs, when available, such that the behaviors are associated, but not causally. Clearly, persons addicted to drugs may be more vulnerable to HIV via trading sex or drugs or money for drugs and can be engaged by clients in especially risky sexual behaviors (Khan et al. 2013). Non-IDUs comprise a variety of populations, particular young adults, MSM, and sex workers. In the USA, racial and ethnic patterns have not suggested one group having much higher non-IDU than another. In a study in Atlanta, Georgia, for example, black MSM had even lower numbers of sexual partners and use of non-injection drugs than white MSM, even though MSM had higher HIV prevalence (Sullivan et al. 2014). While the patterns of non-IDU and their relative contributions to HIV transmission risk differ by regions and socio-demographic characteristics globally, research suggests that interventions to reduce non-IDU drug use could play a role in reducing subsequent HIV exposure. The NIH has highlighted a need to (a) reduce risk of drug-associated and sexual transmission HIV among injecting and non-injecting users; (b) develop and refine primary and secondary interventions for HIV-infected drug users; and (c) develop interventions to overcome structural and community-level barriers to accepting and implementing effective HIV prevention strategies (NIAIDS 2014). Comprehensive HIV prevention interventions for drug users must provide the information, skills, and support necessary to reduce the drug-related and behavioral risk. The effort requires cross-disciplinary partnerships including community health workers, government officials, policy makers, peers and peer organizations, and public health authorities to dissemination of prevention

interventions and evaluate their effectiveness (Khan et al. 2013).

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Non-neutralizing Antibody Responses and Protection Against HIV-1

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Definition

Non-neutralizing antibodies against conserved regions of the HIV-1 envelope glycoprotein (Env) appear early after infection, preceding neutralizing antibodies, and appear to persist for the life of the infected individual. Some non-neutralizing antibodies recognize internal Env epitopes that never become antibody accessible during either virus acquisition or virus production. Such antibodies are of no consequence to protective immunity against HIV-1. By contrast, it is clear that other non-neutralizing antibodies recognize Env epitopes that become antibody accessible during viral acquisition, virus production, or both. In this case, non-neutralizing antibodies can theoretically block transmission by several mechanisms including aggregation of virions, blocking epithelial transport of virions, Fc-mediated effector function, complement-mediated lysis, or various combinations of these mechanisms. A growing body of literature suggests that these responses contribute to protective immunity against HIV-1 transmission.

Neutralizing Versus Non-neutralizing Antibodies

There is strong evidence from passive immunization studies in nonhuman primates (NHPs) that antibodies can protect against transmission by neutralization alone, although protection can be stronger if Fc-mediated effector function is also present (Hessell et al. 2007; Burton et al. 2011).

Similar compelling evidence from passive immunization studies in NHPs is lacking for non-neutralizing antibodies. By contrast, the RV144 vaccine trial provides evidence that non-neutralizing antibodies specific for the C1 region of the outer envelope glycoprotein, gp120, of HIV-1 contributed to against HIV-1 transmission, possibly by antibody-dependent cell-mediated cytotoxicity (ADCC) (Haynes et al. 2012). Before going further, it is important to operationally define neutralizing and non-neutralizing antibodies. Neutralizing antibodies block infection of susceptible target cells *in vitro* without any additional cellular effector function, such as phagocytosis or ADCC. The mechanism may be the simple stoichiometric blocking of virion binding to the target cell or by interfering with virus entry at a post-binding step. By contrast, non-neutralizing antibodies fail to neutralize consistently by these mechanisms using standardized target cells. If they are protective, non-neutralizing must be capable of mediating effector activities such as virion aggregation, blocking virion transcytosis across epithelial cells, phagocytosis, ADCC, complement-mediated lysis and opsonization, or a combination of these mechanisms. There can be great variation among neutralization assay formats but there are now standardized, validated assays that provide good discrimination between neutralizing and non-neutralizing antibodies. Further, neutralizing antibodies can also possess effector activities, such as ADCC or phagocytosis, whereas non-neutralizing antibodies must rely solely on one or more effector functions for protection, which is a fundamental distinction between these two categories of antibody. The following section describes the sites at which non-neutralizing antibodies need to work and the range of effector mechanisms that can be tapped at these sites to prevent the transmission of HIV-1.

Where Do Non-neutralizing Antibodies Work?

It is useful to consider the general anatomical locations where non-neutralizing antibodies need

to work while recognizing that the distinct micro-environments will dictate the specific effector mechanisms required for protection. There are four routes of HIV-1 infection, parenteral, largely through injection drug use; vaginal and anal, through sexual intercourse; and oral or gastrointestinal, through breast-feeding. Surprisingly little attention has been paid over the years to the mechanisms of parenteral infection and this area is in need of renewed scrutiny. For this reason, this route will not be considered further. The other three routes share the commonality of being mucosal and they will be discussed with respect to their shared features.

To a first approximation, each route can be considered as a two-compartment system with a lumen, lined by an apical epithelial surface, and a submucosal lamina propria, lined by the basolateral surface of the same epithelium. When HIV-1 enters the lumen of each of these tissues, it first encounters mucus of varying compositions depending on the precise site. In an immune individual, the mucus will also contain antibodies that can bind free virions. This can be because the antibodies recognize native Env on the virions or because they recognize mis-folded Env that is usually present (Moore et al. 2006). Thus, virion aggregation is potentially the first line of defense by a non-neutralizing antibody, possibly also with contributions from luminal mucus. If a virion makes it past this initial barrier, its next barrier to hurdle is the intestinal epithelium itself where the virion is actively transported from the apical to the basolateral surface. Antibodies can interdict this step (Bomsel et al. 1998). If a virion makes it across the epithelium into the lamina propria, non-neutralizing antibodies must rely on Fc-mediated effector functions or complement activation to prevent an established infection. These functions include phagocytosis, ADCC, or its related function antibody-dependent cell-mediated viral inhibition (ADCVI) (Forthal et al. 2007), and complement-mediated lysis of antibody-coated virions. Any or all of these mechanisms may be operative but they all involve the recognition of antibody-coated virions or infected target cells by either Fc receptors on the effector cells or by complement components. In addition

to the proximal effector sites, non-neutralizing antibodies can exert activity at systemic sites, but, by the time that HIV-1 infects CD4+ cells in secondary lymphoid tissues, it is likely to have too strong of a foothold to be eradicated, although the infection could still be blunted. There are significant variations among the local humoral microenvironments, effector cell types, and Fc-receptor expression patterns that are particular to the anatomical locus, but each route of transmission holds these steps in common.

Mechanisms by Which Non-neutralizing Antibodies Might Block Transmission

Virion Aggregation

Circulating antibodies are bivalent (IgG, IgE, and monomeric IgA), tetravalent (dimeric IgA), or decavalent (IgM) and can cross-link multivalent antigens such as bacteria and virions. Additionally, humans have two forms of IgA, IgA1 and IgA2, which differ in hinge length, IgA1 being the longer. Thus, IgA1 might have a greater ability to cross-link virions and confer protection. This was confirmed recently in a passive immunization study using a neutralizing antibody (Watkins et al. 2013). Although it has never been formally demonstrated, it is possible that aggregated HIV-1 virions are less infectious than single virions. This is because the interior virions in a large aggregate will not have the same access to susceptible CD4+ target cells as the same number of free virions. Regardless of whether this is so, large virion-antibody complexes will certainly have decreased diffusion coefficients relative to individual virions, possibly with increased retardation by mucins (Shukair et al. 2013). Thus, virion aggregation by antibodies is potentially a first line of defense in the luminal sites of mucosal tissues. In this regard, a recent passive immunizations study using non-neutralizing anti-gp41 monoclonal antibodies showed a statistically significant postinfection control of SHIV162p3 viremia (Moog et al. 2013), and, among other activities, these monoclonal antibodies (mAbs) efficiently aggregated infectious virions in vitro (Moog et al. 2013).

Virion Transport Across Epithelial Layers (Transcytosis)

HIV-1 must pass from the apical surface to the basolateral surface of luminal epithelia to gain entry to the lamina propria and susceptible CD4+ target cells. This process is termed transcytosis. At this site, non-neutralizing antibodies can possibly exert two antagonistic activities. First, mucosal epithelial cells have the neonatal Fc receptor that binds IgG with high affinity at luminal pH favoring the trans-epithelial transport of IgG-virion complexes. In the case of a non-neutralizing antibody-virion complex, the virion could theoretically retain infectivity and such a complex would favor transmission rather than protection, although this has not been demonstrated in vivo. Second, some non-neutralizing antibodies can clearly block virion transcytosis (Matoba et al. 2008). Thus, non-neutralizing antibodies that bind to virions can potentially block the transcytosis of HIV-1 from the luminal to the basolateral surface of mucosal epithelium in vivo. On the other hand, depending on specificity and isotype, virion binding by non-neutralizing antibodies could also favor transcytosis providing access to susceptible CD4+ target cells in the lamina propria. The relative ability of HIV-1 to pass across mucosal epithelia is likely to be determined by a balance between these two activities.

Phagocytosis

Once HIV-1 passes the mucosal epithelium, Fc-mediated effector functions become prime mechanisms through which non-neutralizing antibodies might block transmission. Antibody-virion complexes can be phagocytosed by a number of different cell types including monocytes, macrophages, dendritic cells, neutrophils, and mast cells. Presence of these cells at the sites of HIV-1 entry varies with the mucosal tissue under consideration as well as the local microenvironment (normal or inflammatory). However, each cell type shares the common features of bearing Fc receptors and being phagocytic. In contrast to other Fc-mediated effector functions, phagocytosis is less studied in the context of HIV-1 infection, although it is known that monocytic cell lines can be infected with HIV-1 and that non-

neutralizing antibodies can block this infection (Moog et al. 2013). This observation correlated with protection in the recent passive immunization study discussed above (Moog et al. 2013). Additionally, there appears to be an inverse relationship between antibody-mediated phagocytosis and disease progression in HIV-1 infected individuals (Dugast et al. 2011), but the precise role of phagocytosis in preventing HIV-1 acquisition is not known.

ADCC and ADCVI

Three lines of evidence suggest that ADCC or ADCVI plays a role in preventing acquisition in humans (Forthal et al. 2007; Haynes et al. 2012; Mabuka et al. 2012). First, there was a significant inverse relationship between ADCVI titers specific for clinical strains and reduced infection risk for a subset of volunteers in the VAX 004 Phase III gp120 vaccine trial in which there was no overall efficacy (Forthal et al. 2007). In that study, each 10% increase in ADCVI activity correlated with a 6.3% decrease in the hazard rate of infection (Forthal et al. 2007). Alleles of the Fc γ RIIa and Fc γ RIIIa receptors influenced this relationship, strongly implicating an *in vivo* role for Fc-mediated effector activity. The precise specificities of the antibodies mediating these effects were not determined, and it is possible that both neutralizing and non-neutralizing antibodies made contributions.

Second, the RV144 vaccine trial, in which modest efficacy was observed after immunization with a combination of membrane-anchored gp120 delivered by canarypox and two Clade B gp120 proteins, was the first to indicate a role for non-neutralizing antibodies in the prevention of HIV-1 acquisition (Haynes et al. 2012). In that study, immune correlates analysis showed that infection risk varied inversely with titers of anti-V1/V2 antibodies and directly with IgA titers against gp120, specifically the C1 region. Secondary analyses in which the top third tier of individuals mounting IgA anti-gp120 titers were excluded, a significant inverse correlation emerged between ADCC, measured by two independent methods, and infection risk. This finding

suggested the possibility that the IgA antibodies specific for the C1 region of gp120, which don't mediate ADCC in the assay formats employed in that study, competitively inhibit ADCC mediated by IgG antibodies specific for this region that is a known potent target for ADCC in infected individuals. This hypothesis was confirmed using matched pairs of IgA and IgG mAbs of the same specificity from the RV144 trial (Tomaras et al. 2013). This study provided the first evidence suggesting that non-neutralizing antibodies of defined specificity play a role in the prevention of HIV-1 acquisition via Fc-mediated effector function.

Third, a role for Fc-mediated effector function in preventing HIV-1 acquisition was indicated by a study in which ADCC activity in breast milk correlated with decreased perinatal transmission by breast-feeding (Mabuka et al. 2012). Although the precise specificities of the antibodies that mediate ADCC in this study were not determined, the decreased transmission did not correlate with neutralization of autologous or circulating HIV-1, suggesting that non-neutralizing antibodies are playing a role in protection against acquisition due to breast-feeding (Mabuka et al. 2012).

Complement-Mediated Lysis and Opsonization

Although HIV-1 virions can bind and activate complement directly, there is clear evidence that complement activation by antibody-coated virions results in virion lysis and/or opsonization (Sullivan et al. 1996). Thus, non-neutralizing antibodies can theoretically block HIV-1 transmission by direct viral lysis or increasing phagocytic activity via opsonization. Although a role for complement in passive protection of NHPs against a SHIV virus challenge by a neutralizing antibody has been questioned (Hessell et al. 2007), there is evidence that complement plays a protective role in HIV-1 infection (Huber et al. 2006). It should also be recognized that complement-mediated opsonization by non-neutralizing antibodies can enhance HIV-1 infection *in vitro* (Willey et al.

2011), which indicates that complement activation is potentially a double-edged sword with regard to HIV-1 transmission. The degree to which complement activation by antibody-coated virions favors protection versus increased acquisition of HIV-1 is currently unknown.

Conclusion

Largely through passive immunization studies in nonhuman primates, it is well accepted that neutralizing antibodies will block HIV-1 acquisition (Hessell et al. 2007; Burton et al. 2011). By contrast, similar compelling evidence is absent for non-neutralizing antibodies. Two passive immunization studies in nonhuman primates have shown that non-neutralizing antibodies can impact the postinfection control of viremia (Burton et al. 2011; Moog et al. 2013), but there is no evidence that such antibodies can block the acquisition of model AIDS viruses in challenge studies. Against this backdrop, three studies in HIV-1 transmission cohorts suggest that non-neutralizing antibodies can block acquisition, possibly by an Fc-mediated mechanism. First, an inverse correlation between infection risk and antibody-dependent cell-mediated viral inhibition was noted for a subset of volunteers in the VAX004 vaccine trial, for which overall efficacy was absent (Forthal et al. 2007). This correlation was influenced by Fc-receptor genotype (Forthal et al. 2007), further indicating a role for Fc-mediated effector function in protection. Second, an inverse correlation between reduced infection risk and antibody-dependent cell-mediated cytotoxicity mediated by non-neutralizing antibodies against epitopes in the N-terminal region of gp120 was found for a subset of volunteers in the RV144 vaccine trial, for which modest efficacy was reported (Haynes et al. 2012). Third, an inverse correlation was found between antibody-dependent cell-mediated cytotoxicity titers in breast milk and risk of transmission to infants of HIV-1 infected mothers by breast-feeding (Mabuka et al. 2012). Taken together, these correlations imply a role of non-neutralizing

antibodies in protection against HIV-1 infection. Although the correlations are provocative, confirmation of this hypothesis awaits definitive passive immunization studies in nonhuman primates showing that non-neutralizing antibodies can block the transmission of model AIDS viruses.

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Non-Nucleoside Reverse Transcriptase Inhibitors for Treatment of HIV Infection

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Definition

Non-nucleoside or nucleotide reverse transcriptase inhibitors (NNRTIs) are a class of antiretroviral drugs that inhibit the reverse transcriptase enzyme and are used in the treatment of the human immunodeficiency virus type 1 (HIV-1) infection.

Introduction

Treatment of HIV is based on blockage of the different stages of the HIV life cycle. Twenty eight drugs classified into six different classes (nucleoside-analog reverse transcriptase inhibitors [NRTIs], non-nucleoside reverse transcriptase inhibitors [NNRTIs], integrase inhibitors, protease inhibitors [PIs], fusion inhibitors, and CCR5 coreceptor antagonists) are currently available for HIV treatment (Pau and George 2014). Initial antiretroviral therapy (ART) consists of a combination of two NRTIs plus either a NNRTI, a PI, or an integrase inhibitor (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015).

NNRTIs affect viral replication by inhibiting the reverse transcriptase (RT) enzyme, responsible for the conversion of viral RNA to complementary viral DNA. Unlike NRTIs, NNRTIs are chemically diverse compounds that non-competitively bind to the same site in the RT enzyme. The binding site is a hydrophobic pocket located near the enzyme's catalytic site in the p66 subunit that induces a conformational change of the amino acid side chains towards the catalytic

Non-Nucleoside Reverse Transcriptase Inhibitors for Treatment of HIV Infection, Table 1 Summary of NNRTIs formulations, characteristics and clinical use

NNRTIs	Formulation	Taken with Food	Available as single tablet ART	Adverse events	Clinical use
Nevirapine	Tablet (200 mg and 400 mg XR) Suspension (50 mg/5 ml)	No	No	Rash hepatotoxicity	Prevention of mother to child HIV transmission
Delavirdine	Tablet (100 mg and 200 mg)	No	No	Rash; CNS side effects	Rarely used
Efavirenz	Tablet (50, 200 and 600 mg) Suspension (30 mg/ml)	No ^a	Yes (Atripla [®])	Rash; CNS side effects	Preferred “single pill” regimen in resource limited settings
Etravirine	Tablet (100 and 200 mg)	Yes	No	Rash; diarrhea headache	Effective as salvage regimen for NNRTI resistant virus
Rilpivirine	Tablet (25 mg) Long Acting IM ^b (300 mg/ml)	Yes	Yes (Complera [®])	Nausea dizziness transaminitis	Active against K103N mutant virus Less CNS side effects Pregnancy category B
Doravirine^b	Tablet (25, 50, 100 and 200 mg)	No	No	Rare	Investigational Potential for less drug interactions

NNRTIs non-nucleoside reverse transcriptase inhibitors, ART antiretroviral therapy, IM intramuscular, CNS central nervous system

^aTaken on an empty stomach

^bInvestigational

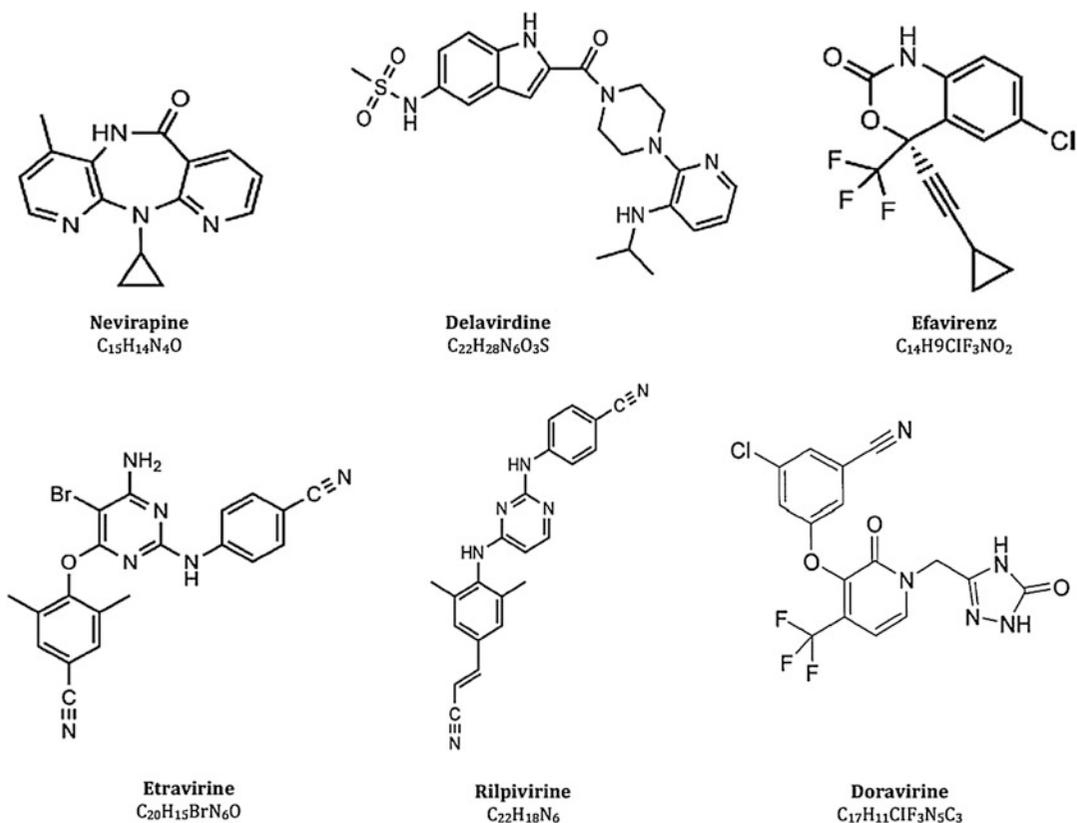
site affecting its activity (Kohlstaedt et al. 1992; Hsiou et al. 1996).

NNRTIs were the third class of antiretrovirals identified for the treatment of HIV. The first drug in the class, nevirapine, was approved by the US Food and Drug Administration (FDA) in 1996. At present, there are five NNRTI drugs available for HIV treatment in the United States. They are all selectively active against HIV-1, but are not active against HIV-2 or animal retrovirus. In addition, NNRTIs do not require additional intracellular phosphorylation to become active and are metabolized to some degree by the cytochrome P450 enzyme. Despite sharing the same mechanism of action, each NNRTI has a unique chemical structure and characteristics and selection of the drug will depend on the HIV resistance pattern, toxicity profile, and drug interactions. In this review, we will describe each drug from the NNRTI class, focusing on their pharmacokinetics, resistance, side effects, and clinical use. Table 1 summarizes key characteristics of each NNRTI drug.

First Generation NNRTIs

The discovery of the 1-(2-(2-hydroxyethoxy-methyl)-6-(phenylthio) thymine (HEPT) and tetrahydroimidazo [4,5,1-jk][1,4]benzodiazepin-2(1H)-one and -thione (TIBO) in the early 1990s as the first compounds to inhibit the RT enzyme led to the development of NNRTI drugs (Baba et al. 1991; Pauwels et al. 1994). Soon after, dipyridodiazepinones, bis(heteroaryl)piperazines and benzoxazinones were used as compounds for the first generation NNRTI. Three first generation NNRTI drugs have been FDA approved: nevirapine in 1996, delavirdine in 1997, and efavirenz in 1998.

- Nevirapine (NVP)
 - Description: NVP is a dipyridodiazepinone derivative discovered in 1990 by researchers at Boehringer Ingelheim (Fig. 1). Oral bioavailability is above 90% and the drug can be administered with or without



Non-Nucleoside Reverse Transcriptase Inhibitors for Treatment of HIV Infection, Fig. 1 Chemical structure and molecular formula of NNRTIs

food. It is highly lipophilic and 60% is bound to proteins allowing penetration of the blood–brain barrier and placenta. NVP has a long half-life of approximately 25–30 h and effective dosing regimen is 200 mg daily for 14 days followed by 200 mg twice a day (or 400 mg daily of the extended release formulation). It is metabolized in the liver and induces the CYP3A4 and 2B6 isoenzymes. Eighty percent of the drug is excreted in the urine and 10% in the feces.

- Resistance: Development of resistance is common with monotherapy or non-adherence. The most common selected mutation is Y181C, which also confers cross-resistance to other NNRTIs.
- Adverse events: Severe hypersensitivity reactions (including Stevens-Johnson

syndrome and toxic epidermal necrolysis) and hepatotoxicity have occurred with NVP. These adverse events are more common in women with CD4 cell counts above 250 cells/mm³ and in men with CD4 cell count above 400 cells/mm³. These adverse events usually manifest during the first 8 weeks of therapy and can be fatal if not recognized. It has been assigned category B for pregnancy and has been used extensively to prevent mother to child HIV transmission.

- Drug–drug interactions: As a CYP3A4 substrate and inducer, NVP can interact with other isoenzyme substrates. NVP has been shown to decrease the serum levels of drugs such as ketoconazole, voriconazole, methadone, carbamazepine, diltiazem, and amiodarone. Other CYP3A4 inducers may

also decrease NVP serum levels. St. John's wort has been shown to significantly reduce NVP serum levels and coadministration should be avoided.

- Clinical trials: The efficacy of NVP is comparable to efavirenz and ritonavir-boosted protease inhibitors when taken with two NRTIs in individuals with HIV infection who are treatment-naïve or have limited exposure to ART. The open label randomized 2NN trial compared efavirenz vs. nevirapine in combination with two NNRTIs (stavudine and lamivudine in treatment-naïve HIV-infected individuals) (van Leth et al. 2004). Results revealed similar efficacy between both NNRTIs but higher treatment failures were observed in patients taking NVP with CD4 cell count <250 cell/mm³ or plasma RNA >100,000 copies/ml as well as higher rates of adverse events. Similarly, the ARTEN and the NEWART studies demonstrated that NVP was noninferior to atazanavir/ritonavir when taken in combination with tenofovir and emtricitabine with comparable rates of adverse events (Soriano et al. 2011; Dejesus et al. 2011).
- Delavirdine (DLV)
 - Description: DLV belongs to the family of bishetero-arylpiperazine compounds (*N*-[2-(4-[3-(propan-2-ylamino) pyridin-2-yl] piperazin-1-yl carbonyl)-1H-indol-5-yl] methanesulfonamide) discovered by researchers at Upjohn Laboratories, now Pfizer (Fig. 1). It has a high oral bioavailability of 85% and is unaffected by food, however it has a short half-life of 5.8 h and requires a dosing of 400 mg every 8 h. DLV is highly protein bound and has a low penetration into the CNS. Metabolism occurs primarily in the liver by the CYP3A4 enzyme. Forty-four percent of the drug is eliminated in the feces and 51% in the urine.
 - Resistance: Resistance to DLV emerges rapidly when used as monotherapy or in sub-optimal regimens. Resistance mutations at codon position 103 [lysine to asparagine (K103N)] and 181 [tyrosine to cysteine (Y181C)] are the most commonly encountered mutations and confer resistance to first generation NNRTIs. Mutation P236L confers high-level resistance to DLV but causes hyper susceptibility to other NNRTIs.
 - Adverse Events: The most common adverse events seen with DLV include skin rash, CNS side effects and gastrointestinal symptoms. The skin rash can be severe and in rare instances erythema multiforme and Stevens-Johnson's syndrome may develop. Headache, anxiety, depressive symptoms and insomnia are the predominant CNS adverse events. DLV has been designated pregnancy category C by the FDA.
 - Drug-Drug Interactions: Unlike other NNRTIs, DLV inhibits the CYP3A4 enzyme thereby increasing the serum level of the enzyme's substrates. Lovastatin, simvastatin, rifabutin, rifampicin, sildenafil, ergot derivatives, quinidine, midazolam, carbamazepine, phenobarbital or phenytoin should be avoided when using DLV. Antacids, histamine-2 receptor antagonists, or proton pump inhibitors may reduce the absorption of DLV.
 - Clinical trials: Early studies using DLV combined with a single NRTI (zidovudine or didanosine) resulted in greater decreases in plasma HIV viral load than did NNRTI monotherapy. Triple therapy combinations of DLV with zidovudine and didanosine showed greater reduction in HIV plasma viral loads and sustained improvements in CD4 cell counts compared to dual therapy, however these differences were not consistent nor statistically significant (Friedland et al. 1999). In a randomized study of treatment-experienced individuals, the addition of DLV to a PI-based regimen showed short-term virologic benefit which did not persist at 48 weeks (Gulick et al. 2000). Due to insufficient evidence supporting DLV's virologic efficacy in combination therapy and availability of better antiretroviral agents, DLV is "not recommended" as a component of antiretroviral regimens for initial treatment of HIV

infection (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015).

- Efavirenz (EFV)

- Description: EFV is a benzoxazinone derivative ((*S*)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one) discovered by Merck researchers (Fig. 1). EFV's oral bioavailability is 40–45% and increases in the setting of a high-fat meal so it is recommended to take the drug on an empty stomach. EFV is highly protein bound (>99%) and is excreted as an unchanged drug primarily in the feces (16–61%) and in the urine as hydrolyzed metabolites (14–34%). As other NNRTIs, EFV is metabolized by CYP3A4 and 2B6 iso-enzymes. Its long half-life (40–55 h) allows for once daily dosing of 600 mg and it was incorporated in the first single tablet formulation in combination with tenofovir disoproxil fumarate and emtricitabine. EFV is the most widely used NNRTI, nonetheless given availability of newer agents with less adverse events and superior efficacy it is now listed as an alternative drug for HIV treatment in ARV naïve individuals.
- Resistance: Like the other NNRTIs, EFV has a low genetic barrier to resistance and selects for HIV-1 reverse transcriptase amino acid substitutions that will lead to resistant mutants. K103N mutation is the most characteristic, which also confers resistance to NVP and DLV. Other mutations such as L100I, K103N, V108I, V179D, Y181C also affect susceptibility to EFV.
- Adverse events: EFV is usually well tolerated but rash and CNS side effects can be significant. Rash has been reported in about 26% of patients and usually resolves on its own without requiring discontinuation. Rare cases of Steven Johnson's syndrome have been reported. Approximately 25–77% of patients on EFV have reported CNS side effects and neuropsychiatric side effects such as headache, dizziness, insomnia, abnormal dreams, impaired concentration, agitation, depression, amnesia, fatigue

and hallucinations. Analyzed data from four AIDS Clinical Trials Group (ACTG) on HIV treatment naïve individuals showed that initial treatment with an efavirenz-containing antiretroviral regimen was associated with a twofold increased hazard of suicidality (suicidal ideation, attempted or completed suicide) compared to a regimen without efavirenz (Mollan et al. 2014). A genetic polymorphism of CYP2B6 has been associated with higher plasma concentrations of EFV and higher risk of CNS adverse events (Ribaudo et al. 2010). EFV should be administered at bedtime and on an empty stomach to minimize the risk of these side effects. CNS side effects usually dissipate within the first 4 weeks of therapy but in certain patients symptoms can persist and discontinuation of therapy should be considered (Clifford et al. 2005). Other less common adverse events include hepatotoxicity, nausea, dyspepsia, lipodystrophy, dyslipidemia, gynecomastia, myopathy, and tinnitus.

- Drug–Drug interactions: Like other NNRTIs, EFV has the potential to cause drug–drug interaction by inducing the CYP3A4 enzyme. Concomitant use with voriconazole should be avoided as EFV significantly decreases the plasma levels of voriconazole and, paradoxically, voriconazole raises the plasma levels of EFV. EFV can also reduce the plasma levels of rifabutin, clarithromycin, atorvastatin, simvastatin, methadone, and carbamazepine. Rifampin increases the plasma levels of EFV generally requiring dose adjustment from 600 to 800 mg daily. EFV has been labeled category D for pregnancy as its use during the first trimester has been associated with neural tube defects in both primates and humans.
- Clinical Trials: Large randomized trials in HIV treatment-naïve patients have demonstrated potent and durable viral suppression when treated with combination ART regimens including EFV and two NRTIs (Gulick et al. 2004). EFV has been shown to have comparable HIV antiviral activity

to nevirapine, lopinavir/ritonavir, and atazanavir/ritonavir based regimens (van Leth et al. 2004; Daar et al. 2011; Riddler et al. 2008), In treatment-experienced HIV-infected individuals with viral suppression, the switch to a single pill fixed dose combination of EFV/emtricitabine/tenofovir disoproxil fumarate was effective in maintaining viral suppression at 96 weeks (DeJesus et al. 2009). Lower dose EFV of 400 mg in combination with emtricitabine and tenofovir disoproxil fumarate was confirmed to be noninferior to the standard dosing of 600 mg with fewer EFV-related adverse events (Study Group 2015). Compared to rilpivirine, a newer NNRTI, EFV has shown to be noninferior with less virologic failure among patients with pre-ART plasma viral load >100,000 copies/ml but with higher rates of therapy discontinuation related to adverse events (Molina et al. 2011; Cohen et al. 2011). When compared to integrase inhibitors, EFV has been shown to be noninferior to the elvitegravir/cobicistat/TDF/FTC combination but raltegravir and dolutegravir have demonstrated superiority to EFV in randomized controlled trials based primarily on discontinuation related to adverse events (Wohl et al. 2014; Lennox et al. 2009; Walmsley et al. 2013).

Second Generation NNRTIs

The need for NNRTIs with a better resistance profile led to the development of the second generation NNRTIs. Second generation NNRTIs currently include etravirine and rilpivirine. These NNRTIs have a higher genetic resistance barrier and can be used in most patients who have HIV-1 virus with resistance to the first generation NNRTIs.

- Etravirine (ETR):
 - Description: ETR was approved in January 2008 by the FDA as a salvage treatment for HIV treatment-experienced individuals.

It belongs to the family of di-aryl-pyrimidine (DAPY) compounds (Fig. 1). ETR's chemical name is 4-[6-amino-5-bromo-2-(4-cyanoanilino) pyrimidin-4-yl]oxy-3,5-dimethylbenzonitrile and this structure allows it to bind to the RT enzyme in more than 1 distinct mode based on changes in the NNRTI-binding pocket. ETR is taken with meals as oral absorption increases by 50% when administered with food. The drug's half-life is approximately 41 h and it is administered at a dose of 200 mg every 12 h. It is highly protein bound (>99%) primarily to albumin and α 1-acid glycoprotein. ETR is metabolized in the liver to mostly inactive metabolites through methyl hydroxylation, glucuronidation, and aromatic hydroxylation. Elimination occurs primarily in the feces (94%), followed by the bile (11%) and to a much smaller degree in the urine (1%).

- Resistance: Unlike other NNRTIs, ETR requires multiple mutations for the development of resistance. In addition, it retains activity against HIV-1 despite emergence of the traditional NNRTI mutations K103N and Y181C. A number of NNRTI mutations including V90I, A98G, L100I, K101E, K101P, V106I, V179D, V179F, Y181C, Y181I, Y181V, G190A, and G190S have been shown to decrease susceptibility to ETR. A score of mutations has been developed to assess the effect of mutations on the activity of ETR. A score of 0–2 confers a 74% response rate (highest response); a score of 2.5–3.5 confers a 52% response rate (intermediate response); and a score of ≥ 4 confers a 38% response rate (reduced response) (Vingerhoets et al. 2010). The E138K mutation, which usually develops after erratic use of rilpivirine, also confers resistance to ETR.
- Adverse Events: ETR is usually well tolerated with rare grade 3 or 4 adverse events leading to drug discontinuations. The most common reported side effects include rash, diarrhea, nausea, and headache. Hyperglycemia and hypercholesterolemia

have also been reported. It does not cause significant CNS side effects as seen with EFV. ETR is a pregnancy category B drug, however due to insufficient data its use is not recommended in pregnancy unless other alternatives are not available. In addition, its safety profile in elderly, individuals with renal or hepatic dysfunction is unknown.

- Drug–Drug Interactions: ETR is a substrate and an inducer of CYP3A4 and an inhibitor of CYP2C9 and CYP2C19. It can decrease the levels of several medications such as antiarrhythmics (disopyramide, lidocaine, quinidine), antineoplastics (vinca alkaloids, docetaxel, paclitaxel), benzodiazepines, calcium channel blockers, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, immunosuppressive agents (cyclosporine, tacrolimus), sildenafil, and tricyclic antidepressants. ETR can also interact with other ART drugs; EFV, nevirapine, ritonavir, and tipranavir can significantly reduce the plasma concentrations of ETR and ETR can lower the concentrations of unboosted PIs, maraviroc, and raltegravir.
- Clinical Trials: Unlike other NNRTIs, ETR was first evaluated in treatment-experienced individuals. The DUET-1 and -2 trials, phase III blinded randomized trials in which highly treatment-experienced individuals with NNRTI mutations were assigned to ETR or placebo in addition to an ART regimen that included darunavir/ritonavir, optimized NRTI combinations, and optional enfuvirtide, demonstrated viral suppression compared to placebo (55 vs. 33%). These studies demonstrated the effectiveness of adding ETR to regimens for individuals who had HIV-1 virus resistant to other NNRTIs (Madruga et al. 2007; Lazzarin et al. 2007).
- Rilpivirine (RPV)
 - Description: RPV is a diarylpyrimidine added to the NNRTI class after its FDA approval on August 2011. Its chemical structure 4-[[4-[[4-(E)-2-cyanoethenyl]-2,6-dimethylphenyl]amino]-2-pyrimidinyl]

amino]benzotrile monohydrochloride allows deeper attachment to the NNRTI binding pocket (Fig. 1). Solubility and systemic absorption are pH-dependent, so RPV has to be administered with a meal at a dose of 25 mg once a day. Antacids and high protein drinks significantly reduce RPV absorption. It is the second NNRTI coformulated with 300 mg of tenofovir and 200 mg of emtricitabine into a single pill form known commercially as Complera. After oral administration RPV reaches peak plasma levels after 4–5 h and the half time to elimination is approximately 50 h. It is highly protein bound (99%), mainly to albumin, and is excreted in the feces (85%) to a lesser extent in the urine.

- Resistance: RPV is active against K103N mutant HIV-1 virus. Fifteen NNRTI mutations have been associated with decreased susceptibility to rilpivirine: K101E, K101P, E138A, E138G, E138K, E138Q, E138R, V179L, Y181C, Y181I, Y181V, H221Y, F227C, M230I, and M230L. The E138K mutation is known to confer resistance against all members of the NNRTI family including RPV and ETR.
- Adverse Events: RPV is generally well tolerated. Nausea and dizziness are the most common adverse events associated with RPV. A lower incidence of rash, neuropsychiatric symptoms, and lipid abnormalities are seen with RPV compared to EFV (Cohen et al. 2012). Depressive disorders have been reported in 4–9% of RPV recipients. Transaminitis can occur and is the most common reason for drug discontinuation (Pozniak et al. 2010). QTc prolongation have been seen with RPV at higher doses but rarely at 25 mg daily. RPV has been classified into a pregnancy category B by the US Food and Drug Administration (FDA); this means that animal studies have failed to demonstrate a risk to the fetus but there are no adequate and well-controlled studies available in pregnant women.
- Drug–Drug Interactions: RPV is primarily metabolized in the liver by CYP3A enzyme;

its plasma concentration may be affected in the presence of CYP3A inhibitors or inducers. Coadministration with anticonvulsants (carbamazepine, oxcarbazepine, phenobarbital, phenytoin), antituberculous drugs (rifampin, rifapentine), proton pump inhibitors, systemic dexamethasone, or St John's wort should be avoided.

- Clinical Trials: Three randomized trials compared RPV with EFV in combination with two NRTIs for treatment-naïve individuals. ECHO and THRIVE showed that RPV met noninferiority criteria at 96 weeks when compared with EFV (Cohen et al. 2013), except in patients with pre-ART plasma viral load of >100,000 copies/ml and CD4 cell count <200 cells/mm³ where higher rates of virologic failure were documented in the RPV group. Fewer drug discontinuations and serious adverse events were observed with RPV compared to EFV. The STaR trial compared the fixed-dose combinations of RPV/TDF/FTC vs. EFV/TDF/FTC and showed that the RPV combination was superior to EFV in patients with pre-ART viral loads ≤100,000 copies/ml (Cohen et al. 2014).

Investigational Agents

New NNRTIs in clinical trials include doravirine and long acting injectable rilpivirine. Doravirine has pharmacologic advantages over other NNRTIs and activity against NNRTI resistant virus. Rilpivirine is being tested in a long acting formulation allowing intermittent dosing by injection. In clinical trials, this drug is being tested for both treatment of chronic HIV infection (in combination with an injectable long acting integrase inhibitor) and for prevention of HIV infection using the long acting rilpivirine injection alone.

- MK 1439 (Doravirine)
 - Description: Doravirine is a potent investigational nonnucleoside inhibitor of HIV-1 reverse transcriptase under development

by Merck & Co., Inc. It is primarily metabolized by oxidation via CYP3A4 and is a P-glycoprotein substrate in vitro. Doravirine may be affected by CYP3A4 inhibitors or inducers but has a low potential to cause drug–drug interactions. The terminal half-life of 12–21 h supports once daily dosing. Administration of 50 mg doravirine with a high-fat meal was associated with slight elevations in AUC_{0-∞} and C_{24hr} with no change in C_{max}. PK was similar between HIV-infected patients and healthy subjects. Doravirine can be dosed without regard to food (Anderson et al. 2015a).

- Resistance: In vitro, doravirine inhibits wild type HIV virus and K103N, Y181C, and K103N/Y181C mutant virus.
- Adverse events: In clinical trials, at doses between 25 and 200 mg, doravirine was well tolerated. There were fewer CNS side effects seen with doravirine compared with efavirenz in a randomized trial (Gatell et al. 2014).
- Drug–drug interactions: Coadministration of ritonavir increases doravirine AUC_{0-∞} 3.5-fold and C_{24hr} 2.9-fold, but increased C_{max} only 30%. Tenofovir had no meaningful effect on doravirine PK (Anderson et al. 2013). Doravirine can be coadministered with dolutegravir and a ketoconazole study showed that strong CYP3A inhibitors can be administered with doravirine (Anderson et al. 2015b, c). However, strong CYP3A inducers should not be administered with doravirine.
- Clinical Trials: In a dose ranging (25–200 mg) PK study in HIV-infected subjects, there was no exposure-response relationship between doravirine plasma levels and both viral load and proportion of subjects with undetectable viral load at week 48 (Gatell et al. 2014). Phase 3 trials are on-going with the 100 mg daily dose.
- Long acting rilpivirine (RPV-LA)
 - RPV-LA is a nanosuspension developed for intramuscular injection. A 300 mg/ml

nanosuspension has been chosen for further investigation. Single dose injections of either 600 or 300 mg RPV-LA given to healthy volunteers showed a median half life of 2.2 and 1.3 months, respectively. Although no serious AEs were reported, grade 1 events included short-lasting pain at the injection site and stiffness of the extremities. A compartment study of RPV-LA demonstrated rilpivirine presence after a single 600 mg IM injection in plasma, rectal fluid and rectal tissue, and in women in cervicovaginal fluid (Jackson et al. 2014). In a healthy volunteer study, RPV-LA and long-acting cabotegravir (GSK1265744) showed that injections were safe and generally well-tolerated (Spreen et al. 2014). A phase 2b study is ongoing in ARV-naïve subjects testing the two injections given every 4 or 8 weeks, after a 24 week induction period with oral daily cabotegravir plus abacavir and lamivudine (Healthcare ViiV 2012).

Clinical Use

First generation NNRTIs EFV and NVP have treated HIV infection successfully for nearly 20 years, most commonly in combination with 2NRTIs for initial infection. EFV with tenofovir and lamivudine (or emtricitabine) is currently considered to be the preferred regimen for initial therapy of adults and adolescents in the developing world (World Health Organization (WHO) 2013). EFV can have significant CNS side effects and therefore some guidelines for resource rich countries list an EFV regimen as an alternative option after first line integrase inhibitor-based or darunavir/ritonavir-based regimens (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015). NVP with zidovudine/lamivudine or tenofovir/lamivudine (or emtricitabine) are alternative regimens after the EFV regimen in WHO guidelines for adolescents and adults. This is due to the higher rate of serious adverse events (rash

and liver toxicity) associated with NVP. EFV based regimens are now considered safe to use in pregnancy (even in first trimester) per WHO guidelines despite many years of concern over fetal neural toxicity. In resource rich areas, other regimens are preferred above one containing EFV, but EFV regimens should be continued if a woman becomes pregnant while taking EFV provided the regimen is well tolerated and achieves virologic suppression. In 2006, a coformulated pill of EFV plus tenofovir and emtricitabine became available, giving patients a one pill once a day option thereby revolutionizing HIV treatment.

Second generation NNRTIs are active against virus with first generation NNRTI mutations and are better tolerated. RPV was coformulated with tenofovir and emtricitabine into a one pill daily regimen in 2011. Higher virologic failure rates were seen in patients with baseline viral loads $>100,000$ or baseline CD4 counts <200 cells/mm³. Emtricitabine/rilpivirine/tenofovir disoproxil fumarate is not recommended for patients with high baseline viral loads. ETR is approved for treatment-experienced patients with NNRTI resistant virus. ETR is not likely to be active for a patient experiencing virologic failure on a RPV regimen due to mutations conferring reduced susceptibility to both drugs.

Doravirine is being studied in phase III trials and appears to be effective, well tolerated, and has minimal potential for drug interactions. A long acting injectable formulation of RPV (given with at least one other injectable ARV) is being tested in phase II studies and, if the study outcomes are favorable, will give patients the option of infrequent dosing.

Conclusion

The NNRTIs are a highly effective and generally well tolerated class of medications, particularly used in treatment-naïve patients. EFV and RPV can be taken in one pill once daily regimens, increasing the ease of administration and

adherence. Doravirine and a long acting injection of RPV may improve treatment options for patients with HIV-1 infection.

Cross-References

- ▶ [Antiretroviral Treatment in Resource-Limited Settings](#)
- ▶ [HIV-1 Preexposure Prophylaxis](#)
- ▶ [Initial Antiretroviral Regimens](#)
- ▶ [Neurotoxic Consequences of Antiretroviral Therapies](#)

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Nonpathogenic SIV Infection of Sooty Mangabeys

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Definition

Nonhuman primates (monkeys) are the most widely used experimental animal model for the study of HIV infection and AIDS. While rhesus macaques are the workhorse system used in the majority of vaccine and pathogenesis studies, significant insight into how AIDS causes immune dysfunction has been made by examining simian immunodeficiency virus (SIV) infection in non-macaque species of monkeys that do not develop disease during SIV infection, referred to as “natural host species.” The purpose of this entry is to highlight the most important findings to come out of research using the sooty mangabey monkey, one of the best studied natural host species, and how these findings have shaped contemporary thinking about HIV infection and AIDS.

Overview: Pathogenic versus Natural Infection of Monkeys with SIV

The use of monkeys as an animal model for the study of AIDS is widely known; however, the particular species used for this research warrant introduction in a more specific manner. The most broadly used species for AIDS vaccine and pathogenesis research is the rhesus macaque (*Macaca mulatta*). Rhesus and other species within the *Macaca* genus ranges are predominantly in Asia, although some macaque species range into northern Africa and southern Europe. The virus used to infect rhesus in this experimental system is the simian immunodeficiency virus (SIV), which is highly related to HIV. SIV infection of rhesus macaques shares similar hallmarks with HIV infection of humans: loss of CD4⁺ T cells,

disruption of lymph node architecture, weight loss, diarrhea, and susceptibility to opportunistic infections. SIV infection of other species within the *Macaca* genus, the best studied being pig-tailed macaques and cynomolgus macaques, also displays AIDS-like symptoms. Prior to the advent of antiretroviral therapy, the time-to-death for an HIV-infected person was estimated to be 10–12 years; in comparison, rhesus macaques typically succumb to AIDS-related complications in 6 months to 2 years, a time frame that is convenient for their use as an experimental model system.

SIV was originally discovered in rhesus macaques within several US primate research centers. Shortly after reports of AIDS in humans had surfaced, a similar disease was noted in captive rhesus colonies in the USA. Subsequent work demonstrated that the etiologic agent responsible for this “simian-AIDS” (sAIDS) was a virus sharing seroreactivity and a high degree of nucleotide sequence similarity with HIV. Surprisingly, similar viruses were also found at a high prevalence in captive sooty mangabey monkeys that did not display AIDS-like symptoms (Murphey-Corb et al. 1986). Follow-up studies showed that the SIVmac strains of SIV viruses (the suffix “mac” given for their origin in rhesus macaques) that infect macaques and cause AIDS could not be found in wild macaque monkeys. Instead, the source of the SIVmac virus was determined to be cross-species transmission of SIVs from sooty mangabeys (referred to as SIVsmm) (Hirsch et al. 1989) that occurred in captivity. Thus, the strains of SIV that are most commonly used for preclinical AIDS vaccine testing in monkeys are not found in the wild naturally, but are in fact a by-product of a zoonotic jump during captivity.

HIV-1, HIV-2, SIV, and the Origins of HIV-Related Immunodeficiency Viruses

HIV-1, HIV-2, and Their Origins in Monkeys

The search for SIV in the wild that occurred in the late 1980s set the groundwork for later research that would identify the origins of HIV and define how it entered the human population. A full

discussion of this topic is not the focus of this entry, but it warrants explanation to avoid confusion. The human immunodeficiency virus can be classified into two major types: HIV-1 and HIV-2. There are four subtypes of HIV-1: M, N, O, and P. HIV-1M is by far the predominant agent responsible for the majority of worldwide infections and main cause of the AIDS epidemic. In contrast, infections with subtypes N, O, and P have only been described in people residing or originating in West Africa. Phylogenetic evidence indicates that each HIV-1 subtype entered into the human population as a result of an independent cross-species transmission event. HIV-1M is thought to have originated from cross-species transmission of SIVcpz, which infects chimpanzee subspecies in West Africa. The other SIV subtypes are thought to originate from either SIVcpz or SIVgor (from lowland gorillas). Molecular dating studies have indicated that HIV-1 likely crossed into the human population as a result of the hunting and consumption of chimpanzee bushmeat at the end of the nineteenth century or early twentieth century.

HIV-2 is endemic to people in West Africa, with the highest prevalence being found in people of Senegal and Guinea Bissau. Typically, the course of infection of HIV-2 is much milder than that of HIV-1, with lower viral loads and less dramatic CD4⁺ T cell depletion. The majority of those infected do not progress to AIDS, but those who do experience similar symptoms to HIV-1 infected patients. At least eight sub-lineages of HIV-2 have been found in people, and each one is likely the result of an independent cross-species transmission. The primary source of most strains of HIV-2 is thought to be from cross-species SIVsmm, from African sooty mangabeys.

SIVcpz Versus SIVsmm and “Natural” SIV Strains

Strains of SIV have been identified in at least 40 different nonhuman primate species in Africa. These monkey species do not display any signs of AIDS when infected with their endemic virus. The majority of SIVs that are found in species such as sooty mangabey, African Green monkeys, and

others have a high degree of similarity with each other and with HIV-2. In contrast, the SIVcpz and SIVgor strains are highly atypical compared to most SIVs, containing different accessory genes and considerable divergence at the nucleotide level. SIVsmm and other “typical” SIV strains have infected their host species for millions of years and are maintained within species at high prevalence. SIVcpz is thought to be the result of recombination of two different strains of SIV in more recent evolutionary history.

In striking contrast to most SIVs, which are benign in their natural hosts, recent evidence indicates that SIVcpz infection in chimpanzees in the wild is, in fact, associated with disease and lower life expectancy.

The considerable divergence between HIV-1 and the SIVmac and SIVsmm strains used for vaccine experiments in rhesus had led to some efforts in the 1990s to develop a chimpanzee model that used HIV-1, with the rationale that it would provide a platform for testing vaccines that could be directly translated into human clinical trials (i.e., without the need to re-engineer from SIV antigens to HIV antigens). While chimpanzees were capable of infection, they did not show an observable disease, and this model failed to gain traction. Recent ethical restrictions on invasive biomedical research performed on chimpanzees have effectively ended any potential use of chimpanzees for this purpose in the foreseeable future.

The goal of this section was to provide the reader with an overview of the different types of HIV and SIV, their animal reservoirs, and the historical and biological reasons underlying the use of different SIVs in contemporary research. Further, the evolutionary history of SIV within their respective species provides perspective on why these “natural” infections are no longer pathogenic. For more detailed information on the origins of immunodeficiency viruses and the molecular mechanisms that affect their cross-species transmission, the reader is directed to an excellent review by Sharp and Hahn (2011). The remainder of this entry will focus on SIV infection of sooty mangabeys and related natural hosts.

Clinical Features of SIV Infection in Sooty Mangabeys

The sooty mangabey (*Cercocebus atys*) is an old-world monkey that ranges in West Africa (Fig. 1). They are notable in that they harbor a strain of SIV, SIV_{smm}, that is the most likely origin of most HIV-2 cross-species transmission events and is the source of the SIV_{mac} strain of viruses, the most widely used strain in monkey (rhesus) vaccine studies. However, the most biomedically important aspect of SIV infection in sooty mangabeys (SMs) and other natural host species of monkeys is that they do not develop any observable signs of AIDS when infected with SIV. It should be noted that although this entry deals specifically with SIV infection of sooty mangabeys, our understanding of natural host/SIV interactions is complemented by studies of SIV infection in other African monkeys; in fact, several of the key findings in SMs have been contemporaneously shown in African green monkeys, which has helped the interpretation of the results. In this regard, within this entry, findings derived equally from natural hosts models will be lumped together; conversely, unique properties of each species will be specifically mentioned. Lastly, should the reader require more in-depth information, they are directed to two

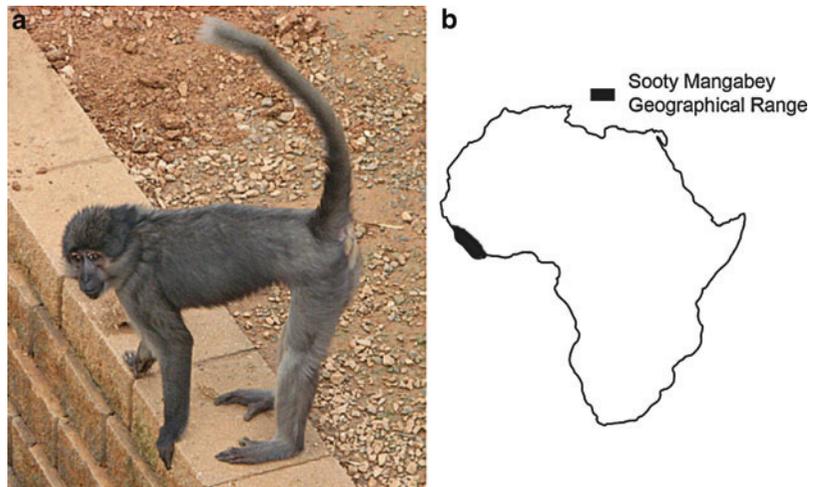
comprehensive recent reviews by Chahroudi et al. (2011) and Sodora et al. (2009).

SIV-Infected Sooty Mangabeys Maintain High Levels of Plasma Virus

Despite the disparity in disease course, nonpathogenic infection of SMs shares many similarities with pathogenic HIV/SIV infection; differences and similarities are summarized in Table 1. Both SMs and pathogenic hosts exhibit high levels of virus in the plasma, at average level of 10^5 RNA copies per ml of plasma. This observation has several important implications for HIV research. First, the observation that SMs (and >40 other African natural host species of NHPs) have, over millennia, evolved to coexist with SIV infection, and have not evolved immune mechanisms that control or eradicate infection, is a sobering lesson for the considerable challenges that need to be surmounted in the development of a vaccine that prevents against HIV transmission. Second, it highlights the contrast between the mechanisms maintaining non-pathogenesis seen in SMs compared to humans that naturally control virus. This rare population of patients, termed long-term non-progressors (LTNPs), comprises <1% of HIV-infected persons and maintains low levels of HIV by immune control mediated by CD8+ “killer T cells.” A graphical comparison of viral

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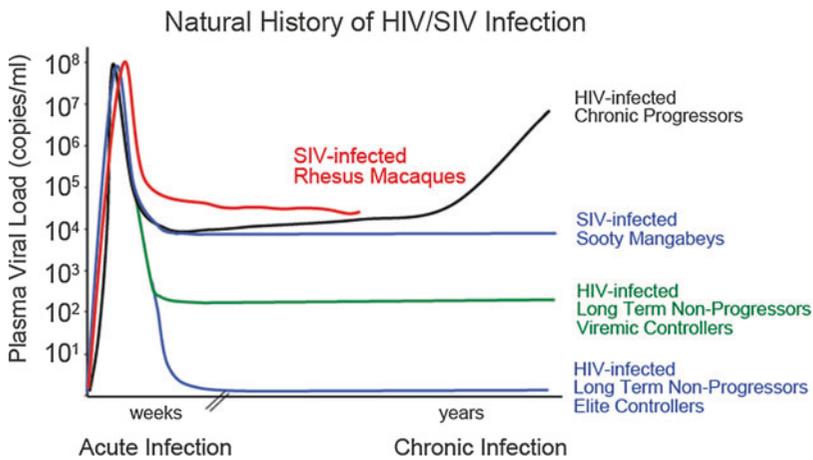
Nonpathogenic SIV Infection of Sooty Mangabeys, Fig. 1 (a) Photograph of a sooty mangabey monkey at the Yerkes National Primate Research Center. (b) Geographical range of sooty mangabeys within West Africa



Nonpathogenic SIV Infection of Sooty Mangabeys, Table 1 Clinical features of SIV infection in natural and pathogenic hosts

Phenotype	Pathogenic hosts (humans, rhesus)	Natural hosts (sooty mangabeys, African green monkeys)
AIDS	Yes	No
Peripheral blood CD4+ T cells	Depleted	Unaffected
Viral load	High	High
Mucosal CD4+ T cells	Progressively depleted	Depleted initially, then stable
Microbial translocation	Yes	No
Immune activation	Perpetual	Acute phase only
CD4+ T cell memory cell infection	Tcm > Tem	Tem > Tcm
Mother-to-child transmission	Frequent:	Rare:
	Humans (~40%)	Sooty (~7%)
	Rhesus (25–75%)	

loads in various HIV and SIV infection settings is shown in Fig. 2. The high levels of plasma viremia are strong evidence that “non-pathogenesis” is not due to an enhanced ability of the immune system in sooty mangabeys to control virus. Nevertheless, to rule out this possibility, follow-up experiments showed that sooty mangabeys do not have superior antiviral CD8+ T cell responses nor more effective antibodies against SIV compared to rhesus macaques. Another early hypothesis to explain the lack of disease in natural hosts proposed that the SIV_{smm} strain was less pathogenic than HIV or SIV_{mac239}. However, infection of sooty mangabeys with the SIV_{mac239} isolate did not cause disease. Additionally, mathematical modeling experiments of SIV_{smm} kinetics in the plasma of sooty mangabeys treated with antiretroviral drugs showed that the mangabey virus replicated in cells with a high turnover rate (i.e., CD4+ T cells) in a manner indistinguishable from HIV infection. Overall, these experiments provided powerful evidence that the lack of disease in sooty mangabeys was not due to a more



Nonpathogenic SIV Infection of Sooty Mangabeys, Fig. 2 Viral loads within models of HIV and SIV infection. During the common course of HIV infection, most patients will experience progressive disease which, left untreated, would result in eventual AIDS. These patients (chronic progressors) typically maintain viral loads in the plasma or 10^4 – 10^5 RNA copies/ml. In contrast, a limited number of patients control virus to varying degrees and are able to maintain stable numbers of CD4+ counts for several years called long-term non-progressors which can be

subclassified based on their plasma virus levels: patients with lowered but detectable virus in the range of 10^2 copies per ml are viremic controllers, and patients that completely suppress virus are elite controllers. In comparison, the plasma viral loads of rhesus macaques, which develop disease, and sooty mangabeys, which remain AIDS-free, are similar to the virus levels in chronic progressors. This suggests that immune control is not likely a mechanism by which SIV-infected sooty mangabeys avoid disease

effective immune response or to defective properties of the SIV_{smm} strain.

Sooty Mangabeys Maintain Blood CD4+ T Cells but Lose Gut CD4+ T Cells During SIV Infection

A key difference that distinguishes SIV infection of sooty mangabeys from pathogenic infections is the absence of depletion of peripheral blood CD4+ T cells, which is the defining clinical feature of HIV infection. Data from SIV + sooty mangabeys at the Yerkes National Primate Research Center have documented infection of more than 200 SMs, many of which have been infected for over 20 years, in which the decline of blood CD4+ T cells does not differ perceptibly from age-matched controls (Taaffe et al. 2010). It should be noted that there is a minor exception to this general observation: depletion of CD4+ T cells was observed in a handful of SMs that were experimentally infected with an atypical strain of SIV_{smm} that utilized the CXCR4 coreceptor (rather than CCR5, which is the most commonly observed tropism in SIV and HIV strains during initial infection) (Milush et al. 2011). Remarkably, despite being CD4 low for many years, the SMs remain disease-free. While the ability of SMs to maintain their peripheral blood CD4+ T cell levels during SIV infection has been firmly established, the immunological mechanism by which this occurs remains elusive and one of the priorities of research into this species.

While the vast majority of SIV-infected SMs maintain stable levels of peripheral blood CD4+ T cells, they undergo a profound depletion (50–90%) of CD4+ T cells in the rectal mucosa within days of infection (Gordon et al. 2007), and similar findings have been shown in natural host African green monkeys. Pathogenic infection of humans and RMs also causes a rapid and severe loss of mucosal-associated CD4+ T cells. Although mucosal CD4+ T cell loss is observed in both nonpathogenic and pathogenic infections, there are, in fact, several features that differ: (1) whereas CD4+ T cell loss in RMs is continual, the levels stabilize in natural hosts; (2) the “quality” of gut CD4+ T cell loss differs – sooty mangabeys maintain a subpopulation of CD4+ T cells

called “CD4+ Th17” cells that are uniquely configured to combat gut infections and maintain the integrity of the gut epithelial barrier; and (3) while the causative link has not been established, the depletion of mucosal CD4+ T cells coincides with a breakdown of mucosal barrier continuity in SIV-infected rhesus macaques, whereas SMs maintain an intact mucosal epithelium even in the face of mucosal CD4+ T cell loss. The consequence of breaches in the mucosal barrier is the appearance of particles from resident flora entering into the systemic circulation. SIV-infected rhesus and HIV-infected humans have been shown to have an accumulation of LPS, a structural component of gram-negative bacteria, in the blood, and blood LPS levels correlate with pathogenic immune activation. This phenomenon, coined “microbial translocation,” has emerged as a major hypothesis to explain the pathogenic immune activation observed in HIV infection. Importantly, no evidence of microbial translocation has been shown in SIV-infected SMs. The ability of SMs to keep their gut architecture intact may allow them to limit the entry of microbial flora to the systemic circulation, thus avoiding immune activation. This hypothesis is an active area of research at present.

SIV-Infected Sooty Mangabeys Avoid Chronic Immune Activation

In the late 1990s, a series of large-scale clinical studies of untreated HIV-infected patients demonstrated that the strongest predictor of time-to-death was not plasma levels of virus or CD4+ T cell counts, but instead was the level of activation markers such as CD38 or HLA-DR on the surface of CD4+ and CD8+ T cells. These studies were important milestones for HIV pathogenesis because they indicated that the loss of CD4+ T cells and the destruction of immune structures were not simply a consequence of the death of infected cells, but rather due to the unrelenting “over-activation” of the immune system. This concept was supported by the observations that: (1) only a small minority of CD4+ T cells are productively infected relative to the number of dying cells and (2) sooty mangabeys did not show disease despite high viral loads. In 2003, a seminal study demonstrated that SMs chronically

infected with SIV did not exhibit any signs of CD4⁺ or CD8⁺ T cell activation (Silvestri et al. 2003). The lack of immune activation in SMs despite high viral loads was compelling evidence that the driving mechanism for CD4⁺ T cell depletion in HIV-infected humans was due to unabated activation, and not due to direct cytopathic effects of virus infecting CD4⁺ T cells. This study further established the utility of using SMs and other natural host species in combination with pathogenic hosts for comparative studies of the immune response to SIV infection to dissect “normal” responses to viral infection from “abnormal” responses that cause disease. The remainder of this entry will focus on studies that have been based on using this comparative approach.

The lack of immune activation observed in early studies suggested that SMs “ignored” the immune stimuli provided by high levels of SIV, and initial follow-up studies supported this view. However, later data demonstrated unequivocally that SMs have a widespread innate and adaptive response to the virus that is rapidly controlled within approximately 30–60 days after infection. Currently, there are several hypotheses to explain the ability of SMs to limit immune activation to the acute phase of infection.

One model proposes that the innate response to SIV is attenuated in an active manner by immunoregulatory genes that arise during the transition to chronic infection and is based off of genome-wide studies of the gene expression that occurs during SIV infection of SMs and AGMs (Bosinger et al. 2009). In these experiments, SIV infection of SMs caused a widespread induction of a class of genes known as “interferon-stimulated genes” (ISGs). Hundreds of diverse genes have been classified as ISGs and are related primarily by (1) having antiviral activities and (2) being turned on by the interferon alpha and beta cytokines. Elevated ISG expression has been detected in rhesus macaques even at 1 year post-infection, but abated quickly in sooty mangabeys and in African green monkeys, coinciding with the upregulation of immunoregulatory genes. In a follow-up experiment, exogenous interferon was administered to sooty mangabeys, and ISG expression was quickly muted, and while there

were changes in immune activation, they were transient (Vanderford et al. 2012). A long-standing hypothesis to explain AIDS pathogenesis is that the continual production of interferon in HIV infection contributes to the disease process, and the observation that both sooty mangabeys and African green monkeys shut off this response has provided additional evidence to this concept.

A second model postulates that the limited immune activation in natural hosts is due to cellular functions of the viral SIV Nef protein. Nef is highly related to disease in SIV and HIV; deletions of the *Nef* gene from the SIV genome render the virus largely apathogenic. One of the many functions of the Nef protein is to lower the amount of CD4⁺ molecule from the surface of T cells. The Nef protein in SIV_{smm} is considerably more efficient than Nef from HIV-1 (Schindler et al. 2006), allowing SIV_{smm} to cause less activation at the cellular level than HIV-1. This hypothesis is currently being tested by infecting NHPs with recombinant SIV viruses that contain the human *Nef* gene.

The models discussed above propose that the ability to resolve immune activation in natural host species is due to either direct immunoregulatory mechanisms by the host or virus. However, as will be discussed below, other recent data suggests that the lack of chronic immune activation in natural hosts is a secondary effect, due to the ability of SMs to avoid microbial translocation and/or by the ability of SMs to sequester viral replication in a manner that minimizes its impact on immune activation.

Sooty Mangabeys Compartmentalize SIV Replication in Different Immunological Niches Compared to Pathogenic Hosts

In both HIV and SIV infection, the primary cellular target of viral infection is CD4⁺ T cells. HIV and SIV both utilize the CD4 molecule as their primary receptor for cellular entry. The viruses also have secondary requirements for fusion, referred to as a “coreceptor”. In HIV/SIV infection the predominant coreceptor is the chemokine receptor CCR5, although other chemokine

receptors are also utilized albeit at a much reduced level. The CD4⁺ T cell population has been demonstrated to be the predominant target cell for HIV/SIV replication in humans and in both pathogenic and natural primate hosts. The primary function of CD4⁺ T cell within the mammalian immune system is to provide “help” to other effector cells of the immune system, such as antibody-secreting B cells and killer CD8⁺ T cells, which kill virally infected cells. Advances in our understanding of CD4⁺ T cell biology over the decade have shown that the CD4⁺ T cell compartment is in fact made up of multiple lineages phenotypically defined by their own distinct pattern of surface markers, residing in diverse anatomical niches and each providing a unique role within a functioning immune system. Simply put, all CD4⁺ T cells are not “equal.” In the sections below, the different classes of CD4⁺ T cells will be reviewed. The subsequent sections will describe how natural and pathogenic hosts differ in the subclasses of CD4⁺ T cells in which SIV is found, and the impact of this different pattern of infection on the function of the immune system.

Basic CD4⁺ T Cell Biology

The progenitors of CD4⁺ T cells move from the bone marrow to the thymus, where they undergo development from immature precursors and emerge as naïve cells. Naïve cells patrol peripherally and migrate throughout inductive secondary lymphoid organs such as lymph nodes until they come into contact with their specific antigen, displayed by MHC II molecules on the surface of antigen-presenting cells (APCs). Interaction of the T cell receptor with its cognate antigen/MHC II complex with appropriate co-stimulatory signals will activate naïve T cells, driving them to proliferate, upregulate cell-surface levels of maturation markers, and, most importantly, differentiate into non-naïve antigen-experienced effector and memory cells. CD4⁺ T cells can be classified in two fundamental ways: (1) according to their exposure to antigen and differentiation (i.e., “memory status”) and (2) based on their specialization to conduct an array of different immune activities (i.e., “functional status”).

Memory Status of CD4⁺ T Cells

During the initial period of an infection, the antigen-exposed pool of CD4⁺ T cells is predominantly comprised of effector cells that produce cytokines that provide “help” to other arms of the immune system, such as enhancing the phagocytic activity of macrophages, enabling the maturation of antibody-producing B cells, or directing the activation of antigen-specific CD8⁺ killer T cells. When the offending pathogen is cleared and the antigenic burden is removed, the pool of antigen-specific effectors rapidly contracts as the cells die. In their stead, a heterogeneous population of memory cells remain, and in the event of a secondary infection by the same pathogen, these pathogen-specific memory cells are capable of activation, expansion, and differentiation to effector cells in a manner much more rapid than naïve cells (hence they provide “memory” of a previous infection).

The memory cell populations are defined in three lineages: effector memory (Tem), central memory (Tcm), and a newly described population, stem-cell memory (Tscm). The populations differ in their anatomic location and regenerative potential and are defined from one another by distinct expression of cell-surface markers. Effector memory CD4⁺ T cells are typically found in peripheral tissues. Accordingly, they lack expression of molecules that drive migration to the lymph nodes (CCR7 and CD62L). They tend to express high levels of the chemokine receptor CCR5 that promotes chemotaxis to peripheral sites (i.e., skin, lung, mucosa) where they reside and can provide an early line of defense against infection by rapidly differentiating into effectors. Effector memory cells are relatively short lived and are capable of differentiating into effector cells with limited self-renewal. In contrast, central memory T cells (Tcm) are long-lived, multipotent cells that are enriched in secondary lymphoid tissues. Reciprocal to effector memory cells, Tcm cells are defined by expression of CD62L and CCR7. Tcm can maintain long-term immunological memory of antigen through their ability to stably self-renew. Upon the reappearance of antigen, Tcm can also divide very rapidly into effector memory cells. Because of their propensity for

self-renewal, T_{cm} are critical for maintaining a stable pool of antigen-specific CD4⁺ T cells. A recently described memory subset of T cells, termed “stem-cell memory” (T_{scm}), are extraordinarily long-lived. T_{scm} share many similar characteristics and surface markers with T_{cm}, however also maintain cell-surface markers associated with naïve cells. T_{scm} are a very small proportion of the overall T cell pool and their function overlaps somewhat T_{cm}, however they maintain a high proliferative capacity similar to naïve cells.

Functional Classes of CD4⁺ T Cells

Whereas memory status reflects the state of maturation, i.e., how far down the maturation path a T cell has gone after coming contact with its cognate antigen, CD4⁺ T cells also differentiate into specialized functional subsets that reside in different anatomical locations and carry out distinct immunological tasks. Functional subsets have been traditionally defined by the major cytokines they produce; however, improved characterization has now identified transcription factors and/or surface markers that discriminate these subsets. The classification of CD4⁺ T cells has yielded a steadily increasing number of functional lineages, and a full description is beyond the scope of this entry; instead the most well-established subsets affected by SIV infection will be discussed.

For many years, CD4⁺ T cells were thought to be polarized into two functional classes: (1) T-helper type 1 (Th1), which function to support the cell-mediated arm of the adaptive immune system by the production of cytokines such as IL2 and IFN γ that enhance the functions of macrophages and cytotoxic/killer CD8⁺ T cells, and (2) T-helper type 2 (Th2) that improve the humoral response (i.e., antibody-mediated response via B cells) through the production of cytokines such as IL4, IL5, IL10, and others. However, novel, more specialized subclasses of CD4⁺ T cells have recently been described. One such subset is CD4⁺ T follicular cells (T_{fh}), which are memory cells that are defined by the surface markers CXCR5 and PD1. These cells colocalize with B cells within the germinal centers

in lymph nodes, and their function is to enhance the antibody response by facilitating class-switching and somatic hypermutation of immunoglobulin genes that improve the affinity and avidity of the resultant antibodies.

Another recently described functional subclass of CD4⁺ T cells are Th17 cells, which were briefly introduced in a preceding section of this entry. While a definitive set of surface markers for this subset have not been described, Th17 are defined by high production of IL17 and IL22 and expression of the transcription factor ROR γ . A major function of Th17 is to provide immunity against fungal and bacterial pathogens; they also support the proliferation of mucosal epithelial cells via the production of IL22, and dysregulation of these cells has been associated with intestinal autoimmune disorders.

Lastly, CD4⁺ T regulatory cells (Tregs) are defined by their immunoregulatory activities, primarily secretion of the suppressive cytokines IL10 and TGF β . The best characterized subset of Tregs are defined by surface expression of the CD25 markers and the transcription factor FOXP3.

Sooty Mangabeys and Natural Hosts Exhibit a Differential Pattern of SIV Infection in CD4⁺ Subsets Compared to Pathogenic Hosts

As new lineages of CD4⁺ T cells were described in mice and humans, research into their depletion, activation status, and in vivo infection within macaques and SMs have yielded important observations that help to explain the natural host phenotype. These results have clearly established that natural hosts are able to maintain SIV replication in a different pattern of cells compared to non-natural hosts. Sooty mangabeys maintain a lower frequency of SIV-infected CD4⁺ T_{cm} when compared to infected RMs (Paiardini et al. 2011). This observation is important because several studies have shown that, consistent with their crucial role in maintaining CD4⁺ T cell homeostasis, depletion of CD4⁺ T_{cm} in RMs predicts the rate of disease progression. The limitation of infection in CD4⁺ memory cells appears to be a consistent theme in natural host infection, as African green monkeys also have limited infection in

their Tcm and Tem compartments. Visualization experiments have shown that SIV infection also differs at the anatomical level; in sections of lymph nodes taken from SIV + RMs and HIV-infected subjects, infected cells were detected at high frequencies in B cell follicles, but were absent from these areas in SMs; instead infected cells were detected in extrafollicular T cell zones. Consistent with anatomical location data, higher levels of cell-associated SIV have been observed in CD4+ Tfh cells from rhesus macaques compared to SMs. The collective observation of natural hosts minimizing infection in vulnerable subsets of CD4+ T cells has led to a model to explain non-pathogenesis in SMs termed the “target restriction hypothesis.” Under this model, natural host shifts the majority of the burden of viral replication away from cell subsets critical for homeostatic maintenance of the CD4+ T cell pool and favors replication in cells such as effector memory cells that are short-lived and, while they are needed to combat infection, are ultimately dispensable.

Sooty Mangabeys Have a Significantly Low Rate of Mother-to-Child Transmission of SIV

Of the 30 million people infected with HIV worldwide, an estimated 3.3 million are children. Mother-to-child transmission (MTCT) accounts for an estimated 370,000 pediatric infections annually. Antiretroviral regimens administered to pregnant mothers have been one of the victories of HIV prevention research, reducing transmission from an estimated 40% to below 5%. Unfortunately, in resource-limited settings, access to ART during childbirth is still underutilized, and thus understanding the mechanisms by which MTCT can be limited immunologically is important. In this regard, natural hosts provide an interesting model. In macaques, the rate of perinatal transmission has been estimated to be 25–75% in various studies; in SMs the rate is significantly reduced at 7% (Chahroudi et al. 2012). Studies of mandrills and African green monkeys have shown that they have similarly low transmission rates.

Interestingly, SMs that are infected by vertical transmission have plasma viral loads that are approximately two orders of magnitude lower than animals acquiring SIV later in life. Research into the mechanisms underlying limited MTCT in SMs is still in early stages. Comparison of SMs and RMs has not observed any differences in the level or infectivity of virus in the breast milk between species. However, infant SMs have a drastically reduced expression of the SIV entry receptor CCR5 on CD4+ cells in tonsils, esophagus, and mucosal lymphoid tissue, suggesting that the low transmission rate may be due to a lower number of potential targets for viral infection/replication. The observation of low CCR5 in both infant and adult natural hosts suggests that modulation of entry receptors may be an evolutionary adaptation that allowed natural hosts to reduce infection. Given the low rate of vertical transmission but the high population prevalence of SIV in wild mangabeys (estimated at approximately 70%), it is likely that the majority of transmission events occur by sexual contact. Overall, this would restrict most transmissions until child-bearing years and limit the impact of SIV infection on the fitness of the species as a whole.

Lessons from Genetic and Genomic Studies of Sooty Mangabeys

The use of natural hosts for detailed immunological comparative studies has been conducted for a little over a decade, but has already proven a useful tool to identify components of the immune system critical to avoiding pathogenic disease. The advent of high-throughput genomic technology should allow for greater insight in coming years. Already, microarray analysis of the immune response to SIV detectable in blood identified the ability of SMs and African green monkeys to resolve immune activation. Currently, two large-scale studies utilizing next-generation sequencing technology to study SMs and natural hosts are underway: (1) the first is using RNA-Seq technology to characterize genome-wide level in multiple classes of immune cells, including the CD4+ T cell memory subsets described here, and

(ii) sequencing of the DNA genome of sooty mangabeys for comparison with human, rhesus and cynomolgus macaques, and African green monkeys to identify potential immune modulating genes under recent evolutionary selection. The unraveling of the genome and transcriptome of SMs will be exciting advances because they will provide the power to examine natural host biology in the absence of preformed hypotheses. Thus, genomic information can be a “roadmap” that leads us to important differences unique to natural hosts.

Other recent studies have not performed genome-wide characterization, but have sequenced individual genes within the majority of animals in the sooty mangabey colony. One such study examined the protein coding regions of IRF7, a signaling molecule that regulates interferon production: these results showed that IRF7 was not appreciably different from human or rhesus IRF7 and likely not responsible for differences in disease. In contrast, the reduced expression of CCR5 in SMs and other natural host species provided a strong rationale for the sequencing of this gene across the colony. Compellingly, approximately 8% of sooty mangabeys are homozygous for one of two mutations in CCR5 (CCR5 Δ 2 and CCR5 Δ 24) that abrogates its ability to be expressed on the cell surface (Riddick et al. 2010). In comparison, an analogous mutation in human CCR5 that provides resistance to infection, named CCR5 Δ 32, is present in homozygosity at about 1% in the Caucasian population. The enrichment of CCR5-null mutations in the species provides a compelling argument for a central role of this coreceptor in dictating pathogenesis. Interestingly, homozygote CCR5-null sooty mangabeys are not infected at lower prevalence, and plasma viral load is only marginally lower (although statistically significant) compared to wild-type animals. This observation, in combination with other studies, have shown that sooty mangabeys have the ability to permit infection by cellular coreceptors other than CCR5. The permissiveness of CCR5-null animals provides another line of evidence that an evolutionary “strategy” employed by sooty mangabeys is to “shunt” infection into dispensable targets,

rather than to block infection altogether. While this “strategy” has the disadvantage of allowing infection, the advantage is that it removes selective pressure for the virus to evolve in unpredictable ways which may ultimately become pathogenic. A compelling line of evidence supports the concept for a central role in CCR5 in permissiveness and disease in HIV infection: the continued, successful “functional cure” (defined as the absence of detectable viremia despite cessation of antiretroviral therapy) for over 5 years of an HIV-positive patient receiving a bone marrow transplant from a donor homozygous for the null CCR5 Δ 32 mutation, known as the “Berlin Patient.” Thus, therapies aimed at reducing the ability of HIV to utilize CCR5 via genetic targeting or pharmacological manipulation may hold real promise for reducing disease in HIV infection. The association between low levels of CCR5 on target cells and reduced MTCT in sooty mangabeys suggests that reduction of CCR5+ targets in viral inductive sites may be a viable strategy for preventative modalities.

Concluding Remarks

Significant progress has been made in characterizing SIV infection in the context of nonpathogenic infection. Despite a wealth of data, the field has not been able to definitively demonstrate that modulation of an immunological pathway can “break” the sooty mangabey phenotype and cause AIDS. This is likely due to the inherent technical difficulties in conducting in vivo interventions in monkeys and the multifactorial nature of the phenotype. Of importance, recent studies have identified a population of HIV-infected patients that recapitulate the natural host phenotype. These patients, termed “viremic non-progressors,” maintain high levels of virus from many years without appreciable CD4+ T cell loss and share many immunologic and transcriptomic features with SIV+sooty mangabeys. The identification of VNPs is strong evidence that research into the natural host model is more than an intellectual curiosity, and will have direct relevance for human health.

Cross-References

- ▶ [Central Memory CD4 T Cells](#)
- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [Microbial Translocation](#)
- ▶ [SIVmac Infection of Macaques, Immunopathogenesis of](#)

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NRTIs

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Definition

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs) are a class of antiretroviral (ARV) medications primarily used to treat HIV infection. Two NRTIs (often referred to as the ARV “backbone”) are commonly included as part of a three- or four-drug treatment regimen. The NRTI class comprises one nucleotide analogue, tenofovir disoproxil fumarate (DF), and six nucleoside analogues: abacavir, didanosine, emtricitabine, lamivudine, stavudine, and zidovudine. These medications act to inhibit the active site of the enzyme reverse transcriptase, which is necessary for the synthesis of viral complementary DNA (cDNA) from viral RNA as part of the HIV replication cycle. Zalcitabine, a nucleoside analogue, was previously used in clinical practice but has since been discontinued by its

manufacturer. A new nucleotide analogue, tenofovir alafenamide (AF), is in phase III clinical trials and has been submitted for approval to regulatory agencies in the United States and Europe.

Mechanism of Action

Each NRTI is an analogue of one of the four nucleosides found in DNA: adenosine (didanosine), cytidine (lamivudine and emtricitabine), guanosine (abacavir), and thymidine (stavudine and zidovudine). Tenofovir, with a chemical structure that includes a phosphate group, is an analogue of the nucleotide adenosine monophosphate. Owing to the poor oral bioavailability of tenofovir, the prodrug tenofovir DF is used clinically.

As a class, NRTIs have activity against both HIV-1 and HIV-2. NRTIs block the activity of the viral enzyme reverse transcriptase, a DNA polymerase that synthesizes viral cDNA from HIV RNA. In order to effectively inhibit reverse transcriptase, NRTIs must first be phosphorylated by intracellular kinases into an active triphosphate form. While NRTIs target viral reverse transcriptase, these drugs have variable affinity for native DNA polymerases, especially mitochondrial DNA polymerase- γ . Inhibition of mitochondrial activity is thought to be responsible for most of the toxicity observed in the earlier medications in this drug class, such as lipoatrophy, peripheral neuropathy, and lactic acidosis.

History

The first five ARVs approved by the United States Food and Drug Administration (FDA) for the treatment of HIV infection were from the NRTI class: zidovudine (1987), didanosine (1991), zalcitabine (1992), stavudine (1994), and lamivudine (1995). The FDA later approved abacavir (1998), tenofovir DF (2001), and emtricitabine (2003). The sale and distribution of zalcitabine was suspended in 2006, owing to comparatively poor efficacy and high rates of toxicity.

NRTIs were initially approved for use as monotherapy against HIV infection, and expert

guidelines recommended zidovudine as first-line therapy for patients with symptomatic HIV infection or CD4+ T-cell counts of less than 200–500/ μ L. Drug-related adverse events complicated the use of the first generation of NRTIs, such as bone marrow suppression from zidovudine and mitochondrial toxicity – primarily from didanosine, zalcitabine, and stavudine – leading to lactic acidosis, peripheral neuropathy, myopathy, hepatic steatosis, and lipoatrophy. While zidovudine monotherapy was shown to reduce HIV mortality, high rates of HIV disease progression and death led many providers to prescribe dual NRTI therapy in the early and mid-1990s. Dual NRTI therapy often delayed disease progression, yet various combinations of early drugs proved problematic due to overlapping toxicities (particularly from didanosine with stavudine) and in vivo antagonism (zidovudine with stavudine). The approval of lamivudine in 1995, however, demonstrated the promise of dual NRTI therapy as combination treatment with zidovudine slowed HIV progression more than either agent alone and was associated with a favorable tolerability profile. The approval of the first drugs in two new classes of ARV medications – protease inhibitors (PIs), saquinavir, indinavir, and ritonavir (1995–1996), and non-nucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine (1996) and efavirenz (1998) – paved the way for triple therapy that included combinations from different drug classes. Early clinical trials of a PI combined with two NRTIs showed improved efficacy when compared to NRTI mono and dual therapy. The first United States Department of Health and Human Services (DHHS) *Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents* in 1998 recommended the combination of one PI and two NRTIs over two NRTIs alone.

The FDA approval of abacavir, tenofovir DF, and emtricitabine was based upon virologic efficacy as well as substantially less toxicity when compared to older NRTIs. Improved tolerability to these medications created renewed interest in combination NRTI therapy alone, this time in the form of three-drug combinations. Clinical trials, however, were disappointing as

combination therapy with abacavir-lamivudine-zidovudine (Gulick et al. 2004) and abacavir-lamivudine plus tenofovir DF (Gallant et al. 2005) demonstrated inferiority to therapy with two NRTIs plus efavirenz. Consequently, clinical practice has continued to favor the inclusion of two NRTIs as the “backbone” of ARV therapy with partner drugs from either the NNRTI, PI, or more recently the integrase strand transfer inhibitor (INSTI) class and occasionally an additional medication to serve as a pharmacologic booster. Many pairs of NRTIs are also available as fixed-dose combinations to improve the convenience

of medication administration. Some combinations also include the partner drug or drugs so that a complete ARV regimen may be taken in a single tablet.

Individual NRTIs and Combination Pills

Individual NRTIs and combination pills, along with key characteristics, are listed in Table 1. Each NRTI is subsequently described with a focus on important toxicities and key clinical features.

NRTIs, Table 1 Nucleoside and nucleotide reverse transcriptase inhibitor (NRTI) characteristics^a

Drug (abbreviation)	Trade name	Year of FDA approval	Usual adult dose	Route(s)	Form(s)
<i>Single NRTIs</i>					
Abacavir (ABC)	Ziagen [®]	1998	600 mg once daily or 300 mg twice daily	Oral	Tablet, suspension
Didanosine (ddI)	Videx [®] , Videx EC [®]	1991	400 mg once daily	Oral	Powder, capsule
Emtricitabine (FTC)	Emtriva [®]	2003	200 mg once daily	Oral	Capsule, solution
Lamivudine (3TC)	Epivir [®]	1995	300 mg once daily or 150 mg twice daily	Oral	Tablet
Stavudine (d4T)	Zerit [®]	1994	30 mg or 40 mg twice daily	Oral	Capsule, liquid
Tenofovir disoproxil fumarate (TDF)	Viread [®]	2001	300 mg once daily	Oral	Tablet, powder
Zalcitabine (ddC) ^b	Hivid [®]	1992	0.75 mg thrice daily	Oral	Tablet
Zidovudine (AZT, ZDV)	Retrovir [®]	1987	300 mg twice daily	Oral, IV	Tablet, capsule, syrup, vial
<i>Co-formulated ARVs with NRTIs</i>					
3TC/AZT (150 mg/300 mg)	Combivir [®]	1997	1 pill twice daily	PO	Tablet
ABC/3TC (600 mg/300 mg)	Epzicom [®] , Kivexa [®]	2004	1 pill daily	PO	Tablet
ABC/3TC/AZT (300 mg/150 mg/300 mg)	Trizivir [®]	2000	1 pill twice daily	PO	Tablet
ABC/3TC/DTG (600 mg/300 mg/50 mg)	Triumeq [®]	2014	1 pill daily	PO	Tablet
TDF/FTC (300 mg/200 mg)	Truvada [®]	2004	1 pill daily	PO	Tablet
TDF/FTC/EFV (300 mg/200 mg/600 mg)	Atripla [®]	2006	1 pill daily	PO	Tablet
TDF/FTC/RPV (300 mg/200 mg/25 mg)	Complera [®]	2011	1 pill daily	PO	Tablet
EVG/Cobi/FTC/TDF (150/150 mg/200 mg/300)	Stribild [®]	2012	1 pill daily	PO	Tablet

DTG dolutegravir, EFV efavirenz, RPV rilpivirine, EVG/COBI elvitegravir/cobicistat

^aThe dosage and use of these medications should be in direct consultation with a medical practitioner

^bWithdrawn from the market

Abacavir

Abacavir is currently one of the most commonly prescribed NRTIs. In contrast to tenofovir DF, abacavir does not have significant adverse effects on renal function or bone mineral density. The primary toxicity related to abacavir is a hypersensitivity reaction characterized by fever and a combination of symptoms that may include rash, gastrointestinal upset, headache, dyspnea, and cough. Continued use of abacavir despite a hypersensitivity reaction (or re-challenge following the reaction) can be fatal. Abacavir hypersensitivity reaction is strongly associated with human leukocyte antigen (HLA) B*5701 allele, and therefore HLA-B*5701 testing is recommended prior to use. Patients who test positive for HLA-B*5701 should avoid abacavir, while those testing negative are at very low risk of having a hypersensitivity reaction. In addition to the risk of hypersensitivity reaction, abacavir use has been complicated by a controversial association with increased cardiac events. This association was first discovered in an analysis of the D:A:D collaboration (an international group of 11 prospective cohorts of HIV-infected patients), which demonstrated an approximately two-fold increased risk of cardiovascular events in those who had recently taken or were currently receiving abacavir (D:A:D Study Group et al. 2008). Although the relative risk for such events was increased in all patients, the absolute risk was highest for those with multiple cardiovascular risk factors. Subsequent observational studies, randomized controlled trials (RCTs), and case-control studies have produced conflicting results with two meta-analyses of multiple RCTs not revealing an association between abacavir and an increased risk for cardiovascular events. Given the discrepancy in results and the lack of an adequately powered RCT, many practitioners avoid the use of abacavir in patients at high risk for cardiovascular events.

Didanosine

The clinical use of didanosine is limited due to the development of other NRTIs with more favorable side effect profiles. Didanosine use has been complicated by gastrointestinal upset, dose-dependent

peripheral neuropathy (17–20% of patients), dose-dependent pancreatitis (1–7%), lipoatrophy, lactic acidosis, and non-cirrhotic portal hypertension. Concomitant administration of didanosine and stavudine greatly increases the rate and severity of these side effects due to the overlapping toxicities of both medications.

Lamivudine and Emtricitabine

Lamivudine and emtricitabine are discussed together as they are structurally similar, inhibit viral reverse transcriptase in the same manner, and have similar side effect profiles. Both drugs are well tolerated, do not affect mitochondrial function *in vitro*, and demonstrate side effect profiles comparable to placebo. Resistance mutations, however, can develop rapidly on therapy, particularly when the ARV regimen has suboptimal potency. Inclusion of one of these drugs is recommended in all first-line ARV regimens for treatment-naïve patients, although they should never be used together. Lamivudine and emtricitabine both have activity against hepatitis B virus (HBV), as does tenofovir DF with which emtricitabine is co-formulated. Inclusion of emtricitabine-tenofovir DF is recommended as part of the ARV regimen of all patients with HBV coinfection in the absence of any contraindications. This co-formulation has also demonstrated clinical benefit when taken as pre-exposure prophylaxis (PrEP), a key HIV prevention measure for high-risk uninfected individuals.

Stavudine

Much like didanosine, stavudine is rarely used due to the development of other NRTIs with more favorable side effect profiles. Stavudine use has been complicated by gastrointestinal upset and mitochondrial toxicity manifesting as dose-dependent peripheral neuropathy (8–21% of patients), dose-dependent pancreatitis, lipoatrophy, and lactic acidosis. Concomitant administration of stavudine and didanosine also exacerbates these side effects due to the overlapping toxicities of both medications.

Tenofovir DF

Tenofovir DF is well tolerated, efficacious, and recommended as part of most first-line ARV

regimens for treatment-naïve patients. Side effects due to mitochondrial toxicity are rare in comparison to earlier NRTIs. Like lamivudine and emtricitabine, tenofovir DF has activity against HBV and is recommended as part of the ARV regimen of all coinfecting patients, barring any contraindications. As above, the co-formulation of emtricitabine-tenofovir DF has demonstrated clinical efficacy when taken as PrEP. Tenofovir DF has the added benefit of improving the lipid profile of patients although the clinical significance of this finding is unclear. The two principal toxicities of tenofovir DF are renal damage and increased loss of bone mineral density. Tenofovir DF-mediated renal toxicity (<5% of patients) comes in the form of proximal tubular dysfunction, acute kidney injury, and chronic kidney disease, most frequently after long-term use and in patients with preexisting kidney disease or those taking concurrent nephrotoxic medications. Early drug withdrawal leads to partial or complete reversal of renal toxicity in many, but not all, patients. Due to this toxicity, tenofovir DF is not recommended for those with an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m² or those who experience a >25% decline in eGFR while taking the medication. An accelerated reduction in bone mineral density, relative to other NRTIs, also complicates long-term tenofovir DF use. While fragility fractures have been reported, it is uncertain to what degree tenofovir DF affects fracture risk and long-term bone health. Regardless, this drug should be used with caution in those patients at high risk of fragility fractures.

Zalcitabine

Zalcitabine was approved in 1992 but removed from the market in 2006 due to toxicity and poor data on virologic efficacy. In addition to the host of side effects mediated by mitochondrial toxicity, zalcitabine was associated with aphthous stomatitis.

Zidovudine

The first approved NRTI, until recently, zidovudine had been widely used in clinical practice. This drug, however, is no longer recommended for most patients because it is less convenient than

other options (dosing is twice daily) and, even more importantly, it is associated with an increased risk of adverse events including hematologic (anemia and neutropenia) and mitochondrial toxicity. While zidovudine is no longer recommended as part of first-line therapy for treatment-naïve patients, it does have a role in the prevention of mother to child transmission, given the extensive clinical experience in this setting. Zidovudine is often included in the antepartum ARV regimen of pregnant women and is given intravenously during the intrapartum period for pregnancies with a higher risk of transmission. It is also administered to newborns during the first 6 weeks of life as postexposure prophylaxis.

NRTI Resistance

Select mutations in the reverse transcriptase gene are associated with resistance to various NRTIs (Wensing et al. 2014). The reverse transcriptase mutations that are most commonly encountered and most likely to affect clinical outcomes are the thymidine analogue-associated mutations (TAMs; M41L, D67N, K70R, L210W, T215Y, K219E/Q), K65R, L74V, M184V, the 69 insertion complex, and the 151 complex.

TAMs arise after exposure to the thymidine analogues (zidovudine and stavudine). Most commonly a series of TAMs is encountered that follows either the TAM1 (M41L, L210W, T215Y) or TAM2 (D67N, K70R, K219E/Q) pathway. TAMs confer reduced susceptibility to all NRTIs, although resistance is greater with the mutations in the TAM1 pathway. The presence of T215Y may increase susceptibility to the NNRTI class.

The M184V mutation is frequently the first mutation seen in patients failing a regimen that includes lamivudine or emtricitabine. This mutation confers outright resistance to both medications, while also reducing susceptibility to abacavir, particularly if at least one TAM is present. The M184V mutation, however, does lead to enhanced susceptibility to zidovudine, tenofovir DF, and stavudine and is associated with a decrease in viral fitness. These benefits, coupled with the fact that lamivudine and emtricitabine are

extremely well tolerated, means that one of these drugs is often continued in treatment-experienced patients who have the M184V mutation as a way of maintaining selection pressure for this mutation.

When emtricitabine is combined with tenofovir DF as first-line therapy, the K65R mutation is often the next NRTI mutation to appear after M184V. The K65R mutation confers reduced susceptibility to abacavir, didanosine, emtricitabine, lamivudine, stavudine, and tenofovir DF. Zidovudine susceptibility is preserved. Patients on lamivudine with abacavir often develop the L74V mutation following M184V and this mutation reduces susceptibility to both abacavir and didanosine.

An insertion complex is when two or more amino acids are inserted at a particular codon. The 69 insertion complex consists of T69S with an additional amino acid added to serine. The 69 insertion complex is typically part of a pattern of mutations that may include A62V and any TAMs (except D67N). In the presence of at least one mutation from the TAM1 pathway, the 69 insertion complex is associated with resistance to all NRTIs. The 151 complex comprises Q151M with up to four other mutations (A62V, V75I, F77L, F116Y). When Q151M is present, there is reduced susceptibility to all NRTIs except tenofovir DF.

Use of NRTIs in Current Clinical Practice

Due to excellent virologic efficacy, tolerability, and the convenience of fixed-dose combinations and single tablet regimens, tenofovir DF with emtricitabine and abacavir with lamivudine are the NRTI backbones of choice for most treatment-naïve patients. The role of abacavir with lamivudine was established in 2004 from the results of a randomized, double-blind non-inferiority trial in treatment-naïve patients. In this trial – where both arms were also given the NNRTI efavirenz – once-daily abacavir with lamivudine demonstrated non-inferior virologic efficacy when compared to twice-daily zidovudine with lamivudine (DeJesus et al. 2004). Two years later, in an open-label non-inferiority treatment-naïve trial also involving

efavirenz, the combination of once-daily tenofovir DF with emtricitabine was found to have superior efficacy and a more favorable side effect profile than zidovudine with lamivudine (Gallant et al. 2006).

When considering tenofovir DF with emtricitabine versus abacavir with lamivudine for treatment-naïve patients, the selection of the optimal NRTI backbone typically depends upon HLA-B*5701 testing, the presence of comorbidities, the partner drug, and the pretreatment plasma HIV RNA level. Among patients with the HLA-B*5701 allele or HBV, and perhaps those with multiple cardiovascular risk factors, tenofovir DF with emtricitabine is favored. In contrast, among patients with chronic kidney disease or osteoporosis, abacavir with lamivudine may be favored.

In the absence of specific comorbidities, clinical trials with different partner drugs can inform the best approach to selecting between tenofovir DF with emtricitabine and abacavir with lamivudine as the NRTI backbone. When these two combinations have been compared in RCTs, results have varied depending on the partner drug and study design. In the first double-blind non-inferiority trial of treatment-naïve patients comparing both combinations, they were found to have comparable virologic efficacy, safety, and tolerability when given with the PI lopinavir with ritonavir (Smith et al. 2009). A later trial compared both combinations with either efavirenz or atazanavir plus ritonavir. Among patients with pretreatment plasma HIV RNA levels of greater than 100,000 copies/mL, the time to virologic failure was shorter in patients receiving abacavir with lamivudine irrespective of the partner drug (Sax et al. 2009). In contrast, there was no difference in time to virologic failure among patients with a pretreatment plasma HIV RNA level of less than 100,000 copies/mL (Sax et al. 2011). The difference in the results between the studies may reflect the difference in the partner drugs used, such as lopinavir and ritonavir in one study and efavirenz and atazanavir plus ritonavir in the other, or study design differences. As a result of these studies, tenofovir DF with emtricitabine is the NRTI backbone of choice when used with

NRTIs,

Table 2 Recommended first-line antiretroviral regimens for treatment-naïve patients with any viral load from three expert panels

	US DHHS ^a 2015	IAS-USA ^b 2014	EACS ^c 2014
ABC/3TC + DTG	✓	✓	✓
TDF/FTC + DTG	✓	✓	✓
TDF/FTC/EVG/COBI	✓	✓	✓
TDF/FTC + RAL	✓	✓	✓
TDF/FTC + DRV/r	✓	✓	✓
TDF/FTC + ATV/r		✓	✓
TDF/FTC/EFV		✓	✓
ABC/3TC + RAL			✓

^aUnited States Department of Health and Human Services

^bInternational Antiviral Society-USA Panel

^cEuropean AIDS Clinical Society

ABC/3TC abacavir/lamivudine, *DTG* dolutegravir, *TDF/FTC* tenofovir DF/emtricitabine, *EVG/COBI* elvitegravir/cobicistat, *RAL* raltegravir, *DRV/r* darunavir plus ritonavir, *ATV/r* atazanavir plus ritonavir, *TDF/FTC/EFV* tenofovir DF/emtricitabine/efavirenz

efavirenz or atazanavir plus ritonavir, particularly in patients with a pretreatment plasma HIV RNA of greater than 100,000 copies/mL.

While tenofovir DF with emtricitabine was the superior NRTI backbone for some regimens, the partner drug plays an important role. For example, the single tablet regimen (STR) tenofovir DF with emtricitabine and the NNRTI rilpivirine was inferior to the STR tenofovir DF with emtricitabine and efavirenz in a subgroup analysis of patients with pretreatment plasma HIV RNA levels greater than 100,000 copies/mL (Cohen et al. 2013). In addition, abacavir with lamivudine is a highly effective NRTI backbone when paired with the INSTI dolutegravir, available as a STR. This three-drug combination was superior to the STR tenofovir DF with emtricitabine and efavirenz in another RCT of treatment-naïve patients regardless of baseline plasma HIV RNA (Walmsley et al. 2013).

Informed in large part by these studies, first-line regimens for treatment-naïve patients (irrespective of pretreatment plasma HIV RNA) from three expert panels are summarized in Table 2. Of note, the most recent World Health Organization guidelines recommend tenofovir DF with emtricitabine (or lamivudine) and efavirenz as first-line therapy for adults in resource-limited settings.

Among patients who have failed first-line therapies, the role of NRTIs is complicated and depends on resistance mutations, medications

involved in the failing regimen, ARV treatment history, and comorbid conditions. In two RCTs of patients who failed therapy with NRTIs and an NNRTI, treatment with lopinavir plus ritonavir and two to three new or recycled NRTIs achieved rates of virologic suppression comparable to lopinavir plus ritonavir and the INSTI raltegravir, despite the presence of substantial resistance to some of the selected NRTIs (SECOND-LINE Study Group et al. 2013; Paton et al. 2014). One of these studies (Paton et al. 2014) also included a study group treated with only lopinavir plus ritonavir. This study group was inferior to the PI with NRTIs study group, suggesting that the NRTIs are an important part of the regimen despite the presence of resistance to multiple drugs in the class. As seen in these trials, the need for NRTIs depends upon how many other active drugs are in the regimen. Similar to the effectiveness seen with the PI plus INSTI study arms, another study demonstrated that if there are more than two fully active drugs used in highly treatment-experienced patient populations, overall efficacy is not impacted by the addition of NRTIs (Tashima et al. 2013).

Future Directions

Despite the successes of NRTIs, bone and renal toxicity (tenofovir DF), hypersensitivity reactions and associations with increased risk of

cardiovascular events (abacavir), and a low barrier to drug resistance (lamivudine and emtricitabine) all provide compelling reasons to develop new NRTIs and to investigate the role for NRTI-sparing regimens. The only NRTI currently in phase III clinical trials is tenofovir AF, another tenofovir prodrug. Compared to tenofovir DF, tenofovir AF achieves higher intracellular concentrations of tenofovir, allowing for reduced dosing and resulting in lower circulating plasma levels. Initial studies of tenofovir AF have demonstrated equivalent virologic efficacy, potentially less renal toxicity and smaller declines in bone mineral density, with a somewhat less favorable lipid profile when compared to tenofovir DF (Sax et al. 2015). Tenofovir AF activity against HBV is being studied. If approved by the FDA, tenofovir AF could be co-formulated with many of the same medications as tenofovir DF, and a new STR with tenofovir AF, emtricitabine, darunavir, and cobicistat is currently under investigation.

Regimens that spare all NRTIs, or at least abacavir and tenofovir DF, have demonstrated favorable results in initial studies, but are not commonly used in clinical practice due to limited data. For example, an RCT of treatment-naïve patients compared ritonavir-boosted darunavir with either raltegravir or two NRTIs. This study demonstrated that NRTI-sparing regimen was less effective in those with fewer than 200 CD4+ T-cells or plasma HIV RNA levels greater than 100,000 copies/mL at baseline (Raffi et al. 2014). Another study of treatment-naïve patients showed that lopinavir plus ritonavir was just as efficacious when given with lamivudine alone as with two NRTIs (Cahn et al. 2014). There are several switch studies that are also pursuing treatment that spares tenofovir DF and abacavir, such as the SALT study where suppressed patients were switched to ritonavir-boosted atazanavir with lamivudine alone or with two NRTIs. Outcomes were similar in both arms (Perez-Molina et al. 2015). In current clinical practice, these NRTI-sparing regimens are mostly used in those for whom there is concern regarding the use of abacavir and tenofovir DF. Further studies will be needed to determine if regimens such as these are appropriate for other patients who are

treatment naïve, desiring a switch to a new regimen or failing therapy.

Conclusions

NRTIs are a vital component of all first-line ARV regimens and many second-line and salvage regimens. As the oldest class of ARV medications, there is a wealth of clinical experience and well-designed RCTs to support the use of NRTIs to treat HIV infection. While the use of the earlier NRTIs such as zidovudine, stavudine, and didanosine were limited by safety and tolerability, relatively newer medications – lamivudine, emtricitabine, abacavir, and tenofovir DF – have markedly improved side effect profiles while retaining excellent virologic efficacy. Current expert guidelines recommend either the combination of tenofovir DF with emtricitabine or abacavir with lamivudine as the NRTI backbone in all treatment-naïve patients. The selection between these two options depends on HLA-B*5701 testing, the presence of comorbidities, the partner drug, and the pretreatment plasma HIV RNA levels and CD4+ T-cell counts. Despite the successes of ARV therapy involving an NRTI backbone, clinically significant toxicities and tolerability issues remain and are the impetus for the development of better medications in the class or regimens that avoid NRTIs entirely.

Cross-References

- ▶ [Antiretroviral Treatment in Resource-Limited Settings](#)
- ▶ [Initial Antiretroviral Regimens](#)
- ▶ [Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis](#)
- ▶ [Treatment Failure and Resistance](#)

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Nuclear Import: HIV-1 Goes NUPs

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Definition

A key feature of human immunodeficiency virus type 1 (HIV-1) and other lentiviruses is their

ability to traverse an intact nuclear envelope and productively infect nondividing cells. The nuclear import mechanisms of HIV-1 appear to involve interactions with several nucleoporin members, the constituent building blocks of nuclear pore complexes that span across the nuclear envelope.

Transport Pathways Between Nuclear and Cytoplasmic Compartments

Eukaryotic cells segregate their genomic information from the cytoplasmic milieu by sequestering the genome within the nuclear envelope, thereby defining the boundaries of the nuclear and cytoplasmic compartments of the cell. The presence of the nuclear envelope necessitates that eukaryotic cells maintain a continuous exchange of proteins and other macromolecules between these distinct cellular compartments to sustain proper cellular function. For example, transcription factors must reach their appropriate gene targets in the nucleus, and mRNA transcripts must locate to the cytoplasm for translation by the protein synthesis machinery.

The regulation of information exchange between the nucleus and cytoplasm must balance the need for high specificity with the need for rapidity and efficiency during transport. This can be exemplified by the rapid transduction of extracellular stimuli into a specific response from the cellular transcription machinery. To achieve this important regulatory balance, eukaryotic cells have evolved an elaborate pathway to mediate appropriate nucleocytoplasmic transport (reviewed in Terry et al. 2007). Key features of the regulatory pathway mediating nucleocytoplasmic transport include peptide signal sequences, or nuclear localization sequences (NLS), that identify proteins as appropriate substrates for transport into the nucleus. Nuclear import receptors belonging to the importin family of proteins recognize and bind to NLS to form complexes competent for nuclear transport. The directionality of importin-mediated nuclear transport is determined by a gradient of nucleotide-bound Ran across the nuclear envelope. High levels of Ran bound to GDP (RanGDP) are maintained in the cytoplasm by a RanGTPase-

activating protein (RanGAP) together with the Ran-binding protein 1 (RanBP1). These proteins enhance the GTPase activity of Ran in the cytoplasm leading to accumulation of RanGDP. Conversely, in the nucleus high levels of Ran bound to GTP (RanGTP) are maintained by the exclusively nuclear guanine nucleotide exchange factor (RanGEF) known as regulator of chromosome condensation 1 (RCC1). When GDP is exchanged for GTP on Ran in the nucleus, disassembly of transport complexes occurs due to the increased binding affinity of RanGTP for importin, effectively displacing the import cargo from the receptor. This entry restricts discussion on pathways pertinent to HIV-1. A more complete discussion on nuclear import pathways can be found elsewhere (Terry et al. 2007).

Bi-directional transport between the nucleus and cytoplasm takes place through the nuclear pore complex (NPC), a large macromolecular complex that forms an aqueous channel that spans the nuclear envelope (Tetenbaum-Novatt and Rout 2010). The NPC functions as the gateway between the nuclear and cytoplasmic domains of the cell by serving as a molecular sieve that allows the passage of small molecules (with a cutoff of approximately 50 kDa) but imposes a strict requirement of active transport on larger molecules (Terry et al. 2007). The structural organization of the NPC is central to its role in nuclear transport. While structural nuclear pore proteins (nucleoporins, NUPs) anchor the NPC in the nuclear envelope, NUPs containing phenylalanine-glycine repeat domains (FG-NUPs) organized at the cytoplasmic face and along the aqueous channel of the complex serve as contact points for assembled transport complexes during translocation through the NPC (reviewed in Terry and Wentz 2009). Models describing the role of FG repeats during transport include “selective gating” where FG-repeat domains provide a transient but specific binding platform for import complexes during passage through the NPC, while function concurrently as a physical barrier to restrict passage of macromolecules in the absence of import receptor binding. Alternatively, the “selective phase” model proposes that FG-repeat domains form a gel-like

barrier through which only receptor-associated cargos can dissolve. In both models, the FG-NUPs present a formidable entropic barrier for entry into the nucleus (reviewed in Tetenbaum-Novatt and Rout 2010).

While receptor-mediated transport of proteins bearing a canonical signal sequence represents the classical nuclear import pathway, non-conventional nuclear import pathways also exist (reviewed in Wagstaff and Jans 2009). These import pathways largely comprise proteins that enter to the nucleus via a receptor-independent mechanism. A common characteristic among proteins that transport via this mechanism is the ability to interact directly with components of the NPC. Specifically, the FG-NUPS, such as NUP62, NUP358 (also known as RanBP2), NUP98, and NUP153, are NPC constituents that facilitate receptor-independent nuclear transport by interacting directly with cargo proteins (Sharma et al. 2012). Proteins that undergo receptor-independent transport are often transcription factors that are released from cytoplasmic retention following a specific signal cascade and then migrate to the nucleus to mediate gene expression in response to the signaling event (reviewed in Wagstaff and Jans 2009).

Exploitation of Nuclear Transport Pathways by Viruses Obligated to Carry Out Replicative Steps in the Nucleus

The nuclear envelope imposes a physical barrier to not only cellular components that carry out necessary processes in the nucleus but also for those microbes that are obligated to carry out part of their lifecycle in the nucleus. For viruses that encode their genomes as single- or double-stranded DNA or utilize a DNA intermediate (retroviral cDNA), access to the nucleus is usually required during replication unless the virus encodes its own RNA polymerase (e.g., poxviruses; reviewed in Cohen et al. 2011). For most RNA viruses, nuclear localization is not necessary for replication except in the case of influenza A. Influenza must transport its segmented genome into the nucleus due to its dependence on the host

splicing machinery. How viruses subvert the restriction of regulated nuclear transport and deliver their genomes into the nucleus is reviewed in detail elsewhere (Cohen et al. 2011). Briefly, five proposed nuclear transport mechanisms account for the diverse strategies employed by viruses: (1) nuclear entry during mitosis when the nuclear envelope is disassembled [murine leukemia virus (MLV)], (2) viral genome release into the cytoplasm followed by nuclear import of the genome through the NPC (influenza A), (3) viral capsid docking at the NPC followed by genome release (adenovirus), (4) nuclear entry of intact capsids (hepatitis B virus), and (5) nuclear entry via virus-induced disruption of the nuclear envelope (parvovirus). These mechanisms indicate that viruses obligated to enter the nucleus during replication have evolved and thus committed valuable genomic resources for the purpose of either interacting with the cellular nuclear transport machinery or manipulating it for their own gain.

HIV-1 Infection and Nuclear Import

Since retroviruses, like HIV and MLV, form a double-stranded DNA copy (cDNA) of the single-stranded RNA genome that must be integrated into the host cell chromosomes for productive replication, these viruses are required to import their genomes to the nucleus. After cell entry and reverse transcription, the viral cDNA is assembled together with viral and cellular proteins into a large nucleoprotein complex termed the pre-integration complex (PIC). Although similar in their requirement for nuclear access, HIV-1 and MLV utilize distinct nuclear import pathways to transport their PICs into the nucleus. For MLV, a member of the *Gammaretrovirus* subfamily of retroviruses, mitosis and dissolution of the nuclear envelope precede PIC nuclear import. In contrast, HIV-1, a member of the *Lentivirus* subfamily of retroviruses, can achieve nuclear import independent of mitosis. Transport of the viral PIC across the interphase nuclear envelope is a distinguishing characteristic shared by all members of the *Lentivirus* subfamily examined thus far and implies

that these viruses have evolved a way to transport their genomes across an intact nuclear envelope. Given the size of the viral genome together with associated proteins, it is assumed that the lentiviral PIC is imported to the nucleus by means of an active nuclear import mechanism.

The significance of the ability of HIV-1 to transport its genome across an intact nuclear envelope is manifold. For example, during transmission to a new host, initial cell targets for HIV-1 infection are macrophage, a terminally differentiated and nondividing immune cell that carries out immune surveillance in the mucosa. Productive infection of macrophage affords the virus a stable foothold in the newly infected host. Importantly, infected macrophage represents a stable, persistent reservoir of virus, facilitating dissemination of the virus across the blood-tissue barrier and into diverse compartments of the host, including the lymphoid tissues where large populations of CD4+ T cells localize.

Current Models of HIV-1 Nuclear Import

Although nuclear transport of the genome is a key feature of the HIV-1 lifecycle, the mechanism that accounts for the delivery of the genome into the nucleus remains poorly understood. It is generally assumed that the large size of the PIC necessitates that the complex be able to engage the cellular nuclear import machinery to achieve active transport into the nucleus. For this reason, considerable effort has been made to characterize the karyophilic determinant in the PIC that would confer this property to the virus. Viral proteins that comprise the PIC, including matrix (MA), Vpr, and integrase (IN), as well as the triple-stranded cDNA reverse transcription intermediate, the central cDNA-flap, have been examined for their ability to mediate nuclear import of the PIC. A detailed discussion of this area of research is beyond the scope of this article, and is referred to a review on this topic (Suzuki and Craigie 2007). To briefly summarize, importin- α family members have been shown to interact directly with PIC constituents, such as MA, and were thus proposed to mediate a functional interaction

between the viral complex and cellular import pathways. However, follow-up studies have failed to support a definitive role for importin- α and MA during PIC transport. Similarly, the central cDNA-flap was shown to have an effect on the steps preceding integration, but whether it functions as a karyophilic determinant is inconclusive. Another candidate viral factor responsible for the nuclear import of the HIV-1 PIC is Vpr, which possesses unique karyophilic properties and is able to interact with components of the NPC (reviewed in Kogan and Rappaport 2011). Vpr has been reported to bind to the central, non-FG region of NUP153, as well as the non-FG region of the nucleoporin hCG1 (Kogan and Rappaport 2011). However, an essential role for Vpr in HIV-1 nuclear import has not been confirmed, and the interaction between Vpr and the NPC may not be essential but serve instead to modulate efficiency of PIC import in certain cell types by promoting PIC association with the NPC.

Two other viral proteins implicated to function as major determinants of HIV-1 nuclear import are Capsid CA and IN. Since the entry mechanism involving either CA or IN during nuclear import of HIV-1 appears to be tightly linked to their interactions with NUPs, the roles played by CA and IN are discussed below under their corresponding NUPs.

Role of NUPs Involved in HIV Import

The potential role of the NUPs in the active transport of the PIC remained largely unknown until recently. Large-scale small interfering RNA (siRNA) screens to identify cellular cofactors for HIV-1 replication have provided important insight to the function of cellular proteins during viral replication and have underscored the pivotal role of the NPC during the step of nuclear transport (Brass et al. 2008; Konig et al. 2008; Zhou et al. 2008). Three such studies identified a total of 842 cellular genes as encoding cofactors for HIV-1 replication. Due to differences in experimental parameters (i.e., cell types, filtering thresholds, timing, and readout), there is little overlap in the identity of cellular proteins identified by these

studies (Bushman et al. 2009). Among all proteins identified within the three studies, eleven were NUPs. The identified NUPs include both structural NUPs (NUP85, NUP107, NUP133, NUP155, and NUP160) and FG-containing NUPs (NUP50, NUP62, NUP98, NUP153, NUP214, and NUP358). Among the 34 proteins identified in two or more of the studies are NUP153 and NUP358 (RanBP2). The fact that multiple NUPs have been identified in separate studies as essential host factors underscores the importance of this supramolecular complex in the HIV-1 lifecycle. The identification of NUP153 and NUP358 by two separate studies further suggests that these NUPs in particular are central to the role of the NPC in mediating nuclear transport of HIV-1. Indeed, subsequent studies have corroborated the importance of the NPC, and both NUP153 and NUP358 (Woodward et al. 2009; Matreyek and Engelman 2011; Schaller et al. 2011; Di Nunzio et al. 2012; Koh et al. 2012; Matreyek et al. 2013), during HIV-1 replication. The remainder of this entry will summarize those studies and extend their importance to not only nuclear import but also ► [integration](#) and viral gene expression.

NUP153

As discussed earlier, many proteins can be transported into the nucleus via direct interaction with the NPC, bypassing the classical nuclear import pathways (Ball and Ullman 2005; Wagstaff and Jans 2009). In the absence of strong evidence supporting a role for importin- α and the classical receptor-mediated nuclear import pathways during HIV-1 replication, the role of NPC for transporting HIV-1 PICs has been investigated. The initial report found that the C-terminal domain (amino acid residues 896–1475) of NUP153, which is rich in FG repeats, binds directly with HIV-1 IN, but not FIV IN (Woodward et al. 2009). In cultured cells, overexpression of the NUP153 C-terminal domain inhibits nuclear import of IN and reduces HIV-1 infectivity by interfering with the nuclear translocation of the viral cDNA. Knockdown studies using siRNA also show the requirement of NUP153 for HIV-1 infection (Brass et al. 2008; Konig et al. 2008; Lee et al.

2010) and nuclear entry of HIV-1 cDNA (Matreyek and Engelman 2011). Further analyses suggest that NUP153 functions downstream of NUP358 and synergistically with transportin-3 (TNPO3/TRN-SR2) (Matreyek and Engelman 2011; Schaller et al. 2011). Importantly, wild-type HIV-1 depleted of cyclophilin A (CypA), a cytoplasmic peptidyl-prolyl isomerase required for optimal viral core stability (Briones et al. 2010), and HIV-1 CA mutants N74D and P90A are insensitive to NUP153 knockdown (Matreyek and Engelman 2011; Schaller et al. 2011). These results suggest that CA is a major determinant of HIV-1 nuclear entry, and NUP153 dependency is linked to uncoating of the viral core. Subsequent studies show the N-terminal domain of CA binds directly to the FG-rich C-terminal domain of NUP153 (Di Nunzio et al. 2012; Matreyek et al. 2013). NUP153 also plays a subsidiary role in targeting HIV-1 PICs to transcriptionally active regions of chromosomes (Koh et al. 2012).

NUP358

A recent study concluded that HIV-1 PICs use NUP358 (RanBP2) and CypA to access the cellular genome and target favored chromosomal regions for integration (Schaller et al. 2011). The way by which the entry pathway is engaged is through the HIV-1 CA. Besides NUP153, HIV-1 CA binds directly to CypA, as well as NUP358. HIV-1 CA-NC complexes assembled *in vitro* can also bind to NUP358 (Di Nunzio et al. 2012). In wild-type cells, CypA molecules are specifically incorporated into virions through CA interactions. The CypA-bound PIC interacts with NUP358, and the complex is then channeled through a specific nuclear entry mechanism involving NUP153 and TNPO3 (Schaller et al. 2011). Under conditions wherein CypA is depleted or inhibited or the infection is done in wild-type cells with CA mutants defective in binding NUP358, NUP358 usage is impaired and the nuclear entry of such PICs becomes less dependent on NUP153 and TNPO3 (Schaller et al. 2011). Importantly, the study shows that the choice of import pathways not only alters HIV-1 viral integration frequency but also affects the targeting preference of HIV-1 to specific genomic regions for integration (Schaller et al. 2011).

The effects of NUP358 and TNPO3 on HIV-1 integration targeting are consistent with the results from an earlier report (Oecwieja et al. 2011). The interaction between HIV-1 cores and NUP358 may form the mechanistic basis for the binding that NUP358 mediates during HIV-1 docking at the nuclear pore (Di Nunzio et al. 2012).

In addition to nuclear import of HIV-1 PICs, NUP358 has been associated specifically with HIV-1 Rev import. Rev is a sequence-specific viral RNA-binding protein that is required for the nuclear export of unspliced or partially spliced viral RNA. Rev shuttles between the nucleus and cytoplasm with its nuclear localization signals and nuclear export signals. These signals utilize typical cellular pathways to enter and exit the nucleus. Depletion of NUP358 led to a dramatic re-localization of Rev to the cytoplasm (Hutten et al. 2009). Moreover, the import rates of several other transportin-dependent proteins are also reduced when NUP358 is depleted (Hutten et al. 2009), suggesting a general role of NUP358 in nuclear import.

NUP98

Results obtained from experiments using vesicular stomatitis virus matrix protein (VSV-M), which binds within residues 66–515 of NUP98 (encompassing most of the FG repeats) and is a specific inhibitor against NUP98, and siRNA have shown that NUP98 has a definite role in HIV-1 nuclear import (Ebina et al. 2004). Expression of VSV-M in infected cells decreases the levels of integrated provirus and 2-long-terminal repeat (LTR) circle formation, an indicator of HIV-1 nuclear entry, but has no effect on full-length cDNA synthesis. The VSV-M-induced impairment of proviral and 2-LTR DNA can be restored by ectopic overexpression of NUP98 (Ebina et al. 2004). HIV-1 infection of NUP98-depleted cells using siRNA also demonstrates a reduction of proviral and 2-LTR DNA but not full-length cDNA (Ebina et al. 2004). The data indicate that NUP98 directly participates in the nuclear import of HIV-1 cDNA following viral entry. However in a separate study, NUP98 can interact with HIV-1 CA-NC complexes, depletion of NUP98 by shRNA only causes a

slight defect in HIV-1 integration (Di Nunzio et al. 2012, 2013).

In contrast to NUP358, NUP98 plays a major role in the export of HIV-1 Rev (Zolotukhin and Felber 1999). Rev actively recruits NUP98 to the nucleolus (Zolotukhin and Felber 1999). As NUP98 can associate with Rev outside of the NPC, this implicates that NUP98 is not solely stationary in the nuclear pore. Treatment of cells with actinomycin D, a polypeptide antibiotic that inhibits transcription and can affect the localization of several shuttle proteins, causes NUP98 to translocate to the cytoplasm in the absence or presence of translation inhibitor cycloheximide (Zolotukhin and Felber 1999) but has no effect on the localization of either NUP153 or NUP214. The result further supports NUP98 acting as a soluble factor (Zolotukhin and Felber 1999). Whether or not NUP98 can engage the PIC in the cytoplasm and affect the nuclear import process requires further investigation.

It is important to note that, in addition to NUP98, Rev export can similarly affect the sub-cellular localization of NUP214 (Zolotukhin and Felber 1999). Although NUP214 has been identified as one of the eleven NUPs important for HIV-1 replication, the role of NUP214 in nuclear import of HIV-1 has not been shown thus far. In fact, depletion of NUP214 may indirectly reduce HIV-1 infectivity by inhibiting general RNA export (Di Nunzio et al. 2012).

NUP62

NUP62 has been shown to be incorporated into HIV-1 and involved in viral RNA genome export, viral gene expression, and infectivity (Monette et al. 2011). A recent study has shown that NUP62 is a chromatin-bound protein that can interact with HIV-1 IN (Ao et al. 2012). Knockdown of NUP62 using short hairpin RNA significantly impairs IN-chromatin interactions and reduces viral DNA integration. Importantly, NUP62 knockdown does not inhibit appreciably HIV-1 cDNA nuclear import. In addition to knockdown, overexpression of the IN-binding region of NUP62 inhibits HIV-1 infectivity by interfering the IN-chromatin association (Ao et al. 2012). Various working models postulate that NUP62 provides the

PIC a direct link from the NPC to the chromosomal DNA. Since HIV-1 prefers to integrate into regions rich in actively transcribed genes, this would suggest the existence of complex communications occurring among NUPs to facilitate shuttling between the nuclear pore and intranuclear transcription sites. Due to NUP62's association to chromatin and transcriptionally active genes, it is hypothesized that IN recruits NUP62 to facilitate chromatin targeting and integration of HIV-1 cDNA (Ao et al. 2012).

It is worth noting that NUP62 knockdown does not completely eliminate IN-chromatin association in HIV-1-infected cells, suggesting that other viral or cellular factors play a similar role in chromatin targeting.

Conclusion

Overall, several NUPs, including NUP153 and NUP358, have been identified as being crucial for HIV-1 infection. How these different NUPs may function together in the context of the macromolecular structure of the NPC to mediate nuclear import of the HIV-1 PIC remains to be elucidated. In fact, the role of the NPC in HIV-1 replication may extend beyond that of nuclear import. The ability of multiple viral proteins to interact with diverse NUPs suggests that the biological properties of the complex are important to other replication steps such as uncoating (Schaller et al. 2011) and viral cDNA integration (Ao et al. 2012; Di Nunzio et al. 2012; Koh et al. 2012). Such a role for the NPC in these distinct replication steps is not surprising given that the complex functions to regulate not only nucleocytoplasmic trafficking but also the structure and organization of genomic DNA and cellular transcriptional activity (Capelson et al. 2010). One possible model that considers the interplay of NUPs with different viral factors proposes that interactions between NUP358 and CA associated with the incoming viral particle allows the docking of the core at the NPC and may cue the virus to uncoat, or functionally alter its CA content, within a specific context in preparation for nuclear transport (Schaller et al. 2011, Di Nunzio et al. 2012).

NUP358-dependent docking and uncoating may then result in interactions between the partially uncoated core, NUP153, and presentation of PIC-associated IN to function as the karyophilic determinant of the viral complex. The reported interaction between the C-terminal domain of NUP153, HIV-1 CA and/or IN would then function to deliver the PIC across the NPC (Woodward et al. 2009; Di Nunzio et al. 2013; Matreyek et al. 2013). After traversing the NPC, NUP153 and NUP62 may provide chaperone activity for the PIC and facilitate chromatin association and integration through its interaction with IN- and chromatin-binding properties (Ao et al. 2012; Di Nunzio et al. 2013; Koh et al. 2012).

Dependency on NUP153 for delivery of the PIC into the nucleus may have important consequences for the integration of the viral DNA into the chromosome. Cellular transcription induces both the dynamic association of NUP153 with the NPC and conformational changes in NPC-associated NUP153 (Ball and Ullman 2005). The dynamic properties of NUP153 may enable contacts with the uncoated viral complex at the cytoplasmic face of the NPC and then facilitate its transport through the aqueous channel of the NPC. Such a transport mechanism would preferentially target the PIC to transcriptionally active regions of the genome. Import of the PIC into genomic regions that are transcriptionally active may offer the advantage of encountering a chromosomal environment that will optimally support both integration and viral gene expression. This model would predict that changing how the virus interacts with the NPC would also change the chromosomal environment into which the virus integrates. Support for this hypothesis comes in the form of integration site analyses carried out after depletion of NUP153, NUP358, and TNPO3 (Ocwieja et al. 2011; Schaller et al. 2011; Di Nunzio et al. 2013; Koh et al. 2012); under these conditions, integration site selection is shifted to genomic regions with low gene density and activity.

Similarly, changes in viral proteins that impact NUP interactions also affect nuclear transport of the virus. CA mutations that result in loss of nuclear import are well documented and may reflect changes in core stability (Dismuke and

Aiken 2006). Some CA mutant can also modulate nuclear import pathways to produce altered integration target site selection. For example, the CA mutant N74D demonstrates impaired use of NUP358 and NUP153 (Schaller et al. 2011) but increases dependency on NUP155 and NUP160 during translocation (Lee et al. 2010). Interestingly, N74D also results in integration into genomic areas of low gene density (Schaller et al. 2011; Koh et al. 2012). Summarily, these findings demonstrate that alterations in the interaction between HIV-1 and NUPs impact the genomic integration site selection and modulate this important step in the viral lifecycle. Therapeutic interventions that target these interactions may represent legitimate goals for drug development.

The extent to which the NUPs play similar roles in the replication of other lentiviruses remains to be fully explored. The CAs of several primate lentiviruses and equine infectious anemia virus also interact directly with the C-terminal domain of NUP153, and NUP153 knockdown reduces their infectivity (Matreyek et al. 2013). Feline immunodeficiency virus (FIV) has neither the same sensitivity to NUP153 knockdown (Matreyek and Engelman 2011; Matreyek et al. 2013) nor the same binding characteristics with NUP153 (Woodward et al. 2009). In still another study (Lee et al. 2010), FIV demonstrates dependency on NUP155 and NUP160 for replication but not NUP153. It is worth noting that the NUP requirements of FIV are similar to that of the HIV-1 CA mutant N74D. Collectively, these findings suggest that lentiviruses have evolved diverse strategies for utilizing the NPC and specific NUPs to mediate this essential step in the viral lifecycle. Deletion of key factors or inhibition of steps along the default pathway of nuclear import opens up HIV-1 for other routes of nuclear entry. These observations emphasize the redundant mechanisms employed by HIV-1 to complete this essential replication step.

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Nurse-Delivered Interventions for Adherence

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Definition

Adherence is key to achieving and maintaining virological suppression in HIV-infected patients in order to achieve optimal health outcomes.

However, not all patients adhere to their medication regimens. In that adherence is a multifaceted behavior, a substantial portion of the literature focuses on interventions delivered by nurses; this chapter offers an overview of nurse-delivered care and its relationship to adherence to antiretroviral medication.

Introduction

Advances in combined antiretroviral therapy have dramatically improved the clinical outcomes and life expectancy of persons living with HIV. These medicines not only enable people living with HIV to live longer and healthier lives but also help to prevent new HIV infections. Yet, these benefits cannot be realized without consistent adherence to maintain drug exposure at levels necessary to achieve durable virologic suppression and to maximize the availability of future therapeutic options.

Adherence has long been recognized as a primary determinant of virological success. Maintaining sufficient levels of adherence to antiretroviral medications has proved to be challenging for many people living with HIV. Although today's regimens are more convenient and better tolerated than those prescribed in the early years, suboptimal adherence with resulting treatment failure is still common. It is therefore essential that measures to support and sustain patients' adherence are a standard component of HIV clinical care.

Maximizing adherence and achieving the full potential of the antiretroviral therapies require attention to complex behavioral issues that compromise the success of HIV therapies. Nurses are well positioned to provide and coordinate adherence care. Since the beginning of the HIV epidemic, nurses have played a critical role in caring for persons with HIV, both as members of care teams and as primary HIV care providers. Nurses are increasingly recognized throughout the world as a significant, largely underutilized asset to health service delivery by virtue of their numbers, distribution in urban and rural areas, educational preparation which emphasizes an integrated

consideration of the patient's physical and psychosocial health-care needs, competence, and their ability to deliver safe and high-quality patient care.

Nurse-Delivered Antiretroviral Adherence Interventions

Numerous behavioral change interventions designed to improve adherence to antiretroviral medications have been tested in randomized controlled trials. Among strategies that have been examined are psychoeducational counseling approaches (e.g., motivational interviewing, behavioral skills development/problem solving), cue-based reminders (e.g., short message prompts via MEMS, text messaging), antidepressant therapy, social support/"buddy systems," directly observed therapy, and reward systems (e.g., payment). Interventions are commonly delivered in 1:1 counseling or group sessions in clinic settings, methadone maintenance programs, at the patient's home or by phone, and computer-assisted or video instruction.

Nurses have delivered the antiretroviral adherence interventions in several of the studies as summarized in Table 1 (Berrien et al. 2004; de Bruin et al. 2010; DiIorio et al. 2008; Holstad et al. 2011; Holzemer et al. 2006; Koenig et al. 2008; Reynolds et al. 2008; Simoni et al. 2011; Wang et al. 2010; Williams et al. 2006). Most of the nurse-delivered adherence interventions have been implemented in HIV clinic settings (de Bruin et al. 2010; DiIorio et al. 2008; Holstad et al. 2011; Holzemer et al. 2006; Koenig et al. 2008; Simoni et al. 2011). A few have been delivered through home visits (Berrien et al. 2004; Wang et al. 2010; Williams et al. 2006), by phone (Reynolds et al. 2008), or through a combination of face-to-face visits and phone calls (Holzemer et al. 2006; Koenig et al. 2008; Wang et al. 2010).

The antiretroviral adherence interventions delivered by nurses have been guided by different theoretical frameworks (e.g., self-regulation, theory of planned behavior, health belief model, and information, motivation, and behavioral (IMB) model). However, each has used a

multicomponent psychoeducational counseling approach that generally involves a combination of tailored knowledge development, motivation, skills building, problem solving, and/or social support. Two have also used electronic feedback (de Bruin et al. 2010) or reminders (Simoni et al. 2011). The frequency of the nurse-delivered adherence intervention sessions has ranged from just a few sessions (de Bruin et al. 2010) to as many as 24 sessions delivered over 12 months (Williams et al. 2006).

The nurse-delivered adherence interventions have shown varying degrees of success. Most have shown some improvement in adherence behavior. Several have demonstrated a significant improvement in adherence behavior (de Bruin et al. 2010; Koenig et al. 2008; Reynolds et al. 2008; Wang et al. 2010; Williams et al. 2006) or a trend toward improved adherence (Berrien et al. 2004; DiIorio et al. 2008; Holstad et al. 2011; Simoni et al. 2011). An effect on viral outcomes has been demonstrated less frequently (de Bruin et al. 2010; DiIorio et al. 2008; Reynolds et al. 2008).

Available evidence suggests that nurse-delivered adherence interventions with even modest effectiveness are likely to provide long-term survival benefit to patients and to be cost-effective. Using data from a randomized controlled clinical trial as input to a computer-based simulation model of HIV disease, the cost-effectiveness of a nursing intervention on antiretroviral adherence was examined by Freedberg and colleagues (Freedberg et al. 2006). Implementing the nursing intervention in addition to standard care yielded a 63% increase in virological suppression at 48 weeks. This produced an increase in expected survival (from 94.5 to 100.9 quality-adjusted life months) and estimated discounted direct lifetime medical costs (\$253,800–\$261,300). The incremental cost-effectiveness ratio for the intervention was \$14,100 per quality-adjusted life year gained compared with standard care.

The relative efficacy of the different nurse-delivered strategies has not been examined. Further, the mechanisms by which the interventions promote adherence are not clear. While delivery

Nurse-Delivered Interventions for Adherence, Table 1 Summary of nurse-delivered antiretroviral adherence interventions

Author (Year) Country	Study population	Mode of delivery	Guiding theory/ mechanism of change	Outcomes	Main findings
Berrien et al. (2004) US	HIV+ children (N = 37) N = 20 intervention N = 17 control	Home visits; individual; 8 structured psycho- educational sessions by registered nurses over 3 months	Health belief model; designed to improve knowledge and identify and resolve barriers to adherence	Adherence – self- report Adherence – pharmacy refill CD4 T-cell count Viral load	Trend (NS) toward better adherence; no difference biologic outcomes
De Bruin et al. (2010) Netherlands	HIV+ adult (N = 133) N = 66 intervention N = 67 control	Clinic based; individual; 2 tailored, psycho- educational and EDM-feedback sessions by clinic nurses	Theory of planned behavior and self- regulation theories; designed to increase knowledge and promote motivation	Adherence – EDM Viral load	Significantly better adherence and viral load
DiIorio et al. (2008) U.S.	HIV+ adults (N = 213) N = 107 intervention N = 106 control	Clinic based; individual; 5 counseling sessions by registered nurses over 3 months	Motivational interviewing; designed to build confidence, reduce ambivalence, and increase motivation	Adherence – EDM CD4 T-cell count Viral load	Trend toward better adherence and viral load
Holstad et al. (2011) US	HIV+ adult women (N = 207) N = 104 intervention N = 103 control	Clinic based; group; 8 counseling sessions led by nurse	Motivational interviewing; designed to empower women in decision- making, use strategies to improve adherence/ risk taking behaviors, reduce resistance/ ambivalence	Adherence – EDM CD4 T-cell count Viral load	Trend (NS) toward better adherence and viral load
Holzemer et al. (2006) US	HIV+ adults (N = 243) N = 118 intervention N = 122 control	Clinic based with phone calls; individual; 3 tailored, nurse- delivered psychoeducational counseling sessions and up to 3 booster phone sessions	Ickovics and Meisler adherence framework, nursing process, tailored health communication; designed to assess areas of adherence risk and target with nursing interventions	Adherence – self- report Adherence – pharmacy refill Adherence – pill count Adherence – EDM	No significant difference in adherence
Koenig et al. (2008) US	HIV+ adults (N = 266) N = 116 intervention N = 120 control	Clinic based with phone calls and support partner; individual and group; 5 nurse-delivered psycho-educational individual sessions over 5 months and 1 booster session at 6 months/5 phone calls/2 group educational sessions/ support partner	Problem-solving theory, five-stage model of problem solving, self- determination theory, social support theory; designed to be tailored, multi-level, multifaceted approach targeting individual and socio-contextual barriers to adherence and enhance social support	Adherence – EDM Viral load	Significantly better adherence. Viral load outcomes inconclusive

(continued)

Nurse-Delivered Interventions for Adherence, Table 1 (continued)

Author (Year) Country	Study population	Mode of delivery	Guiding theory/ mechanism of change	Outcomes	Main findings
Reynolds et al. (2008) US	HIV+ adults (N = 109) N = 54 intervention N = 55 control	Phone calls; individual; tailored, nurse-delivered psycho-educational counseling by phone over 16 weeks	Self-regulation theory (Leventhal); tailored approach designed to improve knowledge, skills, and affective support to recognize, self- manage, and problem solve threats to adherence	Adherence – self- report Viral load	Significantly better adherence; trend toward lower risk of viral failure
Simoni et al. (2011) China	HIV+ adults (N = 70) N = 36 intervention N = 34 control	Clinic-based; individual; Choice of 3 bachelor- level nurse delivered cognitive-behavioral counseling sessions and/or electronic reminder and/or adherence partner	Information, motivation, and behavioral (IMB) model; adapted life- steps (Safren) protocol in which education, problem- solving, and rehearsal strategies used to develop better adherence skills	Adherence – self- report Adherence – EDM CD4 T-cell count Viral load	Trend toward better adherence; no difference biologic outcomes
Wang et al. (2010) China	HIV+ adult heroin users (N = 116) N = 58 intervention N = 58 control	Home visits and phone calls; individual; 4 nurse-delivered home visits combined with phone calls over 8 months	Palo Freire; community-based social support; designed to provide tailored information, build skills, reinforce motivation, and enhance family support	Adherence – self- report Quality of life depression	Significantly better adherence, quality of life and depression outcomes
Williams et al. (2006) US	HIV+ adults (N = 171) N = 58 intervention N = 58 control	Home visits; individual; 24 nurse and community support worker home visits with social and educational components over 12 months	Palo Freire; community-based social support; designed to target individual and social level barriers to adherence with reflective problem solving, self-directed learning, and skills building	Adherence – EDM CD4 T-cell count Viral load	Significantly better adherence; no difference biologic outcomes

by nurses appears feasible generally, this research does not demonstrate whether delivery by the nurses contributes uniquely to the efficacy of the intervention. Nurse communication, nurse-patient relationship, and patient responses have not been explicitly examined as potential active ingredients over and above the a priori hypothesized mechanisms of action.

Nurse-Managed Care and Adherence Outcomes

In other research, the quality of nurse-managed HIV care has been compared to physician-delivered care. Adherence and viral load outcomes are among outcomes that have been examined as quality indicators in these studies.

Global efforts to improve HIV care through prevention and decentralized treatment have led to implementation of models of care that rely heavily on nurses with specialized training. The impetus to use nursing personnel with specialized training in extended roles has developed, in a large part, because several assessments have shown only limited capacity to scale up physician-oriented HIV service models, particularly in settings with low ratios of physicians to population and high rates of attrition among medical staff. The World Health Organization has estimated that there are 57 countries facing critical shortages of health workers. Over half of them are in Africa.

In an effort to reduce dependence on highly trained physicians, many countries now use nurses to undertake a wide range of interventions in community-based primary care settings, including central roles in HIV prevention and treatment. For example, in ART center settings in countries such as Lesotho, Malawi, Kenya, and Rwanda, nurses are routinely trained to provide services that were previously delivered by doctors, such as diagnosis and treatment of opportunistic infections, clinical monitoring, responding to new signs and symptoms, and, in some circumstances, initiating first-line ART regimens in patients who do not have complicating conditions. In such models of care, nurses generally perform tasks customarily provided by physicians per standardized protocols under district medical officers' supervision. They refer patients to medical clinicians when treatment does not seem to control the disease, if the patient experiences severe medication toxicity, or to manage complex and serious conditions.

Use of nursing personnel with specialized training in extended roles is also a reflection of their ability to perform in the roles effectively. The educational preparation of nurses emphasizes autonomous and collaborative care of individuals of all ages, families, groups and communities, sick or well, and in all settings. Nursing includes the promotion of health, prevention of illness, and the care of ill, disabled, and dying people. Advocacy, promotion of a safe environment, research, participation in shaping health policy and in patient and

health systems management, and education are also key nursing roles that equip nurses to be ideal vehicles for medication adherence interventions. Nurses are also educated to provide palliative care, manage clinics, and supervise staff and outreach workers.

Nevertheless, the transition to nurses in expanded roles in HIV care has been accompanied by questions about the safety, competence, and effectiveness of nurses in comparison with physicians. A number of studies have thus been conducted to address this concern. Findings from this body of work provide a substantial, unfailingly positive, evidence base that clearly demonstrates the effectiveness of nurses in these roles.

For example, Humphreys et al. examined the effectiveness of nurse-led care in comparison with usual hospital care in a typical rural sub-Saharan African setting (Humphreys et al. 2010). Clinically stable adults with a CD4 count of less than 100 cells/ μ L and on antiretroviral treatment for at least four weeks at the district hospital were assigned to either nurse-led primary care-based antiretroviral treatment care or usual hospital care. Findings showed that those receiving primary care based on nurse-led care treatment were less likely to miss an appointment compared with those continuing to receive hospital care (RR 0.37, $p < 0.0001$), were more likely to be satisfied in the ability of staff to manage their condition (RR 1.23, $p = 0.003$), and average travel cost was half that of those receiving hospital care ($p = 0.001$). Further, there was no significant difference in loss to follow up and health-related outcomes were equivalent. The authors observed that the patients who received services in the nurse-led primary care setting were more satisfied in the ability of staff to manage their condition in the local clinic setting than the hospital setting in spite of the lack of diagnostics, such as radiology, or medical opinion available on demand. Reasons for satisfaction with the nurse-led clinic included reduced cost, being nearer to home, a shorter queue, being treated better by staff, receiving better care, and "not be talked about at the clinic."

Likewise, in a randomized, non-inferiority trial (Sanne et al. 2010), the health outcomes of nurse-managed ART were compared with physician-

managed ART (Sanne et al. 2010). No significant differences were found. HIV-positive individuals with a CD4 cell count of less than 350 cells/ μ L or WHO stage 3 or 4 disease were randomly assigned to nurse-monitored ART care (N = 404) or doctor-monitored ART care (N = 408) and followed for a median follow-up of 120 weeks (IQR 60–144). Among the patients that reached an endpoint of treatment failure (N = 371), 192 (48%) were in the nurse group and 179 (44%) in the doctor group (hazard ratio 1.09, 95% CI 0.89–1.33). Deaths (10 vs 11), virological failures (44 vs 39), toxicity failures (68 vs 66), and program losses were also similar in nurse and doctor groups (70 vs 63), respectively.

Findings from an observational cohort study (Cohen et al. 2009) conducted in rural Lesotho lend additional support for community-based, nurse-driven ART management for adults and children. Despite a lack of resources for health, Lesotho implemented state-of-the-art antiretroviral treatment guidelines and nurse-initiated and managed HIV care (including ART management) at the primary health-care level. The study found that between 2006 and 2008, the annual enrollment more than doubled for adults and children, with no major external increase in human resources. The proportion of adults arriving sick (CD4 < 50 cells/mm³) decreased from 22.2% in 2006 to 11.9% in 2008, and at 12 months, 80% of adults and 89% of children were alive and in care (loss to follow up, 8.8%), and at 24 months, 77% of adults remained in care.

Consistent with findings from these individual studies, a meta-analysis comparing nurse-initiated and managed ART to physician-initiated and managed ART (Emdin et al. 2013) also found that adherence, viral failure, and mortality outcomes were similar between nurses and physicians.

Conclusions

Nurses offer a valuable, but often underutilized, resource for providing care to support adherence to antiretroviral therapy and strengthen HIV prevention and treatment delivery. Nurses have educational preparation that provides them with a

range of health-care competencies (e.g., therapeutic communication skills, knowledge about psychosocial and pathophysiologic aspects of illness) to deliver comprehensive, theory-based adherence intervention approaches with fidelity. Further, their presence in most health-care settings is an advantage. The available body of evidence shows that safe and effective management of HIV can be provided at the primary care level by nurses in expanded roles. Taken together, the evaluations of nurses in adherence counseling and expanded HIV patient care roles demonstrates that the use of nurses in expanded HIV patient care roles is feasible and well accepted by patients; nurses can provide a range of services safely and effectively (e.g., initiating and managing ART to adults and children, patient education, and adherence counseling); outcomes (e.g., adherence, treatment failure, mortality, toxicity, loss to follow up, patient satisfaction) of nurse-led management of uncomplicated HIV adults and children are as good as doctors and sometimes better. While nurse-delivered adherence interventions have demonstrated efficacy, the relative efficacy of the different nurse-delivered strategies has not been examined, and the mechanisms by which the interventions promote adherence are not clear.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Antiretroviral Treatment in Resource-Limited Settings](#)
- ▶ [Behavioral Interventions for Adherence](#)
- ▶ [Behavioral Science Highlights of Evidence and Research](#)
- ▶ [Healthcare Workers, Shortage and Task Shifting of](#)

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O

Origin and Distribution of HIV-1 Subtypes

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Definition

HIV-1 displays a high genetic diversity that can be classified into clades, called subtypes. HIV-1 subtypes represent separate epidemics with independent evolutionary histories that occasionally overlap to generate recombinant forms. The stratification of HIV-1 strains into subtypes and circulating recombinant forms (CRFs) is useful to study the impact of HIV-1 genetic diversity on pathogenesis, disease progression, treatment including resistance development, molecular epidemiology, and vaccine design. Furthermore, such a classification provides a way to monitor

the evolution of different subtype epidemics, its geographic distribution, and its spread across countries, continents, and risk groups.

HIV Genetic Diversity

HIV accumulates mutations at an alarming rate, mainly due to its error-prone replication cycle, vast virus burden, and high recombination rate. This fast evolution allows the virus to adapt quickly upon attack by the immune system and to resist drug treatment and, thus far, prevents successful vaccine development. However, such a fast evolution greatly facilitates understanding the details about how HIV spreads, providing a window on potential means to better contain the pandemic. No two infected individuals have the same virus, and divergence has accumulated to such an extent that the pandemic HIV-1 group M can be subdivided in different lineages, called subtypes. Each of these subtypes has their own history and epidemic peculiarities, and the accumulating divergence within each subtype allows a fine-grained tracking of the movement of the virus.

Origin and Dissemination of HIV Types and Groups

Immediately after the discovery of the etiological agents of AIDS, HIV, it was clear that there were two forms of AIDS, one associated with a relatively fast progression caused by HIV-1 and the

other associated with a much slower progression caused by HIV-2. The genetic divergence between these two AIDS-causing viruses was so huge, and their geographic and risk group association so different, that it soon became clear that these were two different epidemics. Later, after the discovery of the different simian origins of HIV-1 (acquired from chimpanzees and/or gorillas) and HIV-2 (acquired from sooty mangabeys), different groups within each type were further identified, each resulting from a separate zoonotic transmission event. Thus, the large genetic diversity within AIDS-causing viruses is in a large part inherited from its distinct zoonotic origins.

Four different groups have currently been identified within HIV-1 (M, N, O, and P), originating from separate transmission events from chimpanzees and/or gorillas. The close relatedness of groups M and N to SIVcpz from *Pan troglodytes troglodytes* (a subspecies of chimpanzees from Central Africa) indicates that this is the subspecies from which humans have acquired these viruses. The fact that phylogenetic HIV-1 groups M and N cluster separately among SIVcpz strains attests that they arose from two separate zoonotic transmissions from this central subspecies of chimpanzee. The origin of group O is however more dubious. HIV-1 group O-like infections found in wild gorillas (SIVgor) suggest that this clade may have been originated from gorillas (Peeters and Delaporte 2012). The fourth group P is even more closely related to SIVgor, making the case stronger that humans can get infected from gorillas. These gorilla strains are however nested inside an SIVcpz clade, suggesting that most probably gorillas got their infection also from chimpanzees. Where and how some of these zoonotic events turned into human epidemics is expanded elsewhere (Sharp and Hahn 2011).

The current pandemic is almost entirely linked to HIV-1 group M. The ancestral SIVcpzPtt strain that crossed the chimpanzee-human barrier and gave rise to HIV-1 group M belonged to a viral lineage that still persists today in *Pan troglodytes troglodytes* living in southeastern Cameroon. Hunting and butchering chimpanzees for the “bushmeat” market are

currently considered the most probable and parsimonious explanation for the zoonotic transmission of SIVcpz to humans. From southeastern Cameroon, HIV-1 appears to have made its way via the Sangha River (or other tributaries) south to the Congo River and on to Kinshasa where the group M pandemic was probably spawned (Sharp and Hahn 2011).

HIV-1 group N (non-M, non-O) was first identified in 1998 in a Cameroonian woman. Until 2011, only 12 cases of group N infections had been identified exclusively in patients living in Cameroon. However, in that year, a new case of a patient probably infected in Togo was identified in Paris indicating a possible dissemination across borders. Even though group N is also associated with AIDS, if it were not for the HIV-1 group M pandemic, this group N might never have been discovered. HIV-1 group O, on the other hand, causes thousands of infections and AIDS cases in West-Central Africa, especially in Cameroon, and its epidemic spread, including to a limited extent to Europe, is of concern. Group P has only been identified in two humans originating from Cameroon, suggesting that its dissemination in humans is rare and not of epidemic potential (Mourez et al. 2013).

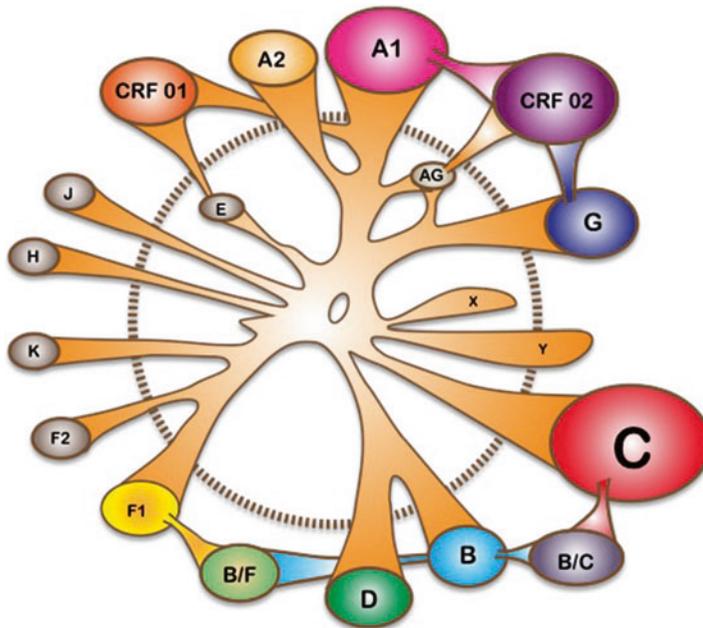
The timing of the origin of different HIV types and groups is still a matter of discussion but different studies and methods have generated a rough time frame. These estimates are based on phylogenetic molecular clock calculations, extrapolating back in time beyond the earliest documented strain. Overall, group N seems to be the youngest clade, having crossed to humans around the 1960s. Going further back in time, the two major HIV-2 clades, HIV-2A and HIV-2B, both arose a few decades earlier around the 1930s/1940s. Perhaps a decade earlier, HIV-1 group O arose around the 1920s (Mourez et al. 2013). The common ancestor of HIV-1 group M has been traced back to the beginning of the twentieth century (Faria et al. 2014). If both HIV-1 group P strains are descendent from the same interspecies transmission, then this is the oldest clade surviving today, with an estimated date of origin of its tMRCA to have occurred in the second half of the nineteenth century (Mourez et al. 2013).

The AIDS Pandemic and HIV-1 Group M

The AIDS pandemic, with an estimated number of 60 million cumulative infections, is caused almost exclusively by HIV-1 group M. The enigma why so many decades passed between the cross-species transmission from simians to humans and the discovery of AIDS has still not been resolved satisfactorily, and only now the factors involved in the early establishment and spread of the HIV-1 pandemic are being uncovered (Sharp and Hahn 2011; Pepin 2011). The earliest serologically and genetically confirmed HIV-1 group M infection has been found in an archival sample from 1959 belonging to an adult Bantu male, with a sickle-cell trait and a glucose-6-phosphate-dehydrogenase deficiency, living in Leopoldville, Belgian Congo (now Kinshasa, Democratic Republic of Congo, DRC). In addition, molecular epidemiological studies have indicated that all of the known HIV-1 group M subtypes, as well as

additional lineages that have remained restricted to this area, circulated in Kinshasa in the mid-1980s. Together, these findings have been taken as evidence that most of the early diversification of HIV-1 group M occurred in and around Kinshasa, making Kinshasa the most probable location of the early HIV-1 group M epidemic.

From the Congo Basin, the virus spread successfully around the world, accumulating significant genetic variability. Nine different, so-called pure lineages have formed, representing the subtypes (A, B, C, D, F, G, H, J, and K). Their variability and relationship can conveniently be illustrated by a phylogenetic tree (Fig. 1). Such a phylogenetic analysis reveals three levels of classification, based on the clustering and distances in the phylogenetic tree and on the recombination patterns: *subtypes*, *sub-subtypes*, and *circulating recombinant forms (CRFs)* (Robertson et al. 2000).



Origin and Distribution of HIV-1 Subtypes, Fig. 1 Phylogenetic origins of HIV-1 group M subtypes and circulating recombinant forms (CRFs). The *dashed circle* differentiates old and contemporary sequences. Inside the *circle*, the old sequences, such as the subtype E clade, may no longer exist in the current epidemic. The ancient presence of subtype E based on CRF01_AE is inferred, a recombinant between subtypes

A and E. “X” represents a hypothetical extinct strain, and “Y” represents a hypothetical old strain that is still circulating in the current epidemic but has not been identified. CRF02 is an old recombinant derived from both old and contemporary subtypes A and G. BF recombinants in South America and BC in China are new, as their parents are contemporary sequences (Adapted from Zhang et al. 2010)

Subtypes refer to the major clades, which supposedly have evolved independently from each other. These form distinct clusters with inter-subtype genetic distances ranging from 25% in *env* (Robertson and et al. 1999) to between 5% and 30% (nucleotide divergence), depending on the genes compared. Continuing evolution has as consequence that diversity keeps increasing over time and slightly differently so for the different subtypes as their evolutionary rates are different.

Sub-subtypes refer to distinct lineages that are very closely related to each other. Subtype A is divided in at least two distinct sub-subtypes (A1 and A2) and subtype F has two sub-subtypes (F1 and F2). From the point of view of genetic distance, subtypes B and D could be considered sub-subtypes rather than different subtypes; however, epidemiological argumentation as detailed below has maintained their status as different subtypes until now.

CRFs represent inter-subtype recombinant lineages that play an important role in the pandemic. Inter-subtype recombinants can form when two epidemics that initially evolved separately meet each other again. When a single individual becomes infected with two viruses from the two epidemics, some cells will be dually infected and spawn inter-subtype recombinants in the next replication round. When such inter-subtype recombinants perpetuate in the epidemic, many strains share an identical mosaic structure; since they are descended from the same recombination event(s), they achieve the CRF status, whereas many others will just disappear and represent unique recombinant forms or URFs. Currently, there are 55 different CRFs described in the Los Alamos database (<http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html>), although most of them have not really taken epidemic proportions. Together, all CRFs account for approximately 20% of all HIV-1 infections. However, CRF01_AE and CRF02_AG are the most important for the world pandemic, causing a significant number of infections in Asia and West Africa, respectively. For this reason, a separate section will be included for these two CRFs.

Clinical and Epidemiological Importance of Subtyping

HIV-1 subtypes have been shown to be highly compartmentalized between risk groups and geographic regions. A study in patients newly diagnosed in Europe between 2002 and 2005 showed a stratification of HIV-1 subtypes according to gender, risk group, and patients' country of origin: subtype B is more prevalent in men who have sex with men (MSM), while most other subtypes are more prevalent in heterosexuals. The fact that HIV-1 subtypes have a different prevalence in different risk groups could suggest that they diverged because of differences in transmission potentials depending on the mode of transmission in risk groups involved. For example, subtype B may have adapted to anal sex practiced by MSM, while other subtypes may be more easily transmissible by vaginal sex in heterosexuals. Subtype C seems particularly adapted to heterosexual transmission. Alternatively, the different prevalence in the different risk groups could simply be a stochastic event, a founder effect and lack of mixing of the different risk groups. Until now, no study has refuted any of these hypotheses and it may be that both are valid.

Some evidence might point toward adaptation to risk groups. For example, the evolutionary rate differs between HIV-1 subtypes. CRF02_AG and subtype G were found to have higher rates, while subtype D had lower rates than the other subtypes. In addition, evolutionary rate is linked to the speed of transmission of the epidemic, i.e., when HIV-1 infections are transmitted faster, HIV-1 evolves slower and vice versa. The speed of transmission is in turn linked to the risk group, for example, HIV spreads much faster by needle sharing than by sexual contact. The differences in evolutionary rate between subtypes were not only at nucleotide but also at amino acid level, varying in roughly similar ways. This could be attributed to both differences in selective pressures (amino acid level) potentially associated with a particular mode of transmission and risk behavior, but also to differences in replication dynamics (nucleotide level) (i.e., mutation rate or generation time) of the strains potentially associated with speed of transmission.

The evidence supporting the founder effect is much stronger and an entire next section below is dedicated to these founder effects. Therefore, although the strongest contributing factor may be founder effects, the jury is still out on whether the mode of transmission has also shaped the genetic divergence among subtypes. Perhaps distinct founder effects were at the origin of the different HIV subtypes, and these genetic clades might have later adapted to the way they were transmitted in the infected population where they were first introduced.

HIV-1 Subtypes and Disease Progression

Several studies in Uganda, Kenya, and Tanzania have compared the disease progression of HIV-1 subtypes A and D, the most prevalent subtypes in that region. The prevalence of other subtypes included in such cohorts is small making it difficult to draw conclusions about the disease progression of other subtypes. All studies agree that people infected with subtype D have a higher risk of progressing to AIDS and to death than people infected with subtype A. One study also found that infection with subtype D, with multiple subtypes (probably subtypes A and D, given the geographic range of the study), and with A/D recombinant viruses resulted in significantly faster progression to AIDS and to death than did infection with subtype A only. Studies also agree that the observed differences in rates of disease progression are most likely explained by differences in coreceptor tropism: in the course of HIV-1 infection, the emergence of CXCR4 (X4) tropic viruses is associated with a more rapid decrease in the CD4⁺ cell count, and subtype D has been shown to have a higher frequency of syncytium formation and X4 tropism compared with subtype A. Another study indicated that Africans infected with subtypes C, CRF02_AG, A, and G had slower disease progression before commencing treatment than Haitians and Canadians infected with subtype B.

Response and Resistance to Antiretroviral Drugs

In terms of response to antiretroviral therapy, one large study indicated that current combination

therapies are very effective regardless of the infecting subtype. The recovery of the immune system and the hazard of therapy failure were similar across subtypes. Despite the many nucleotide and amino acid differences, most resistance mutations are similar across subtypes. However, several differences have been noted in the mutational pathways to resistance development. These differences could be explained through a combination of several factors (Camacho and Vandamme 2007).

Inter-subtype amino acid differences are involved in minor structural changes in the targets of therapy. The presence of such polymorphisms can influence the emergence of drug resistance mutations. Different mutations can emerge under the same drug pressure. Sometimes, different mutations arise in different subtypes. Different and mutually exclusive pathways to the development of resistance to the protease inhibitor nelfinavir occur in subtypes B and G or C. While subtype B preferentially develops the mutation D30N, subtype G strains exclusively and C strains preferentially develop the mutation L90M.

On the other hand, inter-subtype differences in codon usage result in differences in nucleotide and mutational motifs that may affect the genetic barrier to resistance. The number of mutations or whether transitions or transversions are needed to develop resistance to antiretroviral drugs all contribute to the genetic barrier for resistance development. An example is the development of the resistance mutation V106M in the reverse transcriptase of subtype C strains due to the usage of codon GTG (the mutation is GTG to ATG) as opposed to other subtypes that more frequently use the GTA codon and therefore evolve more easily to the V106A mutation (the mutation is GTA to GCA). Other such positions include protease codon 82 where subtypes C and G have an increased genetic barrier to the development of the resistance mutation V82A when compared to subtype B and protease codon 89 where the subtype B wild-type 89L develops few mutations, while subtypes C, F, and G use wild-type 89M and frequently develop 89I/V mutations (Santoro and Perno 2013).

Another result from such differences in mutational motifs is the type of mistakes made by replication enzymes. Some common mistakes by the viral reverse transcriptase favor the development of particular drug resistance mutations. For example, the K65R mutation that confers resistance to tenofovir is more frequent in subtype C than in subtype B, and D67N, contributing to the resistance to several nucleoside reverse transcriptase inhibitors, occurs more often in subtype B than in subtype C. This seems to be due to the nature of the subtype C RNA template. Differences in nucleotide sequence in this region cause a difference in RT pausing which in turn results in different errors made (Santoro and Perno 2013).

Most of these differences can be related to genetic differences between subtypes. However, genetic differences also exist within a subtype where slighter differences in evolutionary pathways to develop drug resistance seem to occur.

Vaccine Development

Several factors pose important obstacles in the development of an effective preventive HIV-1 vaccine, most notably the high degree of antigenic and genetic viral diversity and the capacity to quickly develop mutations that allow HIV-1 to escape neutralization by antibodies. In fact, a successful vaccine against HIV-1 needs to target a quasispecies of viruses and antigens that are constantly changing and not a single or a small number of viral proteins. In addition, an effective vaccine will need to target all circulating subtypes and recombinant forms simultaneously.

Currently, it is thought that a successful HIV-1 vaccine will need to elicit both humoral immune responses (Env-specific neutralizing antibodies) to block HIV-1 acquisition and cellular immune responses through Gag-specific CD8 T cells to control viral replication for breakthrough HIV-1 infections. However, it is not clear which antigens should be used to deal with HIV-1 sequence diversity. Major strategies have been proposed that include the design of (1) vaccines specific for each geographic region that include antigens matching local circulating strains and (2) a single vaccine broadly neutralizing all subtypes that could either target highly conserved epitopes

shared by all subtypes or elicit highly diverse HIV-1-specific responses (Stephenson and Barouch 2013).

Independently of the strategy followed, vaccine trials have shown very discouraging results. Some optimism returned with the RV144 trial that used a vaccine candidate based on a recombinant viral vector boosted with a protein-based vaccine VaxGen gp120 including subtype B and CRF01_AE immunogens. In this trial, a significant but modest and transient 31% reduction in HIV-1 acquisition was observed. However, the latest large-scale clinical study HVTN505, which tested a multiclade HIV-1 DNA plasmid (EnvA, EnvB, EnvC, gagB, polB, nefB) boosted by a recombinant adenovirus vector (Ad5 EnvA, EnvB, EnvC, gag/polB), was interrupted because the vaccine regimen did not prevent HIV infection nor reduce viral load (the amount of HIV in the blood) among vaccine recipients who became infected with HIV (Kwong et al. 2012) (<http://www.niaid.nih.gov/news/newsreleases/2013/Pages/HVTN505April2013.aspx>).

Recent studies on neutralizing antibodies are again encouraging. Broadly neutralizing antibodies have been found in the sera of some HIV-1-infected individuals. Monoclonal antibodies generated with peripheral blood B cells from donors with potent serum neutralizing antibodies were found to broadly neutralize currently circulating HIV-1 isolates. These antibodies are being used to design an effective vaccine eliciting protection against most HIV-1 variants. Vaccine efforts are now directed more at developing therapeutic than preventive vaccines, although not all hope for a protective vaccine has been lost yet (Klein et al. 2013).

Vaccine efforts used to be the major driving force behind subtyping efforts, while nowadays most subtyping is done in the context of drug resistance testing and epidemiological monitoring.

The Origin of HIV-1 Group M Subtypes

It might not seem logical to call the firstly discovered HIV-1 clade subtype B, while subtype A was only discovered in 1990. However, at that time the term subtype was not yet in use. Gerald Myers and

collaborators at the Los Alamos database (currently <http://www.hiv.lanl.gov/>) were the first to group the growing divergence of HIV-1 into subtypes, and they happened to have started the nomenclature following the appearance of the five main clades of that time in a phylogenetic tree, subtypes A through E (Myers et al. 1992). Subsequently, newly discovered subtypes had consecutive letter codes, while subtypes E and I were later renamed when they were identified as CRFs.

It is currently commonly agreed that subtype clusters are in a large part the result of a combination of incomplete sampling and founder effects. Incomplete sampling because samples from West and Central Africa have been collected that fall between subtypes and bridge the divergence between clusters (Rambaut et al. 2004). Founder effects because of different studies on the origin of the most important HIV-1 subtypes, B and C, indicate that the burst of those subtype epidemics occurred after migration out of the Congo Basin.

Postcolonial circumstances were responsible for the migration of at least one subtype D strain out of the Congo Basin into Haiti, where it diverged into an early subtype B clade around 1962–1970, before a single branch dispersed to the USA around 1966–1972 and from there globally. One study also showed that the spread of this epidemic was driven essentially by bottlenecks, chance events, and ecological interactions, rather than driven by evolutionary changes associated with selection (Rambaut et al. 2004). Historical information supports that hundreds of Haitian teachers and doctors were actively recruited after the independence of the DRC in July 1960 to fill in the gaps left by the previous Belgian rulers. Whereas at that time the virus might have been mainly spreading in heterosexuals, the only successful (from the point of view of the virus) link between Haiti and the USA was gay sex tourism (Vangroenweghe 1997), which seems to have ignited the “gay” AIDS disease that was discovered in the early 1980s in the USA (Centers for Disease Control (CDC) 1981). In most countries worldwide, HIV-1 subtype B still predominantly infects MSM.

According to recent data, subtype C originated around the 1950s in the southern DRC and subsequently dispersed south and east along the

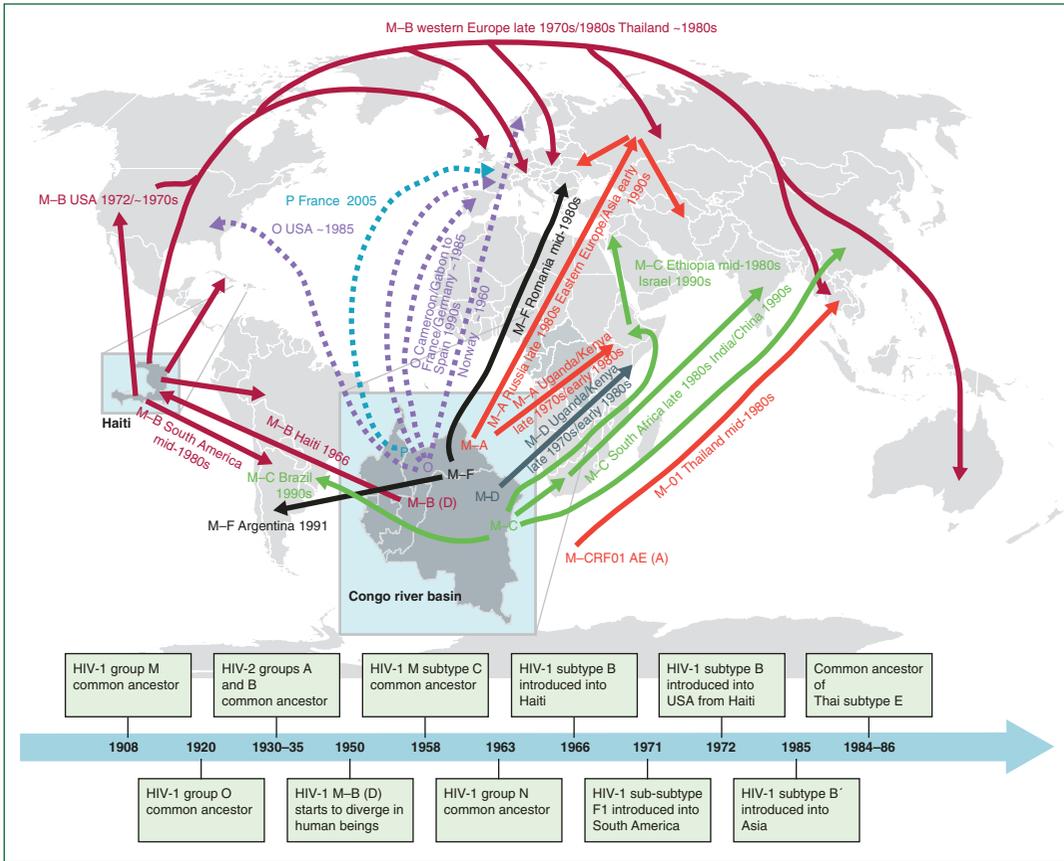
Copperbelt corridor (Faria et al. 2014). Phylogenetic analysis indicated that for several of the well-supported African subtype C clusters, there is a match with specific geographic regions and thus they presumably resulted from additional founder events. Also for other subtypes, further founder effects gave rise to well-supported clades linked to specific geographic regions or risk groups.

Geographic Distribution of Different Subtypes

Subtype A

Subtype A is divided in two sub-subtypes. Sub-subtype A1 originated around the 1950s with a local epidemic in West-Central Africa, followed by an exponential growth phase around the 1970s and a leveling off period during the mid-1980s/early 1990s. It has spread worldwide, causing a significant burden in Africa, Europe, and Asia (Hemelaar et al. 2011). It is currently thought that sub-subtype A1 was exported from the Congo Basin to Uganda and Kenya around the 1980s, to Russia in the late 1980s, and from there to Eastern Europe and Asia in the early 1990s (Tebit and Arts 2011) (Fig. 2). Sub-subtype A2, on the other hand, is limited to West and Central Africa.

In the 2004–2007 period (Hemelaar et al. 2011) (Fig. 3), HIV-1 sub-subtype A1 caused 12% of all HIV-1 infections worldwide, with its highest proportion among HIV-1 infected in Eastern Europe and Central Asia (up to 80%) and in East Africa (up to 50%). It can be found however throughout the entire Africa. In a cohort of newly diagnosed patients from Western Europe, sub-subtype A1 was found to cause 6.9% of infections in patients newly diagnosed between 2002 and 2005. In West and East Africa, subtype A infections are linked to heterosexual transmission. On the other hand, in Eastern Europe and Central Asia, injecting drug use is the main route of transmission for subtype A. As a consequence of the co-circulation of subtypes A1 and B in Eastern Europe and Central Asia, a recombinant form of these subtypes (CRF03_AB) circulates in injecting drug users in this geographic region (Bobkov et al. 2004).



Origin and Distribution of HIV-1 Subtypes, Fig. 2 Global spread of HIV outside its heartlands. In the upper part, arrows denote the pathways, along with estimated dates of introduction, of HIV-1 groups M, N, O, and P subtypes and circulating recombinant forms (CRFs) from the Congo River Basin to elsewhere. In the lower

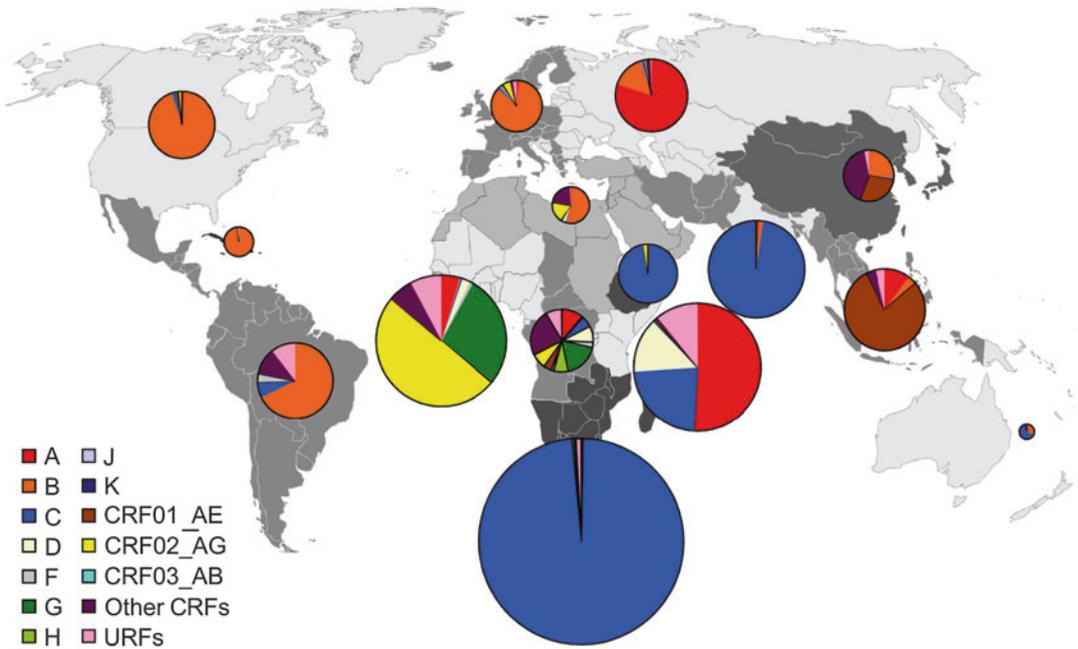
part, a time line of the estimated common ancestors of HIV-1 and HIV-2 groups, together with estimated introductions that subsequently led to some of the main circulating subtypes, is shown (Adapted and reproduced with permission from Tebit and Arts 2011)

Sub-subtype A1 infections have recently spilled over to the MSM risk group, and this is taking epidemic proportions in Greece.

Subtype B

Subtype B was the first variant of HIV-1 that was identified. It was initially found in homosexual men in San Francisco and New York and soon after in MSM and intravenous drug users (IVDUs) in Europe (Centers for Disease Control (CDC) 1981). As described above, it is now known to be an expansion of a subtype D strain that was exported from Africa to Haiti and from there to the USA in the 1970s (Fig. 2). From the USA, subtype B was exported to Western Europe and

Thailand in the 1970s/1980s and to South America somewhere in the mid-1970s. The subtype B strain that circulates in Asia has been named “B” because of its clear distinction from the “American” subtype B, another example of a founder effect followed by geographic separation. Although subtypes other than B were unknown for some time after the discovery of HIV-1 and despite the fact that it was virtually the only subtype circulating in developed countries for some years, this subtype causes only 11% of infections worldwide (Hemelaar et al. 2011). Subtype B is the main genetic form in North America (95% of all infections) and the Caribbean (98%), Western and Central Europe (85%), Latin America (68%),



Origin and Distribution of HIV-1 Subtypes, Fig. 3 Distribution of HIV-1 subtypes and recombinant forms per global region (2004–2007). Countries belonging to the same region are shaded identically. Pie charts represent the proportion of infections caused by

subtypes, circulating recombinant forms (CRF), and unique recombinant forms (URFs), as indicated in the legend on the left, during 2004–2007 in each geographic region (Reproduced with permission from Hemelaar et al. 2011)

and Middle East and North Africa (54%) (Fig. 3). In these geographic regions, this subtype infects all risk groups, with a predominance for MSMs and IDUs. It is also common in several countries of Eastern Europe and Central Asia (15%) and East Asia (26%), where it infects mostly IDUs. Finally, it causes 31% of HIV-1 infections in Oceania.

Subtype C

HIV-1 subtype C was first noticed in East Africa; however, recent findings show that subtype C arose from a founder effect from the Congo Basin into the Copperbelt of Southern and Eastern Africa (Faria et al. 2014). The subtype C epidemic then invaded Ethiopia, and from there it spread to Israel along with the return of “the lost tribe,” and that is the reason why Israel has two HIV-1 epidemics of almost equal proportion, one of subtype B mainly in Caucasians and one of subtype C mainly in black Africans. The virus also spread

from Eastern Africa to Brazil (possibly through the UK) and then to Argentina and Uruguay (Fig. 3). The epidemic has also spread to South and Central China, India, and Brazil. There is significant evidence of overall geographic clustering but no evidence of geographic clustering within South Africa. Significant clusters from a single geographic region are from Ethiopia, South America, India, and Botswana.

Subtype C is the most prevalent subtype worldwide. It causes 48% of all HIV-1 infections. This high prevalence is caused by its predominance in countries where the prevalence of HIV-1 is extremely high, such as Southern Africa, India and Ethiopia, where it causes over 95% of all local infections. It is transmitted almost exclusively by heterosexual transmission. It is also increasingly prevalent among heterosexuals in other world regions, such as Western Europe, Oceania, and Latin America. Subtype C recently entered the MSM population in the UK.

Subtype D

HIV-1 subtype D is only a minor player in the pandemic, even though it was at the origin of subtype B. This may be related to its higher malignancy compared to other subtypes, giving its host fewer opportunities to spread the virus. Between 2004 and 2007 (Hemelaar et al. 2011), subtype D accounted for only 2% of worldwide infections. These infections were spread mainly over East Africa (13.8% of local infections), Central Africa (8.5%), West Africa (1.5%), and Middle East and North Africa (2.2%) (Fig. 3). A higher proportion is found in Sudan and Uganda (up to 50%). In competition with other subtypes, subtype D seems to lose foot, for example, its proportion in Uganda decreased by 8% in 8 years (between 1994 and 2002). Also, in East Africa, a 3% decrease in proportion was observed between 2000 and 2007.

Subtype F

Subtype F causes only 0.45% of all HIV-1 infections, distributed mostly between South America (3.53%), Eastern Europe (1.14%), and Central Africa (2.69%) (Fig. 3). Except for Central Africa, where there was a small increase in the proportion of infections caused by this subtype, in the other geographic regions, its prevalence decreased between 2000 and 2007 (Hemelaar et al. 2011).

Subtype F is divided in two sub-subtypes: F1 and F2. However, only sub-subtype F1 was exported from Africa to other geographic regions. Sub-subtype F2 is restricted to Cameroon, with very few cases outside Africa and some cases found in West and Central Africa (Equatorial Guinea, Gabon, DRC, Central African Republic).

Sub-subtype F1 on the other hand has been widely exported from the Congo Basin to Eastern Europe (around 1985) and to South America and was introduced in Argentina around 1991 and in Brazil in the late 1970s (Bello et al. 2007) (Fig. 2). In Romania, the first HIV-1 cases were reported in 1985 and were followed by a dramatic increase in the number of infections primarily in children. These infections, caused by nosocomial transmission, were largely caused by sub-subtype F1 strains. F1 is still the most prevalent subtype in Romania, although newly diagnosed patients in 2007 were more infected by other subtypes than

by sub-subtype F1. In other Balkan countries, such as Bulgaria, Slovenia, and Hungary, a small proportion of infections are also caused by sub-subtype F1. However, as opposed to what happens in South America and Italy, F1 does not seem to have spawned BF-related CRFs in the Balkan epidemic. In Italy, sub-subtype F1 accounts for 2.7% of local infections and BF recombinants are also described in 1.1% of infections.

From all South American HIV-1 sequences deposited in the Los Alamos database, sub-subtype F1 amounts for 7.4% of strains and BF recombinants account for 16% of all sequences. This bias in the public database is related to intensive HIV and AIDS research in Brazil, where F1 infections and BF recombinants are major players in the epidemic, including in the surrounding countries (Paraguay, Bolivia, Uruguay, Argentina, and Chile). While sub-subtype F1 is contributing at a high proportion to infections in Brazil, this is based on partial genomic fragments, and one study with full genomes found that very few isolates were full-length subtype F: most of them had some B and C fragments and were thus in fact recombinant strains. The reported prevalence of subtypes does indeed depend on the genetic region analyzed, and full genomes are rarely sequenced to verify the claims. The BF recombinant pattern seems to increase the epidemic fitness of the virus, and this may explain its success.

Subtype G

Subtype G accounted for 4.6% of worldwide infections between 2004 and 2007. Subtype G is a typical West and Central African virus where it accounts for 27.6% and 18.2% of infections, respectively (Hemelaar et al. 2011). In Europe, subtype G is found in Western and Central European countries where it causes around 1% of local infections, with the exception of Portugal, where it has spread substantially causing a local epidemic. This epidemic was probably caused by an importation of a strain from West Africa to a network of Portuguese IVDUs that propagated the epidemic in the 1980s/1990s. Nowadays, subtype G circulates also among Portuguese heterosexuals. In 2003 it already accounted for nearly 30% of new infections. CRF14_BG originated

from this epidemic around 1990 and circulates in Portugal and Spain. A similar but much smaller subtype G epidemic among IVDUs has also been identified in Italy and Spain.

Subtype G causes substantial epidemics in many Central and Western African countries: Cape Verde (48% of HIV-1 infections), Nigeria (39%), Republic of Congo (21%), Togo (12%), Gabon (10%), Cameroon (4.5% in blood donors), Cote D'Ivoire (4%), and Chad (4%). Despite its low proportion in Cameroon, genome segments of subtype G are present in 67% of all infections and 80% of infections are due to inter-subtype recombinant strains.

CRF01_AE

CRF01_AE is a recombinant form for which the parental "subtype E" has never been identified. Globally, it causes an estimated proportion of 5% of all infections; however, in Southeast Asia it causes 79% of infections. It is now also spreading in East Asia, with the last estimate being 27.6% (Hemelaar et al. 2011). Phylogenetic studies have shown that CRF01_AE originated in Central Africa in the 1970s and spread to Thailand in the 1980s through heterosexual transmission. It was first identified among female sex workers in Northern Thailand in 1989. Later, it expanded to Southeast and East Asia and to other risk groups. It was introduced in China in the 1990s through multiple strain introductions in different risk groups and geographic regions. In a nationwide survey in 2006, CRF01_AE was found to cause 28% of infections in China. A study based on PRO-RT sequencing in Northern Thailand identified 95% of infections as CRF01_AE. Other studies, also based on PRO-RT sequences, found 98% of CRF01_AE in Vietnam and in Malaysia 57% of infections. However, based on the PRO-RT genomic region, it is not possible to discriminate CRF15_01B from CRF01_AE. In Vietnam, CRF01_AE was found in 96% of patients from Jakarta based on *gag* and *env* sequences.

CRF02_AG

CRF02_AG causes 5.5% of all HIV-1 infections worldwide. These infections occur mainly in West Africa, where it causes 50.1% of infections, and in

Middle East and North Africa with 18.1% of infections (Hemelaar et al. 2011). One study indicated that CRF02_AG diverged in the DRC around 1973, although CRF02_AG infections in this country are rare, and the predominance of this clade in Cameroon is related with at least two chance exportations of the virus from DRC to Cameroon.

Studies have shown a predominance of CRF02_AG in Cameroon (~58%), Cote D'Ivoire (82%), Senegal (64%), Togo (~50%), Burkina Faso (~50%), Benin (39–66%), Equatorial Guinea (54–64%), Gabon (~47%), Gambia (47%), Ghana (66%), Guinea (89%), Mali (~70%), Niger (54%), and Nigeria (~45%). All these studies indicate the importance of CRF02_AG in the HIV-1 epidemic in West and Central Africa.

In Northern Africa, in Libya, despite the lack of molecular epidemiology studies, CRF02_AG sequences were found to be responsible for the outbreak of nosocomial HIV-1 infections that occurred in Libya in 1998. This HIV clade is also present in Algeria, especially in the southern part of the country, probably due to importation events from West African countries.

Out of Africa, CRF02_AG has had an increasing impact in Western and Central Europe, where it caused 4.5% of infections between 2004 and 2007. These infections occurred mostly in the heterosexual risk group and in people originating from Africa. According to a recent survey, the European countries with the highest prevalence of CRF02_AG are Belgium and the Netherlands.

One study in Central Asia indicated 4.7% of HIV-1 infections in Kazakhstan were caused by CRF02_AG and these strains clustered with sequences from Uzbekistan, where 9.2% of infections were caused by this CRF, reflecting its spread in this geographic region.

Also in the Balkans CRF02_AG has been found: one study indicated that it causes 6.8% of infections in heterosexuals and IVDUs from Bulgaria and 9% of infections in heterosexuals from Croatia.

Other Subtypes/CRFs

Subtype H, J, and K infections are mainly restricted to the DRC and other West/Central

African countries (Fig. 3). Yet, as only very few full-genome sequences are available for these subtypes, it is not known if many of the identified sequences are pure subtypes H and J or descendant recombinant sequences. As these subtypes do not cause HIV-1 epidemics in other countries, they cannot be considered taking part in the pandemic. These subtypes are however included in the genomic structure of several recombinant forms, such as CRF04_cpx and CRF06_cpx.

Importantly, the global proportion of all CRFs combined increased by 4.5% between 2000–2003 and 2004–2007. Some cause epidemics in a specific geographic region. For example, CRF06_cpx is a complex recombinant that includes genomic segments of subtypes A, G, J, and K. It mainly circulates in Western Africa and is the predominant clade in Burkina Faso where it accounts for 40–50% of HIV-1 infections. This country seems to have been the main hub for the CRF06_cpx dissemination at regional level, continuously exporting the virus to other countries in West and West-Central Africa. Other neighboring countries have a lower proportion: 10–15% in Mali and Niger; 3–8% in Benin, Ghana, Côte d'Ivoire, Nigeria, Senegal, and Togo; and 1% or less in Guinea-Bissau and Guinea-Conakry. CRF06_cpx has been exported to Europe, mainly to Russia, and probably from there it has found its way during the 1990s into the IVDU population in Estonia, where it is now causing a major epidemic.

In South America, several recombinant forms originated from the co-circulation of subtypes B and F. CRF12_BF was first identified in Argentina and Uruguay, and other isolates have been identified in South America, Spain, and Italy. Other BF recombinant forms are currently circulating worldwide, albeit causing epidemics of limited proportions. These include CRF17_BF that has been isolated in Argentina; CRF28_BF, CRF29_BF, CRF39_BF, and CRF40_BF that have been identified in Brazil; CRF38_BF in Uruguay; CRF42_BF in Luxembourg; CRF44_BF and CRF46_BF in Chile; and CRF47_BF in Spain.

More recently, recombinants between subtypes B and C were found to circulate in Brazil. The

analysis of HIV-1 strains from Southern Brazil found that many presented the same genomic structure, with a 240-base pair fragment of subtype B in the middle of the reverse transcriptase pol region. This CRF (CRF31_BC) accounted for 11% of infections in the south of Brazil, indicating an important role in the epidemic.

The co-circulation of CRF01_AE and subtype B in Southeast Asia since the early 1990s already generated several CRFs. CRF15_01B was initially identified in heterosexuals from Thailand, but it was soon found to be circulating also in IVDUs. Its genomic structure includes a subtype B *env* and the rest of the genome is CRF01_AE. CRF33_01B was identified in Malaysia. In this chimera, two short subtype B segments are inserted into the gag-RT region in the backbone of CRF01_AE. By 2006, the proportion of CRF33_01B in Kuala Lumpur was 19.0%. Although its proportion was particularly high among IVDUs (42.0%), it was also detected in a substantial proportion of homo-/bisexual males (18.8%) and heterosexuals (9.8%). CRF34_01B was identified in 2007 in IVDUs from Northern Thailand, CRF48_01B in Malaysian IVDUs, CRF 51_01B in Singapore, CRF52_01B in Thailand and Malaysia, CRF53_01B and CRF54_01B in Malaysia, and CRF55_01B in MSMs from China. In China, also CRF07_BC and CRF08_BC were found to circulate among local IVDUs and are an important part of the local epidemic.

CRF03_AB represents a subtype A/B recombinant that was first found in Kaliningrad and is circulating in Russian and Ukrainian cities, primarily in IVDUs. The circulation of this strain appears to have been accelerated by intravenous injection of a locally produced opiate contaminated with HIV-infected blood.

Finally, different recombinant forms between subtypes B and G have been identified in Portugal and parts of Spain, due to the simultaneous epidemics caused by these two subtypes in these countries. The most important of these is CRF14_BG, first identified by Delgado et al. (2000) in IVDUs from Galicia (a Spanish region bordering Portugal). This CRF circulates widely in these two countries and other

recombinant forms including fragments of CRF14_BG have been identified subsequently. Other BG recombinant forms (CRF20_BG, CRF23_BG, and CRF24_BG) have been identified in Cuba, where subtypes B and G also co-circulate.

Many unique recombinant forms (URFs), recombinants that were not found to infect three epidemiologically unlinked individuals, have also been found in distinct geographic regions.

What Is a “Pure” Lineage and What Is a CRF?

There is some discussion on whether the so-called “pure” lineage classification of subtype G and the CRF classification of CRF02_AG are in fact justified. It is true that both have been discovered around the same time, and originally there was some doubt whether subtype G was indeed a “pure” subtype since it had several regions that were rather closely related to subtype A (Robertson et al. 2000). Later on, an in-depth analysis of the diversity within subtype G and within CRF02_AG suggested that subtype G was in fact the circulating recombinant form (including also fragments that were closest related to subtype J), while CRF02_AG could be considered a divergent lineage within subtype A - (Abecasis et al. 2007). Later this was again refuted (Zhang et al. 2010). Also, the fact that CRF01_AE and other complex CRFs were discovered soon after the initial discovery of HIV, and that sometimes the parent subtypes have actually never been sampled, clouds the early history of subtype origins. Moreover, in the Congo Basin, HIV-1 group M diversity is so large, and many strains do not belong to any pure subtype (some with and some without recombination signal), suggesting that the early history of subtype origins may not properly be represented by separate lineages. In fact, it is possible that HIV-1 group M was diverging and recombining locally in the Congo Basin, and what are known today as pure subtypes did not exist up until the exportation of distinct clades out of their epicenter, possibly Kinshasa, after the 1960s (Kalish et al. 2004; Rambaut et al. 2001). As argued above, subtypes are the result of a migration event to a new susceptible population, and one should not try to “enforce”

treelike behavior to this early period of the epidemic. The classical phylogenetic analysis software has no proper way of displaying this initial history of HIV-1 group M before subtypes arose. Perhaps it is better to leave this early divergence period as an unresolved network or cloud, as was done in Fig. 1.

Now that the different HIV-1 subtypes and CRFs are co-circulating in various countries as described above, the number of new CRFs and URFs is increasing. Some of these CRFs are difficult to discriminate from their founder “pure” subtype or CRF, especially in genetic regions where there is no recombination breakpoint. Reporting of subtypes and CRFs is therefore becoming confusing. Robust classification of HIV-1 divergence into distinct CRFs may gain a higher resolution once full-genome sequencing becomes an affordable standard, for example, in drug resistance testing practice. Another issue is that some subtypes are diverging to such an extent, such as sub-subtypes A1 and A2, that it can be disputed whether some of them should be renamed as separate subtypes. It is thus already clear that the existing subtype and CRF nomenclature might not be sufficiently flexible to accommodate the global diversity of currently circulating strains and that perhaps molecular epidemiologists should start talking about “phylotypes,” naming only those phylotypes that have a serious impact on the HIV-1 pandemic.

How to Subtype?

Given the clinical and epidemiological importance of HIV-1 subtypes, and despite the rising confusion about subtypes and CRFs, it remains useful to subtype HIV-1 isolates. The general principle to subtype HIV-1 sequences is to compare the query sequences to a set of reference sequences that includes representatives of all HIV-1 subtypes and/or CRFs. To guarantee that possible recombination events are detected, sequences are split in segments of ~400 base pairs and each one of these segments is compared to the positional homologous segment of the reference sequences. The analysis then moves in steps of around 50 base pairs to find another

sequence fragment that is compared again to the reference set. This procedure is followed for the whole length of the sequences and the results are summarized. If across the genome all sequence fragments are similar to only one reference subtype, then this query sequence corresponds to a pure subtype. If, on the other hand, some sequence fragments are similar to one subtype and others are similar to another subtype and at a certain point in the genome an abrupt and significant drop in the support for belonging to one subtype and a rise in the support for another subtype are detected, then this genomic region most likely corresponds to a recombination breakpoint, and the sequence is either a CRF or a URF.

Ideally, HIV-1 sequences should be subtyped using phylogenetic analysis of the full genome of the query sequences compared to reference sets. This type of analysis includes building phylogenetic trees and making bootscanning analysis across the full genome of the query sequences (Salminen et al. 1995). However, due to the scarcity of full-genome sequences and the high number of sequences that are subtyped, this approach is not practical. Often, and because a large number of pol sequences are generated for HIV-1 drug resistance testing, subtyping is only performed for this genomic region, and assignments are based on automated subtyping methods available in different software packages. Methods available for automated subtyping include similarity-based tools (NCBI subtyping tool, Stanford, Geno2pheno, and EuResist) (http://engine.euresist.org/data_analysis/viral_sequence/new), statistical-based tools (COntext-based Modeling for Expeditious Typing (COMET) which uses partial matching compression algorithms, STAR which uses position-specific scoring matrices, and jumping profile Hidden Markov Models (jpHMM)), and phylogenetic-based tools (REGA and Subtype Classification Using Evolutionary ALgorithms (SCUEAL)). A recent comprehensive comparison between these methods suggests that there is no single method that outperforms all others for all potential uses. Rather, a combination of two well-performing methods can be advised, such as COMET and REGA.

Conclusion

Several studies have shown the importance of taking HIV-1 genetic diversity into account while investigating its clinical and biological properties. There is substantial difference in disease progression among patients infected with different subtypes. Despite reported differences in the pathways to the development of antiviral drug resistance, overall response to treatment seems not to vary between HIV-1 subtypes. Vaccine development, on the other hand, has been severely compromised by the high level of HIV-1 genetic diversity, with several clinical trials showing very low levels of protection against infection. To understand and take into account these differences, it is important to look at the origin of subtypes. After the establishment and early spread of HIV-1 group M in the Congo Basin, different subtypes arose through independent exportation events. This fact generated a disparate prevalence and distribution of HIV-1 subtypes in different geographic regions. The distribution of HIV-1 subtypes is also highly compartmentalized among risk and demographic groups. Although HIV-1 subtype B is historically the best characterized because it was the first clade to be identified in the Western world, it is subtype C that is the most prevalent subtype worldwide, causing around 50% of global infections, most of these in South Africa and India where the prevalence of HIV-1 is now extremely high. Because of the general interest in subtypes for huge datasets, automatic tools have been developed, which are however less accurate than classical manual analysis.

Cross-References

- ▶ [HIV-2 Transmission](#)
- ▶ [HIV-2, Phylogeographic Insights into the Origins and Epidemic History](#)
- ▶ [Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk](#)
- ▶ [Transmission HIV-2: Origin, Epidemiology, and Phenotype](#)

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Other HPV-Associated Cancers (Oropharyngeal and Penile)

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Definition

- ▶ **Human papillomavirus (HPV)** has been recognized as an etiologic agent in malignancies at several anatomic sites, including the oropharynx and the penis. The overwhelming majority of oropharyngeal and penile cancers are squamous cell carcinomas. It is estimated that approximately two-thirds of oropharyngeal cancers and half of penile cancers can be attributed to HPV infection. The incidence of HPV-associated oropharyngeal cancers is rapidly increasing in developed

countries. Penile cancer remains rare in developed countries but constitutes a major cancer burden among men in developing countries. Compared with the general population, patients with HIV/AIDS are at increased risk for HPV-associated cancers, including oropharyngeal and penile cancers.

Introduction

► **Human papillomavirus (HPV)** is the cause of 5.2% of cancers worldwide and has been identified as an important etiologic agent by the International Agency for Research on Cancer (Chaturvedi 2010; Gillison 2008). Virtually all ► **cervical cancers** are attributable to HPV infection, as are 90–93% of anal cancers, 12–63% of oropharyngeal cancers, 36–40% of penile cancers, 40–64% of vaginal cancers, and 40–51% of vulvar cancers; HPV16 is the main viral type involved in carcinogenesis (Chaturvedi 2010). The main risk factors for HPV-positive compared with HPV-negative noncervical cancers are high-risk sexual behaviors, immunosuppression (including immunosuppression due to HIV and immunosuppressive drugs required after organ transplant), and a prior HPV-associated cancer (Chaturvedi 2010). HPV-positive noncervical cancers tend to be diagnosed at earlier ages than HPV-negative noncervical cancers (Chaturvedi 2010). The ratio of noncervical to cervical HPV-associated cancers differs by geographical region. Developed countries with established ► **cervical cancer** screening programs in place have higher proportions of noncervical than cervical HPV-associated cancers, while developing countries without such programs have a high burden of ► **cervical cancer** that far outweighs the burden of other HPV-associated cancers (Chaturvedi 2010).

Oropharyngeal Cancer

The oropharynx contains the tonsils and base of tongue (lingual tonsil) and refers to the mid-portion of the pharynx immediately behind the

oral cavity and above the larynx. Along with smoking and alcohol use, HPV is a major etiologic agent of oropharyngeal cancer, and oropharyngeal cancers may arise from either of two distinct etiologic pathways, one associated with HPV and one associated with tobacco exposure (Gillison 2009; Sturgis and Ang 2011). The incidence of oropharyngeal cancer is increasing in the United States despite a decrease in smoking prevalence (Sturgis and Ang 2011). The prevalence of HPV in oropharyngeal cancer has increased significantly over time to the current prevalence of 70–80% (Sturgis and Ang 2011). HPV16 is the chief type of HPV associated with oropharyngeal cancer, and more than 90% of HPV-positive oropharyngeal cancers are positive for HPV16 (Sturgis and Ang 2011).

Approximately 60–80% of HIV-positive individuals smoke compared with fewer than 20% of individuals in the general population. Between 25% and 40% of HIV-positive individuals have a prevalent oral HPV infection, compared with only 5–10% of individuals in the general population (Gillison 2009; Gillison et al. 2012). However, it remains unclear what proportion of oropharyngeal cancers among HIV-positive individuals should be attributed to HPV and what proportion should be attributed to smoking and alcohol use.

HPV-positive oropharyngeal cancer is often of nonkeratinizing histology (basaloid, lymphoepithelial, or poorly differentiated carcinoma), and patients typically present with late-stage disease characterized by small primary tumors and advanced nodal metastases (Gillison 2009; Sturgis and Ang 2011). These patients also have distinct demographic characteristics: compared with patients with HPV-negative oropharyngeal cancer, patients with HPV-positive oropharyngeal cancer typically present at younger ages and are more likely to be white men of high socioeconomic status (Gillison 2009; Sturgis and Ang 2011). Sexual behaviors, including younger age at first sexual intercourse, a history of sexually transmitted diseases, and high number of sex partners have all been associated with increased risk of HPV-associated oropharyngeal cancer. High number of oral sex partners is particularly strongly associated with HPV-positive cancers (Gillison

2009). In addition to distinct demographic, behavioral, and clinical characteristics, patients with HPV-positive oropharyngeal cancer have outcomes not typical of those in patients with HPV-negative oropharyngeal or other head and neck cancers. In particular, patients with HPV-positive oropharyngeal cancer have much better overall, disease-specific, recurrence-free, and second primary tumor-free survival rates than those with HPV-negative cancer (Sturgis and Ang 2011). Additionally, when recurrences do occur in patients with HPV-positive oropharyngeal cancer, these recurrences are more commonly distant recurrences rather than local-regional recurrences, which are more typical in patients with head and neck cancer.

The increasing incidence of oropharyngeal cancer in the United States is limited to the tonsils and base of the tongue, oropharyngeal sites typically associated with HPV (Sturgis and Ang 2011). From 1973 to 2004, the age-adjusted incidence of oropharyngeal cancer at HPV-associated sites (base of the tongue/lingual tonsil and tonsils) increased significantly, and this increase was especially pronounced from 2000 to 2004, when the annual percent change was 5.22% ($p = 0.016$) (Chaturvedi et al. 2008). Conversely, the incidence of cancers at sites not associated with HPV (oral cavity) remained stable through 1982 (annual percent change, 0.82%; $p = 0.186$) and decreased from 1983 to 2004 (annual percent change, -1.6%; $p < 0.001$) (Chaturvedi et al. 2008).

That the increase at sites associated with HPV is in fact due to an increase in the prevalence of HPV infection has recently been confirmed using oropharyngeal cancer tumor tissue from participants in the Surveillance, Epidemiology, and End Results Residual Tissue Repositories Program (Chaturvedi et al. 2011). In this study, the prevalence of HPV in oropharyngeal tumors increased from 16.3% in the 1980s to 72.7% in the 2000s, and between 1988 and 2004, there was a 225% increase in the incidence of HPV-positive oropharyngeal cancer (from 0.8 cases per 100,000 individuals to 2.6 per 100,000), accompanied by a 50% decrease in the incidence of HPV-negative oropharyngeal cancer (from 2.0 cases per 100,000 individuals to 1.0 per 100,000) (Chaturvedi

et al. 2011). If these trends continue, it is expected that HPV-positive oropharyngeal cancer cases will constitute the majority of head and neck cancers in the next few decades and that the number of HPV-positive oropharyngeal cancer cases will surpass the number of ▶ [cervical cancer](#) cases by 2020 (Chaturvedi et al. 2011).

It is estimated that the odds ratio (OR) for HPV-positive oropharyngeal cancer among individuals with a prevalent oral HPV infection ranges from 3.6 to 230. Studies measuring evidence of a previous HPV infection as indicated by HPV seropositivity have reported odds ratios (ORs) in the range of 2.3–182 for HPV16 L1 antibodies and ORs in the range of 9.2–231 for HPV16 E6/E7 antibodies (Chaturvedi 2012). In contrast to the understanding of cervical HPV infections, large prospective studies confirming these risk estimates for oropharyngeal cancer are lacking, as is documentation of the natural history of oral HPV infections with data on clearance, reinfection, and malignant conversion. Additionally, identification is still awaited of a precursor HPV-positive oropharyngeal lesion analogous to cervical or anal intraepithelial neoplasia, which would allow for screening for oropharyngeal cancer as well as early diagnosis and treatment.

Penile Cancer

Penile cancer is relatively rare in developed countries, where incidence rates range up to 1.5 cases per 100,000 men per year, but is much more common in developing countries, where incidence rates reach 4.4 cases per 100,000 men per year in Uganda and as high as 6.8 cases per 100,000 men per year in Brazil (Anic and Giuliano 2011; Backes et al. 2009; Bleeker et al. 2009). Penile cancer accounts for less than 1% of cancers in adult men in Europe and North America and up to 10% of cancers in adult men in less developed regions (Anic and Giuliano 2011; Bleeker et al. 2009; Miralles-Guri et al. 2009). Penile cancer is usually diagnosed among older men; the mean age at diagnosis is 60 years, and incidence is highest at 70 years (Bleeker et al. 2009). It is estimated that about 40% of

penile cancers can be attributed to HPV, and HPV16 accounts for 63% of HPV-positive cases (Backes et al. 2009; Miralles-Guri et al. 2009).

As with oropharyngeal cancer, penile cancer is thought to arise from either of two distinct etiologic pathways, one related to HPV and one related to other factors. Risk factors for penile cancer include higher number of sex partners and a history of sexually transmitted diseases, factors that are also associated with HPV infection (Bleeker et al. 2009). Other risk factors for penile cancer include smoking, lack of circumcision, poor penile hygiene, phimosis, and inflammation (Backes et al. 2009; Bleeker et al. 2009). HPV-associated penile cancer is thought to follow the same carcinogenic pathway as ► [cervical cancer](#), including a persistent infection with high-risk HPV and genetic alterations necessary for malignancy to develop in an HPV-infected cell (Bleeker et al. 2009). However, the lower incidence rates and later age at onset of penile cancer compared with ► [cervical cancer](#) suggest that tissue- and hormone-specific differences may exist between cancers at the two sites (Bleeker et al. 2009).

Virtually all (95%) penile cancers are squamous cell carcinomas, which can be further classified into at least four histological subtypes, keratinizing, basaloid, warty, and verrucous, with the keratinizing type being the most common (50–60%) (Backes et al. 2009; Miralles-Guri et al. 2009). Several retrospective reviews have estimated the prevalence of HPV and the distribution of HPV types in invasive penile cancer (Backes et al. 2009; Bleeker et al. 2009; Miralles-Guri et al. 2009). These studies found that almost half of invasive penile cancer cases were associated with HPV and that the prevalence of HPV infection differed by histologic subtype (Backes et al. 2009; Miralles-Guri et al. 2009). The highest HPV prevalence was found among basaloid and warty types and the lowest prevalence among verrucous types. Miralles-Guri et al. estimated that 76% of basaloid, 59% of warty, 44% of keratinizing, and 25% of verrucous types were HPV positive (Miralles-Guri et al. 2009). Backes et al. found that keratinizing tumors had an HPV prevalence of 48% and that

warty/basaloid types were 3.5 times as likely as keratinizing tumors and verrucous types were less than half as likely as keratinizing tumors to be associated with HPV (Backes et al. 2009). Although HPV prevalence differed by geographical region, at least 40% of all cases in all regions were positive for HPV (Backes et al. 2009; Miralles-Guri et al. 2009). The most common HPV types found in penile cancer were HPV16 (75%), HPV18 (12%), and HPV6/HPV11 (5%) (Backes et al. 2009; Miralles-Guri et al. 2009).

Genital HPV infection in men is common: prevalence estimates range from 1.3% to 72.9% (most >20%), with the most common types detected being HPV6 and HPV16 (Anic and Giuliano 2011). Higher prevalence rates are reported in studies using more sensitive sampling techniques (i.e., a prewetted Dacron swab) and when multiple anatomic sites are sampled (Anic and Giuliano 2011). A US study reported higher prevalence rates in samples taken from the shaft (50%), glans (36%), and scrotum (34%) than in samples taken from the perianal area (20%), anal canal (18%), urethra (10%), and semen (5%) (Anic and Giuliano 2011).

Penile intraepithelial neoplasia (PIN) is heterogeneous, and without histological confirmation, benign conditions are often misclassified as PIN (Anic and Giuliano 2011). It is estimated that 60–100% of PIN lesions are HPV positive, and one large case series reported prevalence rates of 41% for HPV16, 22% for HPV6, 15% for HPV52, and 4% for HPV11 (Anic and Giuliano 2011). High-grade PIN is considered penile carcinoma in situ, and development to this stage of PIN is rare; however, whereas the risk of progression to invasive disease in patients with cervical and anal intraepithelial neoplasia is known, the risk of progression to invasive penile cancer once high-grade PIN occurs is still unknown (Anic and Giuliano 2011).

Increased Risk of HPV-Associated Cancers Among Patients with HIV/AIDS

HIV-positive patients have an increased risk of HPV-associated cancers, including oropharyngeal

and penile cancers, compared with the general population. Moreover, the risk of these cancers appears to increase with time since AIDS onset. The increased risk is consistent with the increased incidence, prevalence, and persistence of HPV infections and increased smoking prevalence among HIV-infected individuals.

Analysis of more than 300,000 patients with HIV/AIDS from the AIDS-Cancer Match Registry documented that these individuals have an increased risk of HPV-associated cancers (Frisch et al. 2000). Patients with HIV/AIDS were seven times as likely as those in the general population to have HPV-associated cancers, and this risk increased significantly with time since AIDS onset. For invasive cancers, the risk of tonsillar cancer among men with HIV/AIDS was 2.6 times higher than among men in the general population, while no cases occurred among women with HIV/AIDS. The risk of penile cancer was almost four times as high among men with HIV/AIDS as among men in the general population, and the risk was especially elevated among men younger than 30 years (in situ penile cancer, relative risk [RR], 16.1; 95% CI, 4.4–41.2 and invasive penile cancer, RR, 37.2; 95% CI, 7.7–108.6) (Frisch et al. 2000). Furthermore, the risk of both in situ and invasive penile cancer was higher among Blacks and Hispanics with HIV/AIDS, while the risk was not statistically elevated among Whites. When individuals were stratified by HIV-exposure category, the risk of invasive penile cancer was highest among intravenous drug users (RR, 7.1; 95% CI, 2.8–14.6) and only slightly elevated among homosexual men (RR, 2.8; 95% CI, 1.0–6.1) (Frisch et al. 2000).

Engels et al. found that among HIV-positive individuals, the overall standardized incidence ratio (SIR) for oral cavity/pharynx cancer was 1.7 (95% CI, 1.1–2.5) and for penile cancer was 5.4 (95% CI, 1.1–16) (Engels et al. 2008). Furthermore, the risk for oral cavity/pharynx cancer increased after diagnosis with AIDS (RR, 3.6; 95% CI, 1.6–8.2). There were too few cases to evaluate incidence trends for penile cancer (Engels et al. 2008). These results were

confirmed in a study by Chaturvedi et al. that included almost 500,000 individuals diagnosed with AIDS during 1980–2004 (Chaturvedi et al. 2009). The SIR for invasive oropharyngeal cancer was 1.6 (95% CI, 1.2–2.1), but no significant increase in SIR was found with time since AIDS diagnosis. The SIRs for both in situ and invasive penile cancers were elevated (SIR, 19.7; 95% CI, 13.2–28.3 and SIR, 5.3, 95% SIR, 3.2–8.2, respectively), and there was a significant increase in in situ cancers (but not for invasive cancers) since time of AIDS diagnosis ($p < 0.001$) (Chaturvedi et al. 2009). When patients were stratified by HIV-exposure category, the risk of invasive oropharyngeal cancer was highest among heterosexual men (RR, 3.2; 95% CI, 1.6–5.7), followed by intravenous drug users (RR, 2.1; 95% CI, 1.3–3.2). For penile cancer, the risk of in situ cancer was elevated among men who had sex with men, intravenous drug users, and heterosexual men (RR > 20 for all subgroups), and the risk of invasive cancer was elevated among men who had sex with men (RR, 4.4; 95% CI 1.9–8.7) and heterosexual men (RR, 14.7; 95% CI, 4.8–34.5) (Chaturvedi et al. 2009).

It is unclear to what extent immunosuppression plays a role in progression of HPV-associated cancers in patients with HIV/AIDS (Chaturvedi et al. 2009; Frisch et al. 2000). While the risk of in situ cancers appears to increase with time since AIDS diagnosis, such an increase was generally not seen for invasive cancers. This may signify that immune suppression facilitates the progression of premalignancies but is not relevant in later progression to invasive cancer (Chaturvedi et al. 2009; Frisch et al. 2000). Progression of disease may be due to other factors among individuals with HIV/AIDS that are as yet unknown (Frisch et al. 2000).

The incidence of HPV-associated cancer among people with HIV/AIDS remains high even after the use of highly active antiretroviral therapy (HAART). One possible explanation may be that HAART may not influence immunity to HPV or that immunologic control of HPV-related tumors is exerted only at early stages of tumor development

(Chaturvedi et al. 2009). At the same time, the longer life expectancy of individuals in the HAART era may also lead to an increased risk of HPV-associated cancer because individuals with HIV/AIDS now live long enough to develop these cancers (Chaturvedi et al. 2009; Gillison 2009).

Opportunities for Prevention

HPV16 and HPV18 are responsible for 70% of ► [cervical cancers](#) and 90% of noncervical cancers. There are currently two HPV vaccines approved by the US Food and Drug Administration to protect against these types. Cervarix (GlaxoSmithKline) is a bivalent vaccine that protects against HPV16 and HPV18, while Gardasil (Merck) is a quadrivalent vaccine that protects against these and two additional viral types, HPV6 and HPV11. Because of the high global burden of HPV-positive cervical lesions and ► [cervical cancer](#), initial clinical trials were limited to women; however, the vaccines have more recently been shown to be highly efficacious in preventing anogenital warts in males and anal precancer in men and women, and the vaccines are expected to prevent HPV-positive oropharyngeal cancer as well.

Initial results from population-based studies are encouraging. Significant reductions in the incidence of genital warts have been observed in Australia, where 65% of eligible girls have been vaccinated. Among women aged 12–26 years, there was a 59% reduction in the incidence of genital warts between 2004 and 2009. Concomitantly, there was a significant 39% reduction in the incidence of genital warts among men aged 12–26 (Donovan et al. 2011). Herd immunity confers protection to heterosexual boys in areas where vaccine coverage is high (e.g., Australia); however, this is not observed when vaccination rates are low and may not extend to homosexual men, unless a substantial percentage of men are vaccinated (Brisson et al. 2011).

The US Food and Drug Administration approved Gardasil for routine use in girls and young women aged 9–26 years in 2006 and for use in boys in 2011. Unfortunately, the US

Centers for Disease Control and Prevention estimates that in 2011, only about half of girls in the United States of the targeted age range had received at least one dose, and only 35% had received all three doses of the vaccine. Among boys in the United States, 8% had received at least one dose, while only 1% had received all three doses (CDC 2011). It is imperative that both boys and girls be vaccinated since HPV-associated disease occurs among both men and women, and in this decade, HPV-positive oropharyngeal cancer is expected to surpass ► [cervical cancer](#) as the most common HPV-related cancer in the United States. In addition, vaccination programs ignoring boys will leave some men outside the proposed protective effect of herd immunity and at risk for oropharyngeal, penile, and ► [anal cancers](#).

Conclusion

HPV is the cause of approximately two-thirds of oropharyngeal cancers and half of penile cancers. The incidence of HPV-associated oropharyngeal cancer is increasing, especially among middle-aged white men, and is expected to surpass the incidence of cervical cancer in this decade. The incidence of penile cancer is rare in developed countries but is high in areas where the burden of cervical cancer is high. Patients with HIV/AIDS are at increased risk for HPV-associated cancers compared with the general population. The HPV vaccine offers an opportunity for prevention of HPV-associated disease, including oropharyngeal and penile cancer; however, it is imperative that both boys and girls be vaccinated to protect all persons at risk.

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Overcoming the Transcriptional Block: The HIV-1 Tat Auxiliary Protein

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Definition

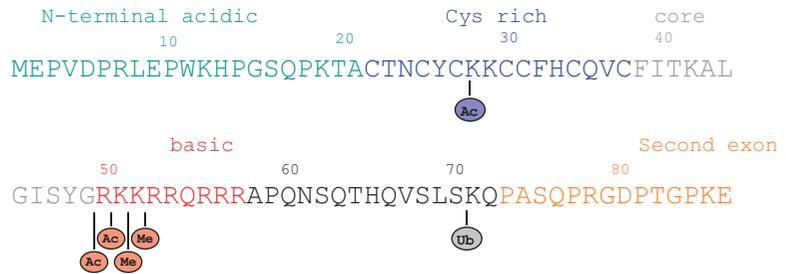
The human immunodeficiency virus type 1 (HIV-1) encodes for the auxiliary protein Tat (*trans*-activator of transcription). Tat is expressed early following HIV-1 infection and dramatically increases the level of transcription from the integrated viral promoter. Before Tat expression, only few RNA transcripts are completed, allowing also for the production of Tat itself. Tat then functions as an adaptor for the binding of cellular proteins to viral RNA, which boosts transcription in a positive feedback cycle. Tat is an essential protein for HIV-1 pathogenesis and for reactivation from post-integrative latency following antiretroviral therapy.

The Tat Polypeptide

HIV-1 is a retrovirus that integrates a DNA copy of its RNA genome into the host chromatin. The HIV-1 promoter is embedded in the long terminal repeat (LTR) of the virus and contains several upstream DNA regulatory elements that act as binding sites for cellular transcription factors. The core promoter includes three binding sites for the SP1 (specificity protein 1) activator plus a TATA box to facilitate RNA polymerase II (RNAPII) transcription. The enhancer region, located upstream of the SP1 binding sites, is formed by two binding sites for NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), which act in concert with Sp1 to stimulate basal transcription following diverse stimuli. However, the unique feature that distinguishes the

Overcoming the Transcriptional Block:

The HIV-1 Tat Auxiliary Protein, Fig. 1 The HIV-1 Tat polypeptide (86 aa) with indicated residues modified by acetylation (Ac), methylation (Me), or ubiquitylation (Ub)



HIV-1 LTR from a cellular promoter is its regulation by the viral transactivator Tat. Essentially, Tat establishes a feedback loop to stimulate HIV-1 transcription. Tat is a small polypeptide (101 amino acids for most primary isolates, 86 amino acids for some laboratory-adapted strains), which is transcribed from a spliced transcript of the HIV-1 genome that overlaps with viral *env* and *rev* genes. Tat is a nuclear protein, but is also secreted as an active polypeptide able to transactivate the viral LTR of neighboring cells. Indeed, Tat-derived peptides are highly efficient in delivering proteins across cellular membranes. The amino acid sequence of Tat comprises four regions in the first exon: the amino-terminal acidic domain (aa 1–21), a domain containing 7 cysteines (aa 22–37), a core region (aa 38–48), and a basic domain enriched in arginine and lysine amino acids, which is highly conserved among different strains (aa 49–57) (Fig. 1). The first exon contains all the minimal functional domain of the protein. The second exon starts at amino acid position 73 and has a relatively more variable sequence. Being dispensable for the transcriptional functions of Tat, the second exon has been largely overlooked, although its conservation points to a specific role in the life cycle of HIV-1 *in vivo*. HIV-1 Tat interacts with a *cis*-acting RNA element (*trans*-activation-responsive region; TAR) present at the 5' end of each viral transcript (nucleotide position +1 to +59 counting from the transcription start site). The basic domain of Tat is a nuclear localization signal but is also required for TAR binding. Through this interaction, the protein promotes the assembly of transcriptionally active complexes at the LTR by multiple protein-RNA and protein-protein interactions. Tat is considered an intrinsically unfolded protein that does

not allow its structure to be easily determined. However, when bound to host factors such as P-TEFb (see below), Tat adopts a resolvable structure that is complementary to the surface of the partners (Tahirov et al. 2010).

Remodeling of Chromatin

Following infection, reverse-transcription, and nuclear translocation of the pre-integration complex, the viral cDNA is spliced into host chromatin. The choice of the integration site is quasi-random, with a preference for actively transcribing genes and highly dependent on the conformation of chromatin (Dieudonne et al. 2009; Marcello et al. 2010). However, independently of the integration site, nucleosomes in the 5' LTR are precisely positioned with respect to *cis*-acting regulatory elements (Van Lint et al. 1996). Nucleosomes form the fundamental repeating units of eukaryotic chromatin, which are used to pack the DNA into the nuclei while regulating accessibility. Nucleosomes carry epigenetically inherited information in the form of covalent modifications such as phosphorylation, ubiquitylation, acetylation, or methylation of their core histones (Jenuwein and Allis 2001). Nucleosome nuc-1, located immediately downstream of the transcription start site, is critical for the control of HIV-1 transcription being specifically remodeled through posttranslational covalent modifications of histone tails and through remodeling complexes that require energy (ATP) to function. In conditions of transcriptional repression, this area of the promoter is enriched with histone deacetylases (HDAC) and with histone methyltransferases (HMT), which actively deacetylate or methylate specific lysine

residues in histone tails, respectively. As a result, chromatin is more compact and less accessible to the RNAPII complex. When the cell is exposed to external stimuli, certain inducible host transcription factors, such as NF- κ B, NFAT (nuclear factor of activated T cells), and AP-1 (activator protein 1), translocate into the nucleus and bind the viral LTR to initiate the transcription cycle and the expression of the first molecules of Tat. Tat is able to recruit to the LTR specific histone acetyltransferases, such as the cAMP-responsive element binding factor (CREB) binding protein (CBP)/p300 and the p300/CBP-associated protein P/CAF (Benkirane et al. 1998; Lusic et al. 2003; Marzio et al. 1998). In addition to acetyltransferases, Tat recruits also the SWI/SNF remodeling ATPase components BRM and BRG1, which contribute to the final remodeling of chromatin at the integrated LTR (Treand et al. 2006). The ability to modulate early events in chromatin remodeling at the viral promoter is at the basis of the most recent attempts to cure the disease. Although combined antiretroviral therapy (cART) is highly efficient in reducing the levels of circulating virus, it is not able to eradicate the infection from cells carrying a transcriptionally silent provirus. Hence, several inhibitors of HDACs or activators of the transcription factors are being tested to drive the virus out once and for all (see paragraph six). These approaches leverage essentially on triggering Tat expression because, once Tat is available, transcription proceeds *sine impedimenta*.

Tat and Elongation

In the presence of suboptimal levels of the viral transactivator Tat, basal transcription from the viral LTR leads to pausing of RNAPII after synthesis of a short RNA that includes the TAR element. Promoter-proximal pausing of RNAPII is a widespread negative regulatory mechanism of gene expression, which is controlled by negative (NTEF) and positive (P-TEFb) transcription elongation factors. A wealth of literature is available on this topic extensively reviewed elsewhere (Marcello 2006; Ott et al. 2011). In the paused state, RNAPII is found associated to the promoter

and to nascent RNA together with the NTEF composed of the negative elongation factor (NELF) multisubunit complex and the 5,6-dichloro-1 β -D-ribofuranosylbenzimidazole sensitivity-inducing factor DSIF, which is a heterodimer, composed of Spt4 and Spt5. Regulation of transcriptional pausing is actively being investigated and has been recently shown to involve RNA-dependent premature termination as well as the integrator complex (Stadelmayer et al. 2014; Wagschal et al. 2012). In order to overcome RNAPII pausing and to enter transcription elongation, P-TEFb is recruited to the promoter-proximal region and phosphorylates NELF, which dissociates from RNAPII, and Spt5. Additionally, P-TEFb phosphorylates the C-terminal domain (CTD) of the RNAPII largest subunit (RPB1), which consists of multiple heptad repeats of the consensus sequence YSPTSPS and serves as a platform for the binding of factors required for the expression of RNAPII-transcribed genes. P-TEFb is composed of the cyclin-dependent kinase Cdk9 associated with cyclin T1 or with one of the other related members of the cyclin T family, including cyclin T2a or T2b. A fraction of nuclear P-TEFb is found in an inactive complex containing proteins such as HEXIM1 (or the homologous HEXIM2), La-related protein 7 (LARP7), and the 7SK-specific methyl-phosphate-capping enzyme (MePCE) associated to the noncoding 7SK RNA (Nguyen et al. 2001; Yang et al. 2001). In physiological conditions, P-TEFb is released from this reservoir and bound by the bromodomain-containing protein 4 (Brd4) to activate genes when cells are exposed to hypertrophic or stress signals. Tat usurps this pathway by competing with Brd4 for P-TEFb binding and its recruitment to the LTR. Whether Tat extracts P-TEFb from the nuclear soluble fraction or from a preloaded fraction on the chromatin at the integrated provirus, or both, remains to be established (D'Orso and Frankel 2010). Tat is such an effective elongation factor because, in addition to P-TEFb, it is able to recruit the so-called super elongation complex (SEC) (He et al. 2010; Sobhian et al. 2010). SEC components such as ELL1 and ELL2 are well-characterized transcription elongation factors that act in concert with P-TEFb to stimulate the

same RNAPII. Tat interacts also with the 19S PAAF1 component of the proteasome to increase transcription in a non-proteolytic mode (Lassot et al. 2007).

Posttranslational Modifications of Tat

Tat functions essentially as a promiscuous adaptor. Not surprisingly, Tat can be post-translationally modified at various residues, thereby creating a variety of configurations for different interacting partners (Fig. 1). Lysine acetylation at Lys28 allows high-affinity binding to P-TEFb (Bres et al. 2002; Kiernan et al. 1999). Lys28 acetylation is reversed by HDAC6 (Huo et al. 2011). Acetylation at Lys50/51 leads to the dissociation of Tat from TAR and promotes the association of the P/CAF acetyltransferase (Bres et al. 2002; Col et al. 2001; Dorr et al. 2002; Kaehlcke et al. 2003; Kiernan et al. 1999; Ott et al. 1999). Lys50 acetylation generates an interaction surface also for the SWI-SNF chromatin-remodeling complex (Mahmoudi et al. 2006). Deacetylation of Tat is mediated by SIRT1 and possibly controls a late step in Tat transcriptional activity, allowing the recycling of the deacetylated protein for next rounds of transcription (Pagans et al. 2005). Lys51 is also methylated by the monomethyl-transferase Set7/9 and demethylated by the action of LSD1 (Pagans et al. 2010; Sakane et al. 2011). Interestingly, both activities are required for Tat function, possibly controlling different steps of process. Finally, polyubiquitination of Lys71 mediated by the proto-oncoprotein Hdm2 does not induce proteasome-dependent degradation but has an activating effect on Tat (Bres et al. 2003).

The Transcriptional Cycle of Tat

Tat is an essential switch that converts inefficient abortive transcription from the viral promoter into a highly robust platform for viral RNA production. The viral promoter appears generally poised for transcription, with a paused RNAPII that is converted to a highly processive enzymatic

complex by Tat-mediated recruitment of P-TEFb and SEC. Control of viral transcription is essential for the success of the infection being post-integration latency a critical step of the virus life cycle, which allows long-term survival also in the presence of cART. In addition to a number of chromatin features at the site of integration and to the spatial positioning of the integration site within the nucleus, triggering of feedback loop of Tat is the final step of the process. In fact, stochastic fluctuations of Tat have been proposed to be sufficient to control HIV gene expression (Weinberger et al. 2005). In excess of Tat, the viral LTR is extremely productive, independently of the site of integration, with an extremely high velocity of RNAPII measured by a fluorescent recovery after photobleaching (FRAP) assay in living cells (Maiuri et al. 2011). FRAP analysis of HIV transcription allowed also the calculation of the dwell time of the various components present at the HIV-1 transcription site: Tat and Cdk9 behaved similarly, while RNAPII remained on the gene for the time required to reach the end of the gene (Boireau et al. 2007; Molle et al. 2007). These data support the idea that Tat and P-TEFb remain associated in the elongating complex, rather than Tat alone being transferred to the elongating polymerase, but that the complex dissociates from RNAPII before termination of transcription.

Pharmacological Modulation of HIV-1 Transcription for Therapeutic Purposes

Due to the emergence of multidrug resistant HIV-1 strains and the inability of cART to completely eradicate HIV-1 from infected patients, there is an urgent need to identify new compounds that act on alternative steps of the viral replicative cycle. To this end Tat-mediated transactivation has been explored as an alternative target for inhibition. Two approaches have been proposed: (i) targeting cellular factors involved in HIV transcription or (ii) inhibition of the Tat/TAR axis.

The principal target for the first approach is the P-TEFb complex. Inhibitors that block CDK9

kinase activity are flavopiridol and derivatives, indirubin-3'-monoxime, DRB, roscovitine, or the R-enantiomer of roscovitine (seliciclib or CYC202). A new class of nontoxic anti-CDK9 compounds based on the 2-phenylquinazolinone scaffold has been recently reported to interfere with Tat-mediated transactivation of the viral promoter and in the inhibition of HIV-1 reactivation from latently infected cells (Sancineto et al. 2013). CDK2 is another potential therapeutic target to inhibit Tat transactivation. CDK2 is reported to phosphorylate CDK9 on Ser90 and thereby contributes to HIV-1 transcription, and this could also provide a new strategy for activation of latent HIV-1 provirus (Breuer et al. 2012). Known CDK2 inhibitors are CYC202, Alsterpaullone, and small peptide inhibitors. Antihuman cyclin T1 intrabodies were also proposed as new agents for HIV-1 gene therapy as they are reported to target cyclinT1/Tat interaction, thereby blocking Tat-mediated transactivation and HIV-1 replication (Bai et al. 2003). However, their delivery represents an unresolved issue.

Quinolone derivatives have been shown to control the latent reservoirs through the inhibition of Tat-mediated HIV-1 transcription. One such molecule is naphthyridone 3 (HM13N), which showed improved solubility and very potent and selective anti-HIV activity in acutely, chronically, and latently infected cells (Massari et al. 2010). To overcome the drawbacks of cART therapy, a valid alternative would be a single molecule able to inhibit multiple viral targets. For instance, a recent study showed that WM5, a quinolone derivative previously identified as HIV Tat-mediated transcription inhibitor, combined either with the tricyclic core of nevirapine or with BILR 355BS (BILR) non-nucleoside reverse transcriptase inhibitors resulted in a hybrid molecule which was sufficient to inhibit both Tat transactivation and reverse transcriptase activity resulting in selective inhibition of HIV-1 reactivation from latently infected cells (Sancineto et al. 2014). Finally, recent work suggested that inhibition of the heat-shock protein 90, a protein chaperone required for the correct folding of client proteins, resulted in inhibition of HIV-1 reactivation from

latency (Anderson et al. 2014). Interestingly, several inhibitors of HSP90 are readily available for testing because of their extended clinical testing for the treatment of certain cancers.

However, once integrated in the host genome, replication-competent viral DNA can become quiescent, with its expression durably suppressed and insensitive to inhibitors. The goal of induction and clearance strategies, which are also known as “kick and kill” strategies, is to induce transcription of these quiescent, replication-competent HIV proviruses (the “kick”), making them susceptible to immune clearance and the effects of cART (the “kill”). Several compounds are being studied that reactivate Tat-mediated transcription of HIV-1 through a variety of mechanisms extensively reviewed in Van Lint et al. (2013). HDACs are implicated in maintaining a quiescent provirus, and their inhibitors (HDACi) are primary candidates for the “kick.” HDACi including valproic acid (VPA), trichostatin A, vorinostat (VOR or suberoylanilide hydroxamic acid or SAHA), sodium butyrate, oxamflatin, belinostat, givinostat, entinostat, and panobinostat have been all reported to activate latent HIV in cell lines and primary cells. Initial results from HDACi clinical trials are promising, although they may not be sufficient for complete reactivation. In addition to HDACi, activation of signaling pathways such as protein kinase C (PKC) and NF- κ B induces expression of quiescent proviruses. Once activated, PKC phosphorylates I κ B α (inhibitor of NF- κ B), which results in the release of NF- κ B. Free NF- κ B then translocates to the nucleus, where it binds to HIV LTR and promotes transcriptional activation. Drugs targeting these pathways, such as tumor necrosis factor- α , prostratin, and bryostatins, are currently under study for the treatment of latent HIV infection. Prostratin, for example, a phorbol ester that targets both PKC and NF- κ B pathways, activates HIV transcription in several models of latency. In addition, prostratin along with HDACi increases the amount of virus produced from cells carrying a quiescent provirus demonstrating the necessity of combining multiple drugs with different targets for improved efficacy. Hence, there is a continuous effort to find novel molecules and targets. For

example, hexamethylene bisacetamide (HMBA) reactivates viral production in stable cell lines as well as in primary infected cells via transient activation of the P13K/Akt pathway, which results in HEXIM1 phosphorylation allowing the recruitment of released P-TEFb to HIV promoter to stimulate transcription. Disulfiram (DSF), an inhibitor of acetaldehyde dehydrogenase that is used for the treatment of alcoholism, was shown to reactivate latent HIV-1 expression in a primary cell model of virus latency and is currently being assessed in a clinical trial for its potential to deplete the latent HIV-1 reservoir in patients on combination antiretroviral therapy. DSF reactivates latent HIV-1 expression via the Akt signaling pathway through depletion of PTEN (phosphatase and tensin homolog) protein level. JQ1, an inhibitor of Brd4, is reported to reactivate latent HIV-1. Brd4 is shown to competitively block the Tat-super elongation complex (SEC) interaction, and this interaction is essential to overcome the limitation imposed by RNAPII pausing at the HIV promoter. JQ1, in contrast, dissociates Brd4 to enable Tat to recruit SEC to stimulate RNAPII elongation and thus enhances transcription.

Conclusions

The study of HIV-1 transcription and the function of Tat have been the subject of intense investigation over the years. In the wake of the AIDS epidemic, research on HIV-1 transcription has become essential to understand the post-integration latency mechanisms that make the virus undetectable by the immune system and insensitive to cART. Current approaches aiming at eradicating viral reservoirs from infected individuals are all based on triggering transcription and derived from basic mechanistic studies. However, the importance of investigating HIV-1 transcription extends far beyond virology into our understanding of cellular pathways of gene expression control. It is expected that such studies will contribute also to uncover disease-related dysregulation of cellular processes.

Cross-References

- ▶ [Acetylation](#)
- ▶ [Cellular Cofactors for HIV-1 Transcription](#)
- ▶ [HIV Life Cycle: Overview](#)
- ▶ [HIV “Auxiliary” Proteins](#)
- ▶ [Transcription \(Initiation, Regulation, Elongation\)](#)

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Overview of HIV CNS Infection

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Definition

Infection of the central nervous system (CNS) is a nearly universal facet of systemic HIV type 1 infection that begins during initial systemic viremia and continues through the subsequent course of chronic untreated infection. Importantly, the character and neurological consequences of this infection vary considerably among individuals and evolve over a protracted chronic course, changing from subclinical, seemingly innocent meningitis in most individuals to severe encephalitis in a substantial minority late in the course of systemic infection. Antiretroviral therapy (ART) is usually effective in suppressing CNS HIV infection and preventing its more severe neurological consequences, though milder neurological

impairment may persist in an appreciable number of patients and, rarely, isolated CNS treatment failure may present with more severe neurological disease.

Introduction

HIV infection can be complicated by an array of neurological diseases afflicting the central nervous systems (CNS) that include not only important opportunistic pathologies (e.g., cryptococcal meningitis, cerebral toxoplasmosis, progressive multifocal leukoencephalopathy, and primary CNS lymphoma) but CNS dysfunction that relate more fundamentally to HIV infection itself rather than another etiology (Del Valle and Pina-Oviedo 2006). However, the connection between HIV CNS infection and disease is complex, involving not only exposure of the brain to HIV but likely changes in the genetic and pathogenic character of virus populations within the individual host along with alteration in host defenses and reactions caused by systemic HIV infection that, in turn, determine CNS disease susceptibility and development. This short review provides an introduction to the general character of untreated CNS infection, its clinical consequences, and the impact of antiretroviral therapy (ART).

Untreated CNS HIV Infection

Untreated HIV infection exhibits three important characteristics: (1) it is a nearly constant facet of systemic infection; (2) its character can change over time as systemic disease progresses; and (3) it has the potential to cause CNS injury, most severely in the context of late systemic disease when blood CD4⁺ T cell counts are low. Much of what we know about CNS infection, particularly in a longitudinal context, has been defined by studies of cerebrospinal fluid (CSF) in which the virus can be measured along with local inflammatory responses and brain injury using biomarkers of these processes. This review therefore draws largely on CSF studies.

Evolution of CNS HIV Infection

HIV RNA can be detected in the CSF of virtually all viremic patients, usually at about one-tenth the concentration of HIV RNA in blood, though the quantitative relation of CSF to blood virus varies among individual patients. General exceptions to this average ratio include both patients studied within the first year of infection and those seen late in disease when blood CD4 T cell counts are below 50 cells per μL ; in both these groups, the CSF HIV RNA concentrations are relatively lower compared to blood, closer to 1:100 copies per μL (Price et al. 2014). While mild CSF lymphocytic pleocytosis is very common in untreated patients, it is usually asymptomatic without headache or other signs of meningitis (Spudich et al. 2005).

However, in some patients, the character and consequences of infection can be more severe, and CNS infection can be invasive with frank HIV encephalitis that presents clinically as HIV-associated dementia (HAD) with debilitating cognitive and motor deficits and high mortality. How can the same CNS virus infection range in its clinical consequences from no symptoms to severe encephalopathy? The answer to this fundamental question is not fully understood in detail, but important factors include the evolution of viral populations within individual patients so that, despite the seeming similarities in CSF viral profiles, the character of the underlying infection likely differs. Changes in the host immune cell responses as systemic disease advances also are likely important in facilitating the different disease outcomes, and indeed virus and host factors are interdependent and likely evolve hand in hand.

For simplicity, Table 1 compares some salient characteristics of typical early and late chronic CNS HIV infections. While CSF HIV may be detected in similar concentrations in these two settings, underneath the seeming similarity are important genetic and biological differences. During *early* chronic systemic infection when blood CD4 T cell counts are high, infection in the CSF generally has two important characteristics. First, the CSF HIV populations are genetically similar to those in blood and referred to as *equilibrated* or *non-compartmentalized*. Thus, if one examines

Overview of HIV CNS Infection, Table 1 Two hypothesized CNS infection prototypes

Timing	Anatomic location	Virus populations	Cell tropism	CSF HIV treatment decay
Early (CD4>350)	Meninges	Equilibrated	T-tropic	Rapid
Late (CD4<200)	Brain (encephalitis)	Compartmentalized	M-tropic	Slow

HIV envelope (*env*) RNA sequences of CSF and blood populations, they are similar, and phylogenetic comparisons show an admixture of related genotypes across the two fluids (Schnell et al. 2011). This implies that CSF HIV populations are recently derived from systemic sources. One explanation for this is that blood lymphocytes traffic into the CSF space (leptomeninges) and release progeny viruses which undergo only limited local replication in uninfected lymphocytes without local evolution. In some of these patients, there may be evidence of limited compartmentalization related to a burst of clonal expansion but without further sustained local evolution. *Second*, when examined for cell tropism determined by their *env* sequences, these CSF viruses, like those in blood, are T-lymphocyte-tropic (T-tropic, requiring high cell CD4 surface concentrations for infection), consistent with this blood origin (Schnell et al. 2011).

By contrast, *late* chronic infection in patients with low CD4 counts more often includes CSF HIV populations that are genetically distinct from those in plasma or *compartmentalized*, indicating independent evolution within the CNS that diverges genetically from blood populations. Additionally, these CSF viral populations show macrophage tropism (M-tropic, able to infect cells expressing low concentrations of CD4) (Schnell et al. 2011). The local selection and expansion of these M-tropic viruses likely are important in the development of frank HIV encephalitis with its characteristic infection of perivascular and parenchymal macrophages and microglia – the main pathological substrate of HAD (Burdo et al. 2013)

These two types of infection are not mutually exclusive within an individual patient, but likely frequently coexist in various mixtures. Because of this variability, CSF HIV isolates vary in their correlation with brain infection and CNS injury,

and the CSF HIV RNA concentration alone does not provide a quantitative index of the severity of neuropathic CNS infection. It remains to be seen if molecular analysis of CSF HIV genotypes and phenotypes (including cell tropism) will prove more helpful indicators of the character of underlying brain infection.

A second major component of the pathogenesis of brain injury and dysfunction relates to the mechanisms that link infection to brain injury. Since productive CNS infection, as outlined above, is confined to lymphocytes in the meninges during early infection and to cells of monocyte lineage within the brain (including the perivascular spaces) in HAD, neuronal injury appears to involve “indirect” mechanisms, whereby intermediary signals and toxic products derived from infected and uninfected (chiefly myeloid) cells impact on neuronal function and, eventually, viability. A number of different molecular mechanisms mediating neurotoxic effects have been described and are reviewed (Spudich and González-Scarano 2012). It should be also added that astrocytes may be infected by HIV, though infection of these cells is generally restricted in its gene expression and does not produce progeny (Dewhurst et al. 1987). Astrocytes therefore do not seem to sustain active infection or viral evolution within the CNS though this has been difficult to examine in detail. However, widespread astrocytic infection could theoretically contribute to brain dysfunction by altering the functions of these important glial elements.

This general picture of CNS infection and neuropathogenic mediation derives mainly from studies focused on more severe infection with HIV encephalitis and HAD. Less well defined are the mechanisms underlying milder CNS dysfunction, so-called asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND). While these clinical forms may involve milder forms of the same processes

causing HAD, this has not yet been clearly established by focused clinical-pathological and clinical-virological correlations.

Treatment of CNS HIV Infection

While the remarkable development of contemporary combination ART has occurred largely without special consideration of the particular effects on CNS infection or disease, treatment has nonetheless had a major impact on brain infection and its consequences. Most notably, the incidence of HAD is now markedly reduced in places where ART usage is widespread to the point that HAD is now uncommon and largely a disease of individuals who are outside the envelope of treatment because of psychiatric, social, or economic obstacles. Unfortunately these same background factors can make diagnosis difficult by obscuring the effects of CNS infection. Thus, in the developed world, contemporary treatment undeniably has had a profound preventative effect on the severe neurological diseases that were common in the early AIDS epidemic and that still linger in resource-poor areas (Chan and Brew 2014). Case studies and common clinical experience also demonstrate a therapeutic effect of ART on HAD with patients improving neurologically after treatment initiation.

However, despite these favorable observations, there are a number of important issues regarding CNS treatment that remain to be addressed. These include question regarding the efficacy of ART in preventing or treating milder neurocognitive impairment which has been reported to remain common in treated populations (Heaton et al. 2011). Also, it is uncertain when and how strategies to treat or prevent CNS infection and dysfunction might diverge from those for systemic infection.

Effect of ART on CNS Infection

The effect of treatment on CNS infection has been examined using CSF HIV RNA as an indicator, and, in general, combination ART has proved very effective in suppressing virus measured in CSF. Most patients with plasma viral suppression

below the clinical detection limits (usually 40–50 copies of HIV RNA per mL) exhibit parallel suppression of CSF HIV (Price and Staprans 1997). Only a small minority of patients may show asymptomatic CSF “viral escape” despite plasma suppression. For example, in one cross-sectional study, CSF escape was detected in about 10% of neurologically asymptomatic subjects undergoing lumbar puncture in the context of cohort studies (Eden et al. 2010). Emerging data suggest that asymptomatic low-level CSF HIV escape is common, characteristically transient, and accompanied by a minor inflammatory response but not by detectable immediate CNS injury or progressive local infection. The lack of symptoms in these individuals suggests that these asymptomatic elevations in CSF HIV RNA may be equivalent to plasma “blips” and not indicative of ongoing CNS viral replication. The low levels of virus in these individuals make studies of genetic evolution difficult, and thus this type of escape has not been characterized in molecular terms.

CSF studies have also examined the dynamics of CSF reduction (decay) with treatment initiation and noted that in some patients CSF decay is rapid and similar to the rapid decay of HIV RNA in plasma, while in others it is considerably slower than that of plasma. This difference supports the concept of different cell sources for CSF HIV (Table 1) with fast decay related to lymphocytic replication, like that of plasma virus in which the rate is determined by the short half-life of lymphocytes (Haas et al. 2003). In contrast, the slow CSF decay is thought to relate to the longer survival of infected macrophages, supporting the presence and importance of the cellular substrate of CNS infections. Patients with lower blood CD4 cells and HAD are more likely to exhibit this slow decay.

In those individuals with undetectable CSF HIV RNA, one study of 45 individuals using the sensitive single-copy assay to measure CSF and HIV RNA down to levels of less than one copy of HIV RNA per mL found that CSF HIV could be detected in some of these individuals but with a lower frequency than that of plasma detection and at lower levels of “residual” virus (Dahl et al. 2014). Together these observations show

that CSF HIV is generally lower than that detected in plasma, with exceptions largely seeming to be transient and with uncertain clinical significance. An important exception to this, neurosymptomatic CSF HIV escape is discussed below.

Effect of Different ART Regimens on CNS Dysfunction

One of the most controversial aspects of treating the CNS revolves around the issues of when and how to optimally target CNS HIV infection. This has been studied more extensively in relation to neurological outcomes assessed by neuropsychological test performance. Most of these studies are retrospective and examine cohorts treated with a variety of regimens. Because of the treatment heterogeneity and the changes in favored drug regimens over time as new drugs are introduced, a common approach in these studies has been to group regimens based on an aggregate “CNS penetration-effectiveness” (CPE) score (Letendre et al. 2008). This approach involves assigning a score, in its present iteration from 1 to 4, to each component drug based on available data regarding CNS pharmacokinetics (mainly of CSF drug concentrations) and virological efficacy (generally sparse since there are few rigorous studies of CNS efficacy that allow one to pick out effects of individual drugs on CSF HIV RNA). While the scale involves some tenuous assumptions and is not strictly quantitative, its great advantage is to provide an easy reference regarding each drug’s CNS pharmacology. A number of studies suggest that higher CPE score is associated with better neuropsychological test outcomes (Fabbiani et al. 2015), though a general difficulty is that the supporting studies are not randomized or prospective; as a result they do not balance potential CNS effects with other properties of the regimens important for systemic efficacy, including potency, tolerability, side effects, and convenience that favor adherence (Libertone et al. 2014).

The importance of this issue has become moot in recent years as newer regimens now suggested for initial therapy also have pharmacological properties that favor CNS drug exposure. For example, among the “Recommended” regimens in the 2015 DHHS Guidelines are two containing

dolutegravir, one with raltegravir, and one with darunavir which have relatively favorable CSF pharmacokinetics (<http://aidsinfo.nih.gov/guidelines>). The relative value of the two nucleoside “backbones,” tenofovir disoproxil fumarate/emtricitabine and abacavir/lamivudine, for CNS treatment remains uncertain. With these choices for the naïve patient beginning treatment, the clinician now does not face a major dilemma of whether to treat the body or the mind, and more than one of these regimens can be recommended for newly presenting HAD.

Neurosymptomatic CSF HIV Escape

An uncommon, but clinically important, exception to the high CNS effectiveness of ART is a syndrome characterized by “discordant” elevations of CSF in relation to plasma HIV RNA in patients presenting clinically with overt neurological symptoms and signs, referred to here as *neurosymptomatic CSF escape*. This was first clearly outlined in a case reported by Canestri and colleagues (Canestri et al. 2010), subsequently confirmed by a second case series and by a number of individual reports (for review, see Ferretti et al. 2015). Some of these cases present an important argument for the need for antiviral drugs to achieve therapeutic levels within the CNS. Dissociation between CSF and plasma virus concentrations is the hallmark of this disorder and indicates that these are examples of isolated CNS treatment failure in the face of systemic success. In those with plasma HIV RNA levels below detection limit, CSF levels >100 copies/mL may be present, while in those with low but measurable plasma VLs, levels at least twice as high are characteristic. Neurological presentations vary and may include focal or non-focal neurological symptoms and signs. Two factors, often in combination, appear to contribute to this: (1) drug resistance within the CNS and (2) poor penetration of drug. Either alone or in combination can lead to antiviral drug concentration inadequate to suppress CNS infection but able to suppress systemic infection. While other factors may also be important (including establishment of latent CNS infection during untreated nadir and CD8+ T cell immunopathology), the inadequacy of local drug

effectiveness seems paramount and supported by reversal of the syndrome when treatment is changed to overcome these two factors.

CNS as an HIV Reservoir

Long-term infection established in the CNS but suppressed by ART may also have implications for HIV cure strategies that are now receiving increased attention. Because of the barriers to drug entry into the CNS and because CNS infection may be supported within myeloid cells (macrophages and related cells) rather than lymphocytes, current cure efforts may not be as effective in reducing or eliminating a CNS HIV reservoir than the systemic lymphocytic reservoir (Fois and Brew 2015).

Conclusions

CNS exposure and infection by HIV is a nearly ubiquitous facet of systemic infection that may evolve in parallel with systemic infection but may also pursue an independent course that eventuates in CNS injury and neurological dysfunction. As antiretroviral treatment has become increasingly effective in suppressing plasma and CSF HIV RNA, current interest has turned to newer concepts of CSF escape, CNS as a reservoir, and ongoing cognitive decline as important areas of investigative and clinical interest.

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Overview: Immunopathogenesis

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Definition

HIV-1 infection causes a chronic disease of the immune system, and almost all clinical HIV-1 disease manifestations stem from immune pathology or immune dysfunction. While initially largely regarded as a disease that primarily affects CD4 T cells and cellular immunity, it is now clear that HIV-1 infection represents a systemic immune disorder that involves almost all innate and adaptive components of the immune system, although they can play diverse and partially opposing roles in disease pathogenesis. This overview briefly discusses four distinct aspects of HIV-1 immune pathogenesis: HIV-1 immune activation, HIV-1 immune defense mechanisms, HIV-1 immune dysfunction, and immune cells that serve as target cells and cellular reservoirs for HIV-1. A detailed discussion of these specific areas is provided in dedicated entries of this encyclopedia.

HIV-1-Associated Immune Activation

Initial infection of the human body with HIV-1 leads to massive, uncontrolled viral replication in lymphoid tissues and causes excessive activation of the immune system that involves stimulation of innate and adaptive effector cells, release of cytokines, and induction of a proinflammatory condition in tissue microenvironments (Appay and Sauce 2008). HIV-1-associated immune activation likely stems from multiple different underlying mechanisms: Persistently elevated levels of circulating HIV-1 antigen can directly stimulate T cells via TCR-dependent immune recognition, and non-HIV-1-specific T cells can be activated via bystander activation (Bangs et al. 2009). In addition, HIV-1 can activate innate immune cells through interactions with pattern recognition receptors, such as Toll-like receptors or cytosolic receptors for HIV-1 DNA or RNA (Beignon et al. 2005; Doitsh et al. 2014). Moreover, HIV-1-associated damage of the enteric immune system can increase microbial translocation and lead to elevated levels of circulating bacterial antigens, which also stimulate and activate the immune system (Brenchley et al. 2006). While possibly playing some role in host protection against HIV-1 progression during early stages of infection, it is now clear that this massive immune activation contributes to disease pathogenesis by increasing cellular susceptibility to HIV-1, inducing exhaustion and accelerated senescence of immune cells, and stimulating fibrotic transformation in lymphoid tissues (Zeng et al. 2012). As such, HIV-1-associated immune activation is responsible for many HIV-1-associated disease manifestations and represents an independent and more accurate predictor of clinical HIV-1 disease progression than levels of HIV-1 replication measured in the blood. Interestingly, immune activation persists at abnormally elevated levels when viral replication is effectively suppressed with antiretroviral combination therapy (Hunt et al. 2003) and seems to be responsible for many clinical problems of HIV-1 patients treated with antiretroviral therapy, including increased frequencies of cardiovascular diseases, accelerated aging, alterations in the bone, lipid and

carbohydrate metabolism, and neurocognitive deficiencies. Abnormally elevated levels of immune activation are also encountered in elite controllers, a group of patients who naturally achieve undetectable levels of HIV-1 replication in the absence of treatment (Hunt et al. 2008). The decisive impact of immune activation on HIV-1 disease progression is highlighted by specific non-human primates who are fully susceptible to infection with simian immunodeficiency virus (SIV) and develop high levels of SIV replication, but do not show signs of SIV-associated immune activation and fail to exhibit clinical signs of HIV/SIV disease typically encountered in humans (Chahroudi et al. 2012). Aspects of HIV-associated immune activation are discussed in the following entries:

- ▶ [Cardiovascular Complications](#)
- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [Collagen Deposition and Fibrosis in the Lymphatic Tissues of HIV-1 Infected Individuals](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [Immune Activation and HIV Transmission](#)
- ▶ [Inflammatory Cytokines](#)
- ▶ [Lymphocyte Apoptosis](#)
- ▶ [Microbial Translocation](#)
- ▶ [T-Cell Homeostasis](#)

Immune Responses Against HIV-1

The human immune response to HIV-1 involves a complex network of innate and adaptive immune cells, but is unable to protect against HIV-1 disease progression in most infected persons. T cell-mediated immune responses represent a principal element of the adaptive immune response against HIV-1 and are generated early during the disease process, typically at a time when the viral load declines to the set point (Walker and McMichael 2012). While there is evidence that cellular immune responses against HIV-1 can contribute to relative control of HIV-1 replication, particularly during early stages of the disease and in carriers of protective HLA class I alleles, the efficacy of HIV-1-specific T cell responses is generally limited by viral escape mutations, which

occur frequently in untreated patients and contribute to shaping viral sequence evolution (Goulder and Watkins 2004). Antiviral activity of HIV-1-specific T cell responses is further limited by T cell dysfunction that likely results from exhaustion and accelerated senescence of cells (Tsoukas 2014). Neutralizing antibodies generated by HIV-1-specific B lymphocytes represent a major aspect of the humoral immune response against HIV-1, but their ability to inhibit HIV-1 replication is also reduced by viral escape mutations in targeted epitopes. Innate immune responses, mediated by, e.g., NK cells, NKT cells, $\gamma\delta$ T cells, macrophages, and interferon-secreting plasmacytoid dendritic cells may also contribute to HIV-1 immune defense in some individuals, and their precise antiviral effects represent an area of active research (Silvin and Manel 2015). Notably, activation of antiviral effector cells can in many cases enhance cellular susceptibility to infection and contribute to viral dissemination and long-term persistence. As such, the physiologic immune response to HIV-1 may paradoxically increase host vulnerability to HIV-1 and may in some aspects benefit the virus more than the host. However, it is noteworthy that small groups of HIV-1-infected patients are able to naturally control HIV-1 infection and maintain undetectable or low level of HIV-1 viral loads in the absence of treatment; this is typical in individuals with strong HIV-1-specific T cell responses, which seem to represent the primary correlate of HIV-1 immune protection in these patients (Walker and Yu 2013). These individuals provide living evidence that the human immune system, at least in very special situations, is able to effectively control HIV-1 infection. Immune defense mechanisms against HIV-1 are discussed in this encyclopedia in the following entries:

- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [Gamma Delta T Cells](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [HIV Antigen Processing and Presentation](#)
- ▶ [HIV-1 Mutational Escape from Host Immunity](#)

- ▶ [Long-Term Nonprogressors and Elite Controllers](#)
- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [Mucosal Immunity to HIV-1](#)
- ▶ [NKT Cells: Bridging Innate and Adaptive Immunity](#)
- ▶ [PD-1](#)
- ▶ [T Follicular Helper Cells in HIV Infection](#)

Immune Cells That Serve as a Reservoir for HIV-1 Persistence

A characteristic feature of HIV-1 infection is that the virus primarily infects CD4 T cells, which play key roles in orchestrating antimicrobial immune defense. When activated during HIV-1 infection, these cells become particularly susceptible to HIV-1, leading to the observation that high levels of HIV-1 are detectable in antigen-specific memory CD4 T cells that specifically recognize HIV-1 (Douek et al. 2002). CD4 T cells infected with HIV-1 are in many cases short-lived and die fast; however, HIV-1 can also infect cells that constitutively have or revert to a functional profile with an extended lifespan and persist in these cells in a transcriptionally silent (latent) form (Finzi et al. 1999). This likely explains why HIV-1 can persist for decades despite very effective antiretroviral therapy. Moreover, studies over the last several years have revealed an enormous heterogeneity of CD4 T cells that differ by phenotype, developmental programs, and functional polarizations (Farber et al. 2014), and it is likely that these characteristics influence the ability of these cells to serve as target cells or cellular reservoirs for HIV-1. Myeloid cells, such as monocytes and macrophages, also express CD4, and many of these cells are susceptible to HIV-1 and may represent a long-term reservoir for HIV-1, although their role in this regard remains less well characterized (Kumar et al. 2014). The investigation of immune cell subsets that serve as target cells for HIV-1 and as reservoirs for HIV-1 persistence despite treatment represents a critical aspect for developing interventions that may lead to at least a transient drug-free remission of HIV-1 infection. Immune cells that serve as a reservoir for HIV-1

are discussed in the following entries of this encyclopedia:

- ▶ [Central Memory CD4 T Cells](#)
- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [T Memory Stem Cells](#)
- ▶ [Post-treatment Controllers](#)
- ▶ [Immunology of Latent HIV Infection](#)

HIV-1-Associated Immune Dysfunction

The clinical hallmark of HIV-1 infection is progressive immune deficiency and immune dysfunction. The numeric decline of CD4 T helper cells was initially identified as the most visible and arguably most important aspect of HIV-1-associated immune deficiency. However, it is now clear that impaired CD4 T cell immune responses only represent one aspect of immune dysfunction during HIV-1 infection. An increasing number of studies demonstrate that during untreated HIV-1 infection, B cells (Moir and Fauci 2014), follicular T helper cells (Cubas and Perreau 2014), dendritic cells (Piguet et al. 2014), NK cells, gamma-delta T cells (Pauza et al. 2014), and many other immune cells are functionally impaired or deficient, leading to dysfunction of many, if not all, immunological networks that ensure immune surveillance and pathogen-specific immune defense. HIV-1-associated immune deficiency can occur as a direct result of viral infection, which can lead to cell death and apoptosis. However, even in patients with progressive HIV-1 infection and AIDS, only a comparatively small number of cells are impaired this way. Indirect mechanisms of HIV-1-associated immune deficiency are substantially more frequent and can result from activation-induced cell death, cellular exhaustion, upregulation of inhibitory immunoregulatory pathways, and altered cell-to-cell interactions in lymphoid tissue microenvironments. Immune dysfunction in HIV-1 infection is complicated by the fact that mechanisms for immune regeneration are impaired during the disease by mechanisms that include thymic and bone marrow dysfunction (Sauce et al. 2011). Different aspects of HIV-1-associated

immune dysfunction are discussed in the following entries:

- ▶ [Gamma Delta T Cells](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [Long-Term Nonprogressors and Elite Controllers](#)
- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [Mucosal Immunity to HIV-1](#)
- ▶ [NKT Cells: Bridging Innate and Adaptive Immunity](#)
- ▶ [PD-1](#)
- ▶ [Role of Regulatory T Cells During HIV Infection](#)
- ▶ [T Follicular Helper Cells in HIV Infection](#)
- ▶ [Tim-3](#)
- ▶ [Thymic Function](#)
- ▶ [T-Cell Homeostasis](#)

Conclusion

Infection with HIV-1 causes a complex perturbation of almost all component of the human immune system and leads to excessive immune activation, immune exhaustion, and accelerated immune senescence, all of which contribute to the typical clinical signs of immune deficiency associated with HIV-1 infection and AIDS. Substantial progress has been made over the recent years in defining and understanding cellular and molecular aspects of HIV-related immune dysfunction and antiviral immune defense, and although our current understanding of HIV-1 immune pathogenesis is far from complete, a unifying theory of how HIV-1 causes disease of the human immune system is emerging. Notably, many of the immune abnormalities observed during HIV-1 infection regress during treatment with antiretroviral agents; however, residual levels of immune dysfunction and elevated immune activation persist despite treatment and fail to normalize even after extended periods of treatment. The development of interventions that eliminate residual HIV-1 disease in patients treated with

antiretroviral agents remains a challenge for the future generation of immunologists interested in HIV-1 pathogenesis.

Cross-References

- ▶ [Cardiovascular Complications](#)
- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [Central Memory CD4 T Cells](#)
- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [Collagen Deposition and Fibrosis in the Lymphatic Tissues of HIV-1 Infected Individuals](#)
- ▶ [Gamma Delta T Cells](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [HIV Antigen Processing and Presentation](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [HIV-1 Mutational Escape from Host Immunity](#)
- ▶ [Immune Activation and HIV Transmission](#)
- ▶ [Immunology of Latent HIV Infection](#)
- ▶ [Inflammatory Cytokines](#)
- ▶ [Long-Term Nonprogressors and Elite Controllers](#)
- ▶ [Lymphocyte Apoptosis](#)
- ▶ [Macrophages in HIV Immunopathogenesis](#)
- ▶ [Microbial Translocation](#)
- ▶ [Mucosal Immunity to HIV-1](#)
- ▶ [NKT Cells: Bridging Innate and Adaptive Immunity](#)
- ▶ [PD-1](#)
- ▶ [Post-treatment Controllers](#)
- ▶ [Role of Regulatory T Cells During HIV Infection](#)
- ▶ [T-Cell Homeostasis](#)
- ▶ [T Follicular Helper Cells in HIV Infection](#)
- ▶ [T Memory Stem Cells](#)
- ▶ [Thymic Function](#)
- ▶ [Tim-3](#)

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PD-1

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Definition

PD-1 (programmed death-1), also called CD279, is a receptor expressed upon activation on innate and adaptive immune cells and is encoded by the gene *Pdcd1*. It recognized two ligands, PD-L1 (programmed death-ligand 1) and PD-L2 (programmed death-ligand 2). Upon ligation of its ligand, PD-1 negatively regulates immune activation and is a key modulator of immune responses. PD-1 deficiency results in the loss of peripheral tolerance and the development of auto-immune phenotypes in mice. In humans, single mutations in *Pdcd1* are associated with autoimmune diseases. Pathogens including HIV exploit this negative regulatory pathway important for peripheral tolerance to evade immune control. Exhausted T cells during chronic infections have been shown to overexpress PD-1. In HIV infection, the inhibitory role of PD-1 has been demonstrated for both CD8 and CD4 T cells. Blockade of the interaction between PD-1 and its ligand restored the survival and proliferation of the exhausted T cells in chronic HIV infection. PD-1 expression is also a marker of ongoing immune activation, as its levels are not normalized after

control of HIV replication by antiretroviral therapy. The PD-1 molecule is therefore a potential target for therapy in HIV infection.

Introduction

PD-1 is inducibly expressed on CD4 T cells, CD8 T cells, B cells, NK cells, NKT cells, monocytes, and dendritic cells (DCs) but has been mostly studied on T cells. PD-1 belongs to the B7-CD28 family of molecules involved in regulating the activation of immune cells (Sharpe and Freeman 2002). Two classes of molecules in this family correspond to co-stimulatory and co-inhibitory molecules. The triggering of TCR concomitant with a co-stimulatory signal through CD28 leads to T cell proliferation and cytokine production. Inversely, the engagement of a co-inhibitory molecule upon TCR triggering leads to a blockade of the TCR signaling cascade and no T cell activation. The first signal through the TCR confers specificity to the response; the second signal through the co-stimulatory or co-inhibitory molecules modulates this response. The fate of the T cell response is shaped by the balance between positive and negative signals from these molecules that regulate T cell activation and tolerance. PD-1 shares structural properties with CD28 and CTLA-4. It is a transmembrane protein of 268aa composed of an extracellular IgV domain followed by a transmembrane region and an intracellular tail. PD-1 is in a monomeric form on the

cell surface as it lacks the membrane-proximal residue required for homodimerization characteristic of other members of the CD28 family. The cytoplasmic tail of PD-1 contains two tyrosine signaling motifs which can be phosphorylated upon receptor triggering. The extracellular domain recognizes two known ligands, PD-L1, also called B7-H1, or CD274 and PD-L2, also called B7-DC or CD273 (Latchman et al. 2001). These two PD-1 ligands are differentially expressed on immune cells. PD-L1 is broadly expressed on cells including B cells, T cells, DCs, and macrophages and is further expressed upon activation. This ligand is also expressed on other cell types throughout the body including parenchymal cells, vascular endothelial cells, glial cells, keratinocytes and pancreatic islet cells, but also tumor cell and virus-infected cells. PD-L2 is inducibly expressed exclusively on DCs and macrophages. The ligation of PD-1 with its ligands leads to the inhibition of T cell proliferation and cytokine production after TCR triggering (Freeman et al. 2000). The PD-1 pathway is likely to affect a large number of cell types *in vivo*. Several other inhibitory molecules besides PD-1 can also be upregulated on activated T cells including the receptors 2B4, LAG3, CD160, and TIM3 (Blackburn et al. 2009). The coexpression of several inhibitory receptors enhances the exhaustion phenotype.

Signaling of PD-1

PD-1 is induced after T cell activation and serves as a negative regulator of TCR signaling to dampen the TCR signaling cascade and prevent excessive T cell activation. PD-1 engagement with its ligands activates a signaling pathway that suppresses T cell activation by inhibiting AKT phosphorylation. During antigen recognition by a T cell, triggering of PD-1 induces the cross-linking of PD-1 and the TCR signaling complex. The cytoplasmic tail of PD-1 contains an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Both motifs can be phosphorylated upon PD-1 engagement. The

phosphorylation of the ITSM motif upon PD-1 cross-linking recruits the tyrosine Src homology phosphatase SHP-2 to the cytoplasmic tail of PD-1 (Okazaki et al. 2001). The recruitment of SHP-2 leads to dephosphorylation of ZAP70 and an inhibition of the TCR proximal signal (Okazaki et al. 2013). The inhibitory mechanisms of the TCR signal by PD-1 and CTLA-4 are distinct. CTLA-4 blocks the costimulation by CD28 via its stronger affinity to B7 molecules. PD-1 does not compete for the ligands of CD28 but acts on the TCR signaling cascade. The inhibitory function of PD-1 is therefore less stringent and slower than the one exerted by CTLA-4. The inhibition of proximal TCR signal prevents CD28-mediated activation of phosphatidylinositol-3-kinase (PI3K), reduced AKT phosphorylation resulting in decrease activation, cytokine production, glucose metabolism, and cell cycle arrest.

Regulation of PD-1

The amount of antigen and the duration of antigen exposure determine the amount of PD-1 expression on the cell surface, but the molecular events that regulate the expression of PD-1 are still largely unknown. PD-1 was shown to be differentially expressed during T cell differentiation as PD-1 expression was higher in early and intermediate CD8 T cell subsets and downregulated in later stages of differentiation (Sauce et al. 2007). PD-1 expression is associated with the expression of other activation markers. Transient PD-1 expression is important to limit excessive immune response during acute infection and might be important for avoiding tissue damage due to the exacerbated immune responses during the acute phase of infection. PD-L1-deficient mice infected with LCMV clone 13 succumb to severe liver damage. PD-1-dependent inhibitory role is important in later phases of the immune response such as sustained activation or secondary responses. However, persistent expression of PD-1 in chronic immune responses has a negative impact on the disease progression. PD-1 is expressed on a significant fraction of CD4 and CD8 T cells under physiological conditions. Common gamma chain

cytokines IL-2, IL-7, IL-15, and IL-21 as well as inflammatory cytokines differentially regulate PD-1 expression on CD4 and CD8 T cells (Kinter et al. 2008). Some transcription factors have been shown to modulate PD-1 expression. The basic leucine zipper transcription factor ATF-like (BATF) has been shown to be upregulated in exhausted CD8 T cells. BATF is a negative regulator of AP-1 activity and is sufficient to inhibit proliferation and cytokine production (Quigley et al. 2010). The B lymphocyte-induced maturation protein 1 (BLIMP-1) transcription factor has been shown to repress PD-1 expression through a feedforward repressive loop regulating PD-1 directly and by repressing NFATc1 expression, an activator of PD-1 expression (Lu et al. 2014). Epigenetic modifications can also modulate the expression of PD-1. The upregulation of PD-1 transcriptional expression during the differentiation of PD-1 from naïve to effector cells is associated with transient DNA demethylation of the PD-1 locus, then remethylation in memory cells when the antigen is cleared. The high expression of PD-1 during chronic HIV infection has been associated with the irreversible demethylation of CpG dinucleotides in the *Pdcd1* promoter due to chronic exposure to viral antigens (Youngblood et al. 2011, 2013). A decrease of viremia results in reduced PD-1 expression, but does not cause a remethylation of the PD-1 locus. The unmethylated locus of PD-1 reaches maximal PD-1 expression much faster than fully functional memory CD8 T cells with a methylated PD-1 locus.

Role of PD-1 in Chronic Viral Infections

During chronic infections, as the virus is not cleared, the continuous exposure to the viral antigens leads to an exhaustion of the CD8 T cells that lose their capacity to proliferate, survive, and exert effector functions. The upregulation of PD-1 expression on exhausted CD8 T cells was first described in the lymphocytic choriomeningitis virus (LCMV) model of chronic infection in mice (Barber et al. 2006). When comparing

functional and exhausted LCMV-specific CD8 T cells in that model, PD-1 mRNA was found to be highly upregulated in exhausted T cells. PD-1 was only transiently expressed in acute infection on effector CD8 T cells when LCMV was controlled. In contrast, PD-1 was sustained and expressed at high levels on virus-specific CD8 T cells in mice with chronic LCMV infection. When LCMV-infected mice were injected with antibodies to block the PD-1/PD-L1 pathway, virus-specific CD8 T cells were enhanced demonstrating the role of this pathway in mediating the dysfunction during chronic infection. After PD-1/PD-L1 blockade, virus-specific CD8 T cells regained proliferative capacity and effector function resulting in a decrease in viral load. Following the LCMV study demonstrating the role of PD-1 in mediating CD8 T cell dysfunction, several studies demonstrated the role of PD-1 in mediating CD8 T cell exhaustion chronic infections in humans such as HIV or Epstein-Barr Virus (EBV).

Role of PD-1 in HIV Infection

PD-1 on CD8 T Cells

Defective HIV-specific CD8 T cell responses are suspected to be a major factor in the lack of immune control in HIV infection. Of note, during chronic HIV infection, PD-L1 is expressed at higher levels on antigen-presenting cells than in noninfected individuals. Three studies demonstrated the role of the PD-1/PD-L1 pathway in the exhaustion of HIV-specific CD8 T cells during chronic HIV infection (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006). They showed that PD-1 was highly upregulated on HIV-specific CD8 T cells during chronic HIV infection. The degree of PD-1 expression was associated with the level of CD8 T cell dysfunction. PD-1 expression was associated with lower proliferation of HIV-specific CD8 T cells. Its levels also correlated with viral load, loss of CD4 T cells, and disease progression. The blockade of the PD-1/PD-L1 interaction in vitro restored the proliferation and survival of these cells as well as their effector functions. PD-1 triggering increased apoptosis in

the CD8 T cells expressing high levels of PD-1. HIV-specific CD8 T cells were shown to have shortened telomeric DNA and a reduced telomerase activity. Blocking the PD-1/PD-L1 pathway increased telomere length and telomerase activity that may contribute to the recovery of T cell functions (Lichterfeld et al. 2008). PD-1 upregulation was also shown on the total CD8 T cell population and was not restricted to HIV-specific CD8 T cells. Viral load and CD4 T cell loss both correlated negatively with the expression of PD-1 on total CD8 T cells. PD-1 expression was found to be higher in lymph nodes and in gut-associated lymphoid tissues than in the peripheral blood. Control of viral load after treatment initiation resulted in decreased PD-1 levels on HIV-specific CD8 T cells. Levels of PD-1 expression correlated with the presence of antigen as they decrease after antiretroviral treatment or on HIV-specific CD8 T cell responses directed against epitopes that undergo mutational escape. In a minority of HIV-infected individuals that control HIV replication called HIV controllers, HIV-specific CD8 T cells are functional, have a preserved proliferative capacity, and express low levels of PD-1. The role of PD-1 expression on HIV-specific CD8 T cells also varies for different epitopes in a given individual. HLA-B-restricted CD8 T cell responses express lower levels of PD-1 and exhibit higher functionality compared to HLA-A-restricted CD8 T cell responses.

PD-1 on CD4 T Cell

One subset of CD4 T cells expresses high levels of PD-1 within the germinal centers: the follicular helper T cells (T_{fh} cells) defined by CXCR5+ PD-1^{hi} FoxP3⁻ (Locci et al. 2013). PD-1 expression on CD4 T cells is involved in the regulation of class-switch recombination and somatic hypermutation of activated B cells. In HIV infection, T_{fh} cells are elevated probably because of the increase in germinal center formation due to extensive viral replication (Lindqvist et al. 2012; Cubas et al. 2013). However, recent evidence suggested that these T_{fh} cells are impaired and are unable to provide B cell help, in part due to PD-1 ligation to PD-1 ligands on germinal center B cells (Cubas et al. 2013). Interestingly, it has

been further shown that T_{fh} CD4 T cells harbor HIV DNA and can serve as HIV reservoir in lymphoid tissues (Perreau et al. 2013). Levels of PD-1 are higher on HIV-specific CD4 T cells in chronic HIV infection and have been shown to correlate with levels on HIV-specific CD8 T cells. As for CD8 T cells, the blockade of PD-1/PD-L1 pathway by monoclonal antibodies was also able to restore the proliferation of HIV-specific CD4 T cells (Porichis et al. 2011). In non-treated HIV-infected individuals, PD-1 is also upregulated on total CD4 T cells. PD-1 expression levels on CD4 T cells correlate with viral load and with loss of CD4 T cells. High levels of PD-1 on CD4 T cells appear to predict suboptimal CD4 T cell recovery after long-term ART (Grabmeier-Pfistershammer et al. 2011).

Therapies Targeting PD-1

The PD-1/PD-L1 pathway has been shown to be critical in tumor-induced dampening of the immune response. As in chronic infections, blockade of the PD-1/PD-L1 interaction resulted in tumor eradication in various cancer models. These results served as basis to develop a fully humanized monoclonal antibody against PD-1 (nivolumab also called NON4538, MDX-1106, or BMS-936558). The phase I clinical trial using nivolumab reported high response rates for several cancers, non-small cell lung cancer, melanoma, and renal cell carcinoma (18–28%) (Topalian et al. 2012). Another phase I clinical trial using a monoclonal antibody against PD-L1 (BMS-936559 or MDX-1105) demonstrated similar response rates (6–17%) (Brahmer et al. 2012). The PD-1/PD-L1 blockade used in these trials resulted in the highest rate of antitumor activity of the many immunotherapeutic approaches tested over the past 30 years. These treatments showed better tolerance than CTLA-4 blockade, even though autoimmune side effects did occur in some subjects. Several groups are conducting safety and efficacy studies with PD-1-blocking antibodies in the simian immunodeficiency virus (SIV) rhesus macaque model (Velu et al. 2009; Amancha et al. 2013). Treatment with PD-1-blocking antibodies resulted

in expansion of SIV-specific CD8 T cells and increase in cytotoxic function of these cells and led to a reduction in viral load. The treatment resulted in expansion of memory B cells and an increase in antibodies against SIV envelope (Titanji et al. 2010). The treatment also showed increased survival and evidence of restoration of the mucosal barrier integrity. These preclinical studies provide the rationale for initiating human clinical trials targeting PD-1 in HIV infection. Future clinical trials in humans will determine if blockade of the PD-1 pathway is capable of improving T and B cell responses beyond what is achievable with ART.

Conclusion

PD-1 is a critical regulator of the balance between tolerance and T cell activation. Studies have demonstrated that exhausted T cells overexpress PD-1 during chronic HIV infection, but blocking the PD-1/PD-L1 pathway leads to an enhancement of T cell survival, proliferation, and function. These studies provided the basis for the development of humanized antibodies that block the PD-1/PD-L1 interaction to enhance immune effector functions for the eradication of tumors and chronic diseases including HIV. The use of PD-1 blockade has been initiated in cancer immunotherapy and led to promising results. The PD-1 pathway might be of interest for intervention strategies in HIV infection as well. PD-1 is an attractive potential target as it is not only responsible for HIV-specific T cell impairment but plays a wider role in HIV pathogenesis.

Cross-References

- ▶ [Cellular and Soluble Immune Activation Markers in HIV-Infected Subjects](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [HIV Infection, Immune-based Interventions for](#)
- ▶ [T Follicular Helper Cells in HIV Infection](#)

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Peer-Based Intervention Approaches

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Definition

Peer-based intervention approaches use community members, as opposed to highly trained specialists, to provide HIV care and prevention services.

Review of Peer-Based Approaches

Since the onset of the HIV epidemic, people living with HIV/AIDS (PLWHA) and those directly affected by it such as high-risk groups and family caregivers of PLWHA, have been an indispensable component of HIV prevention, care, and treatment. Yet it has not been until recently that efforts have been made to systematically define peers, describe their roles, and evaluate the efficacy of their contributions.

Simoni et al. (2011a) offer the following comprehensive definition of “peers”: individuals who (a) share key personal characteristics, circumstances, or experiences with the people they serve; (b) contribute services whose value emanates from the peers’ status as a peer; (c) lack

formal expertise but may have short-term and competency-based training; and (d) provide services according to standard protocols, rather than as a natural result of informal social engagement.

Peers can function as leaders of formal meeting/structured groups, in one-on-one interactions, as opinion leaders, and in outreach activities. Formal meetings/structured groups typically range from 1 to 12 sessions and involve a manualized curriculum led by one or two peers. One-on-one interventions are often scripted but have the flexibility to be more individually tailored and run anywhere from 1 to 12 sessions. Peer opinion leaders are taught to endorse risk-reduction behaviors through casual, one-on-one conversations with members of their own social network in the hopes of changing community norms around HIV risk behaviors. General peer outreach interventions typically involve teaching a group of peers specific talking points and giving them educational material to take into the community.

Peer-based interventions are often targeted at specific at-risk populations such as men who have sex with men (MSM), school-based youth, non-school based youth, sex workers, substance users, people living with HIV/AIDS, and at-risk women. Outcomes typically targeted and assessed include sexual risk behaviors, substance use, HIV knowledge, and attitudes and cognitions, while biomarkers and other non-self-reported outcomes (e.g., condom distribution counts, needles exchanged) are much less frequently targeted or assessed.

Two reviews have systematically evaluated the efficacy of peer-based interventions in the area of HIV/AIDS (Medley 2009; Simoni et al. 2011b). These reviews collectively assessed the efficacy of peer-based interventions from 147 studies in developing and developed countries. Both of these reviews concluded that while peer-based interventions had moderate effects on behavioral outcomes, including increasing HIV knowledge, reducing equipment sharing among injection drug users, decreasing sexual risk behavior, and decreasing substance use, they were not associated with any changes in biological outcomes (e.g., sexually transmitted infection rates) or other non self-reported outcomes (e.g., condom

distribution counts, needles exchanged), which are perhaps capable of offering a less subjective, more rigorous test of efficacy. These findings suggest that peer interventions to promote the health and well-being of persons living with or at risk for HIV/AIDS are widespread and potentially efficacious. Unfortunately the majority of current research lacks a rigorous study design, limiting the extent to which causality can be inferred. Further, work in this area often lacks a clear theoretical basis for the use of peers in these interventions, limiting our understanding of why and under what circumstances peers may be useful.

Simoni et al. (2011a) have argued that optimal integration of peers into health-related interventions requires a consideration of how and why they might be helpful. Specifically, they outline a two-step process involving, first, specification of the main goal of the intervention and, next, identification of a theory that justifies the use of peers to attain that goal and might explain the specific impact of the peers. Below is a list of potential targets and theories that might justify the choice of peers as interveners.

Most peer-based interventions involve peer education and communication to affect HIV/AIDS related attitudes and knowledge, which are thought to influence behavior change. Multiple health behavior, social impact, and social comparison theories posit that peers may be particularly effective communicators of information, primarily because of their similarity to targeted individuals. For example, Dynamic Social Impact Theory (DSIT) emphasizes that the success of communication about behavior change relies on the communicator being similar and credible; that the communication occur at critical high-risk moments (e.g., immediately following an HIV diagnosis or in high-risk situations such as those involving drug use); and that the new behavior is communicated by multiple persuasive sources. Peers are well suited to provide communication in such a fashion.

A second common intervention using peers attempts to bolster social support, which has been shown to help people cope with stressful events, improve physical health, and increase beneficial health behaviors. In addition to DSIT and

social comparison theories, mutual support group and self-help theories provide a conceptual basis for the potential efficacy of peers as resources of support. Specifically, peers are posited to be particularly good resources of support because of their shared identity, experiences, and/or social capital. For example, sharing personal experiential knowledge concerning safe sex and substance use practices when providing social support is likely to increase the credibility of the peer, the sense of closeness felt between the peer and the person or people they are supporting, and the perceived strength of the social support which in turn can help improve health related outcomes.

Peers have also been used to change perceived social norms associated with high HIV risk behaviors. Such interventions are often grounded in the theory of reasoned action, which posits that perceived social norms are powerful predictors of health related behaviors. Social network theories argue that the social capital of peer opinion leaders in high-risk communities is crucial to their success in modeling safer sex and HIV-prevention behaviors.

Peers have also been used in interventions targeting individuals' self-efficacy or perceived ability to execute desired health behaviors. Self-efficacy has been shown to be a powerful predictor of engagement in healthy behaviors. The rationale for using peers to influence self-efficacy is based in social cognitive theory, which proposes that self-efficacy develops through mastery experiences, vicarious or observational learning, and social persuasion, each of which is amenable to peer-based approaches. Peers can provide realistic opportunities for individuals to practice skills and gain mastery, model self-efficacy for desired health behaviors, and cultivates an environment in which individuals perceive support and encouragement with regard to their abilities to change their behaviors.

Peers have also been used to promote advocacy interventions, which are targeted at empowering traditionally marginalized, stigmatized, and oppressed populations – those generally at higher risk for HIV/AIDS. They aim to engage targeted populations in participatory learning processes to

empower them and enable them to promote their collective health interests beyond a specific behavior change. Based on empowerment theory's focus on societal-level factors and connections with others, peers are optimally suited to empower others, helping to foster self-development, decisions-making skills, and a sense of community. Further, a supportive peer relationship can also allow for the development of a more critical understanding of how individuals are personally connected to social and political struggles, which may proactively enhance social justice and enact social change.

Successful implementation of peer-based approaches into HIV care and prevention requires careful consideration and systematic application. On the one hand, peers must be comprehensively trained; on the other hand, organizations must be ready to embrace trained peers into a multi-disciplinary structure of care and prevention intervention delivery. Various successful initiatives to adopt peer-based approaches have revealed several essential lessons learned ([Peer Education and Evaluation Resource Center 2012](#)). First, to integrate peers in HIV care and prevention it is important for someone within the existing organization or community to strongly believe in and champion the benefits of integrating peers into HIV care and prevention. Second, implementation strategies need to be tailored to each organization's specific needs and continuously monitored to increase the likelihood of a smooth integration. Third, it is important that the partner organization engender a supportive environment that considers peers as equal in status to other care and prevention providers, which can be accomplished through (a) the development of policies and procedures that are peer-inclusive; (b) obtaining support from existing members of the community at all levels during both planning and implementation stages; (c) training peers not just in their area of focus, but also in their role as peers within the organization; and (d) providing supervision for peers and monitoring their progress. Fourth, systematic evaluation of the peer implementation initiative is crucial to fully understand the contribution to service provision. Lastly, focusing on

program sustainability through obtaining consistent funding, creating an ongoing network of shared resources to improve learning, and provision of supportive tools, will allow for maximum success of a peer-based initiative.

Conclusion

To maximize the utility of peer-interventions in the area of HIV/AIDS, it is important to understand the theoretical and conceptual justifications for using peers, ways to effectively implement and integrate peers into HIV care and prevention services, and what the current research reveals about the efficacy of peer interventions. Incorporating a clear theoretical conceptualization and rationale for an intervention before implementation allows for the examination of constructs that might explain its effect, such as increased self-efficacy, empowerment, improved knowledge, or skills acquisition. Furthermore it is important to learn from previous work in this area both in terms of the logistical aspects of effective and efficient implementation and also what types of peer-based interventions have proven to be effective in the past. Peers are increasingly recognized as potentially powerful agents in promoting health and well-being. Future research and implementation work in this area, if carefully conducted, can contribute to their success.

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PIMT/TGS1

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Definition

PIMT, *PRIP-interacting protein with methyltransferase domain*, is also known as trimethylguanosine synthase (TGS1), nuclear receptor coactivator six interacting protein (NCOA6IP), hepatocellular carcinoma-associated antigen 137 (HCA137), and CLL-associated antigen KW-2. PIMT catalyzes two serial methylation steps for the conversion of the 7-methylguanosine (m7G) RNA cap to a 2,2,7-trimethylguanosine (TMG) cap. PIMT was first isolated as a peroxisome proliferator-activated receptor interacting protein (PRIP) (Zhu et al. 2001). PIMT contains a methyltransferase motif (9-amino acid VVDAFCGVG) and an invariant segment (GXXGXXI) found in K-homology motifs of RNA-binding proteins (Zhu et al. 2001; Misra et al. 2002). The PIMT gene is localized on human chromosome 8q1, spans more than 52 kb, and contains 11 exons; it is conserved in human, chimpanzee, rhesus monkey, dog, cow, rat, chicken, zebra fish, mosquito, and *Saccharomyces cerevisiae*. The PIMT mRNA is ubiquitously expressed, with high expression in heart, skeletal muscle, kidney, liver and placenta.

Organization of the PIMT Protein

The PIMT human cDNA encodes an approximately 90 kDa protein of 853 amino acids (<http://www.uniprot.org/uniprot/Q96RS0>). PIMT/Tgs1 proteins in human and other metazoan cells are much larger (e.g., varying from 491 aa in *Drosophila* 889 aa in chicken) than the corresponding Tgs

enzymes of yeast, *Saccharomyces cerevisiae*, and *Giardia* (315 and 239 amino acids, respectively), which mainly contain a C-terminal methyltransferase catalytic domain (Mouaikel et al. 2002) (<http://www.ncbi.nlm.nih.gov/homologene/32608>). The N-terminal extension of PIMT/TGS1 found in higher eukaryotes contains an RNA-binding domain and is also responsible for interactions with PRIP, CREB-binding protein (CBP), p300, and PPAR-binding protein (PBP) (Misra et al. 2002). The C-terminus of PIMT binds S-adenosyl-L-methionine (AdoMet), and mutagenesis of PIMT shows that the C-terminus spanning amino acids 504–853 are sufficient for its methyltransferase activity (Misra et al. 2002; Enunlu et al. 2003; Yedavalli and Jeang 2010). Additionally, the C-terminal methyltransferase domain of human PIMT/TGS1, consisting of the amino acids 576–853, is able to complement the cold-sensitive phenotype of a yeast *tgsl*Δ strain, demonstrating that the human TGS1 methyltransferase domain is a true functional ortholog of the yeast enzyme (Hausmann et al. 2008). PIMT/TGS1 in human cells is distributed diffusely in the cytoplasm and is also found in the nucleus, where it is concentrated in Cajal bodies (Yedavalli and Jeang 2010); however, in yeast cells, it has been reported that TGS1 localizes to the nucleolus (Mouaikel et al. 2002). There is evidence that PIMT/TGS1 in human cells is a nucleocytoplasmic shuttling protein, which uses a CRM1 (see ► CRM1)-dependent nuclear export pathway to exit the nucleus; indeed, treatment of HeLa cells with leptomycin B (LMB – a potent inhibitor of CRM1-mediated nuclear export of proteins) was found to result in nuclear accumulation of PIMT (Yedavalli and Jeang 2010).

Other motifs found in human PIMT include a classical AdoMet-binding site composed of ⁶⁹⁴VVDAFCGVGGN⁷⁰⁴ and ⁷¹⁷IAIDI⁷²¹ and a m⁷G-binding site comprised of ⁷⁶³SPPWGG⁷⁶⁸ (Misra et al. 2002; Hausmann et al. 2007, 2008; Monecke et al. 2009).

Functions of PIMT/TGS1

Two roles ascribed to PIMT are those of a transcriptional cofactor (Zhu et al. 2001; Misra

et al. 2002) and a trimethylguanosine synthase (Zhu et al. 2001; Mouaikel et al. 2002; Enunlu et al. 2003; Yedavalli and Jeang 2010). PIMT was first identified as a nuclear receptor coactivator (PRIP/NCOA6)-interacting protein containing RNA binding and methyltransferase motifs suggesting dual functions in transcription and RNA hypermethylation (Zhu et al. 2001). Little more is known about the role of PIMT/TGS1 in transcription, other than its interaction and regulation of PRIP, CBP/p300, and PBP (Misra et al. 2002). However, the identification of trimethylguanosine synthase activity for PIMT and yeast TGS1 has spawned additional studies exploring its functions as an RNA trimethylguanosine transferase. In eukaryotes, an m⁷G cap is added to newly transcribed RNA polymerase II (RNAP II) transcripts (Topisirovic et al. 2011). A subset of RNAP II-transcribed cellular RNAs, including small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), and telomerase RNA (TLC1), acquires two additional methyl modifications to the m⁷G cap at exocyclic N₂ position to form the TMG cap (Mouaikel et al. 2002; Maxwell and Fournier 1995; Gallardo and Chartrand 2008). In yeast, deletion of the *TGS1* gene abolishes the conversion of the m⁷G to TMG caps for both snRNA and snoRNA (Mouaikel et al. 2002; Hausmann et al. 2007).

In genetic screens of yeasts, *Tgs1* was shown to be nonessential for growth of budding and fission yeasts (Mouaikel et al. 2002; Topisirovic et al. 2011). This result is surprising given that TMG capping is found on so many important yeast cellular RNAs including snRNA, snoRNA, and telomerase-associated TLC1 RNA. The *tgsl*Δ *Schizosaccharomyces pombe* grows normally, notwithstanding the absence of TMG caps on its U1, U2, U4, and U5 snRNAs (Mouaikel et al. 2002; Hausmann et al. 2007). The equivalent *tgsl*Δ mutation of *S. cerevisiae* causes a growth defect at cold temperatures, although *tgsl*Δ cells grow as well as TGS1 cells at 34 °C. Additionally PIMT/Tgs1 depletion by RNA interference in HeLa cells, which have reduced Tgs1 mRNA levels to 8% of the control value and Tgs1 protein levels to below the limit of detection, had no effect on cell growth (Lemm et al. 2006), suggesting that

mammalian somatic cells, like fungi, do not require TMG modifications for viability (Mouaikel et al. 2002; Hausmann et al. 2007; Lemm et al. 2006). However, TMG synthesis plays an essential role during *Drosophila* and mouse development (Komonyi et al. 2005; Jia et al. 2012). Mutations in the PIMT/Tgs1 methyltransferase active site caused lethality at the early pupal stage, which is consistent with findings seen upon the depletion of TMG-containing RNAs in *Drosophila* (Komonyi et al. 2005). Also embryonic lethality at E3.5 was observed in mice disrupted for PIMT gene with failed development around the blastocyst stage and at time of uterine implantation (Jia et al. 2012). These embryos show reduced expression of Oct4 and Nanog transcription factors with a failure to form an inner cell mass (Jia et al. 2012).

The 7-Methylguanosine Cap of HIV-1 Unspliced RNA is a Substrate of PIMT

The capping of viral RNAs has not been investigated as extensively as that of cellular RNAs. It has been generally assumed that, like cellular mRNAs, many viral RNAs have a 5' m7G cap, and viruses that replicate in the nucleus use the cellular capping machinery while viruses that replicate in the cytoplasm encode their own capping enzymes (Banerjee 1980; Ferron et al. 2012). However, the limited number of viral RNA capping studies point to a surprisingly diverse range of cap modifications (Yedavalli and Jeang 2010; Banerjee 1980; Ferron et al. 2012; HsuChen and Dubin 1976; van Duijn et al. 1986).

HIV-1 expresses both unspliced and spliced RNAs (see ► [HIV-1 Rev Expression and Functions](#)). The export of spliced and unspliced HIV-1 RNAs from the nucleus to the cytoplasm is a complex process, because eukaryotic cells normally choose to retain unspliced RNAs in the nucleus. To overcome this retention, HIV-1 encodes a Rev protein (see ► [HIV-1 Rev Expression and Functions](#)) that participates in the export of unspliced/partially spliced viral RNAs from the nucleus to the cytoplasm by binding to a cis-RNA element (RRE, *Rev response element*), which is present in unspliced (9 kb) and partially spliced

HIV-1 transcripts (4 kb) but is not found in spliced (1.8 kb) viral RNA. Several cellular factors including CRM1 (see ► [CRM1](#)), Ran, FG-repeat nucleoporins, RIP/RAB protein, DDX3, DDX1, RNA helicase A (RHA), and matrin 3 cooperate with Rev/RRE to facilitate the export of unspliced/partially spliced transcripts (see ► [DDX3, Cofactors, and RNA Export](#)) (Nekhai and Jeang 2006; Yedavalli and Jeang 2011). It was recently reported that PIMT hypermethylates selectively the RNA cap of unspliced and partially spliced HIV-1 transcripts to increase their cytoplasmic distribution (Yedavalli and Jeang 2010). PIMT is recruited by Rev to RRE-containing transcripts and then the Rev-recruited PIMT hypermethylates the m7G cap on these RNAs to a TMG cap. The activity of PIMT appears to be selective for only RRE-containing HIV-1 RNAs, while fully spliced viral transcripts, which do not contain the RRE sequence, are not TMG capped. How the TMG cap acts to facilitate the cytoplasmic expression of the RNA remains not understood, but one suggestion is that the TMG cap may be recognized by CRM1 which along with PHAX (Yedavalli and Jeang 2010; Gallardo and Chartrand 2008; Yedavalli and Jeang 2011), then directs the transcript for nuclear export. CRM1 and PHAX are involved in the nucleocytoplasmic and intranuclear trafficking of TMG cap-containing RNA, the nuclear export of snRNA and TLC RNA, and the nucleolar localization of snoRNA (Gallardo and Chartrand 2008; Yedavalli and Jeang 2011; Verheggen and Bertrand 2012). Multiply spliced HIV-1 RNAs, rather than using the CRM1-dependent route, exit the nucleus through the NXF1/TAP pathway.

Conclusion

The study of PIMT has added to the understanding of the complex nucleus to cytoplasm export processes for HIV-1 unspliced and partially spliced RNA. The recognition of m7G cap by eIF4E is a requirement for mRNA-ribosome association and the translation of the mRNA (Topisirovic et al. 2011). PIMT has been shown to increase the expression of proteins encoded by

HIV-1 unspliced transcripts such as RRE-containing HIV-1 Gag mRNA. Additional published data suggest that TMG capping per se is not a barrier to ribosome translation of RNAs (Yedavalli and Jeang 2010; Banerjee 1980; Ferron et al. 2012; Van and Hirsh 1990; Maroney et al. 1995) and that mechanism(s) may exist for translating TMG-capped RNA. More recently it was found that translation of HIV-1 unspliced and partially spliced RNA is sustained under conditions which suppress the expression of cellular and viral spliced transcripts (Sharma et al. 2012). Thus, HIV-1 unspliced and partially spliced transcripts predominantly associate with nuclear cap-binding complex (CBC)/cap-binding protein 80 (CBP80) in contrast to the finding that fully spliced viral and cellular mRNAs are associated with eIF4E for translation in the cytoplasm. Accordingly, PIMT-mediated TMG cap modification can provide a mechanism for HIV-1 to synthesize structural proteins during Vpr-induced suppression of eIF4E-dependent translation. One can speculate that TMG cap modification by PIMT/TGS1 provides a mechanism that can also be exploited by selected cellular mRNAs during conditions of cellular stress to bypass the down-regulation of eIF4E-dependent translation.

TMG capping of HIV-1 RNA by PIMT/TGS1 can also be a potential drug target for inhibiting HIV pathogenesis (see ► [Cellular Cofactors of HIV as Drug Targets](#)) (Ferron et al. 2012). RNA methylation inhibitors in some instances have been used successfully to inhibit the replication of herpes simplex virus, vesicular stomatitis virus, flavivirus, and other viruses (Issur et al. 2011). Emerging evidence also indicates that inhibitors of PIMT-mediated RNA cap hypermethylation can also suppress HIV-1 replication (Yedavalli and Jeang 2010).

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PKR and HIV Replication

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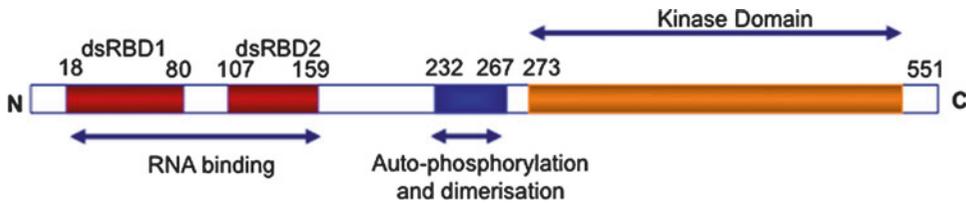
Definition

The protein kinase activated by RNA, PKR, is an interferon-induced cellular restriction factor for HIV. It phosphorylates its downstream target, the

alpha subunit of the eukaryotic translation initiation factor 2 (eIF2 α), which blocks translation initiation. PKR is expressed and activated at the beginning of HIV infection, which contributes to a block in virus expression and replication. PKR is activated by low levels of the HIV transactivation response (TAR) RNA element. During HIV replication, PKR activation is counteracted in part by the viral Tat protein and by large amounts of the TAR RNA. PKR activation is also strongly inhibited by cellular factors such as the TAR RNA-binding protein (TRBP), the adenosine deaminase acting on RNA (ADAR1), and the PKR activator (PACT), which form a multiprotein complex around PKR during HIV replication.

Introduction

Following HIV infection, interferon (IFN)- α and interferon- β are produced mainly in plasmacytoid dendritic cells through innate immune detection of viral RNA. IFNs are secreted and inhibit HIV replication in primary macrophages and T cells by inducing the production of IFN-stimulated genes (ISGs), which block several steps of the viral replication cycle. PKR was originally characterized as one of these ISGs induced by viral infection (Meurs et al. 1990). It is a 551-amino-acid-long double-stranded RNA-binding protein (dsRBP) that has two dsRNA-binding domains (dsRBDs) and a serine/threonine kinase domain (Fig. 1). PKR is central to innate cellular defense strategies with strong antiviral and antigrowth activities. PKR is present in the cell as an inactive monomer. It becomes activated by autophosphorylation after binding to low levels of dsRNA through its two dsRBDs and by dimerization. Once active, PKR phosphorylates several substrates including eIF2 α . Phosphorylated eIF2 α blocks the ability of eIF2B to renew the eIF2-GTP-Met-tRNA_i ternary complex required for protein synthesis initiation and is a critical component of antiviral and cell growth pathways (Sadler and Williams 2007). Viruses and cells have evolved to counteract the PKR antiviral and antigrowth pathway by a variety of mechanisms. In the context of HIV replication, the direct



PKR and HIV Replication, Fig. 1 Schematic representation of PKR. PKR is a 551-amino-acid-long serine/threonine kinase. It has two dsRBDs, an autophosphorylation and dimerization domain and a kinase domain

activity of viral components is not sufficient to prevent PKR activation. Instead, cellular proteins and virus-induced cellular mechanisms contribute to PKR inhibition to allow HIV production (Clerzius et al. 2011).

PKR Activation During HIV Replication

PKR is transcriptionally induced in lymphocytic T cells after HIV infection and activated at the beginning of an infection, but it is deactivated when the virus replicates actively (Clerzius et al. 2009, 2013). PKR is best known to be activated by dsRNAs longer than 30 base pairs, but other natural compounds like heparin, certain cytokine mRNAs, and its activator PACT also induce its phosphorylation. In the cell, PKR acts as a dsRNA sensor in response to viral infection. Low amounts of dsRNA promote PKR dimerization, autophosphorylation, and activation of the kinase function. Activated PKR subsequently phosphorylates several targets, including eIF2 α , whose phosphorylation on serine 51 prevents its recycling for ongoing translation (Sadler and Williams 2007).

In cell culture, PKR overexpression blocks HIV expression and viral replication very effectively, which shows its activity as a restriction factor (Benkirane et al. 1997; Adelson et al. 1999; Daher et al. 2001). HIV mRNAs all start with the structured stem-bulge-loop TAR RNA at their 5' end. PKR is activated by low amounts of the TAR RNA *in vitro*, whereas high concentrations inhibit its kinase function (Bannwarth and Gatignol 2005). Astrocytic brain cells provide an example of cells that do not replicate HIV efficiently due in part to a

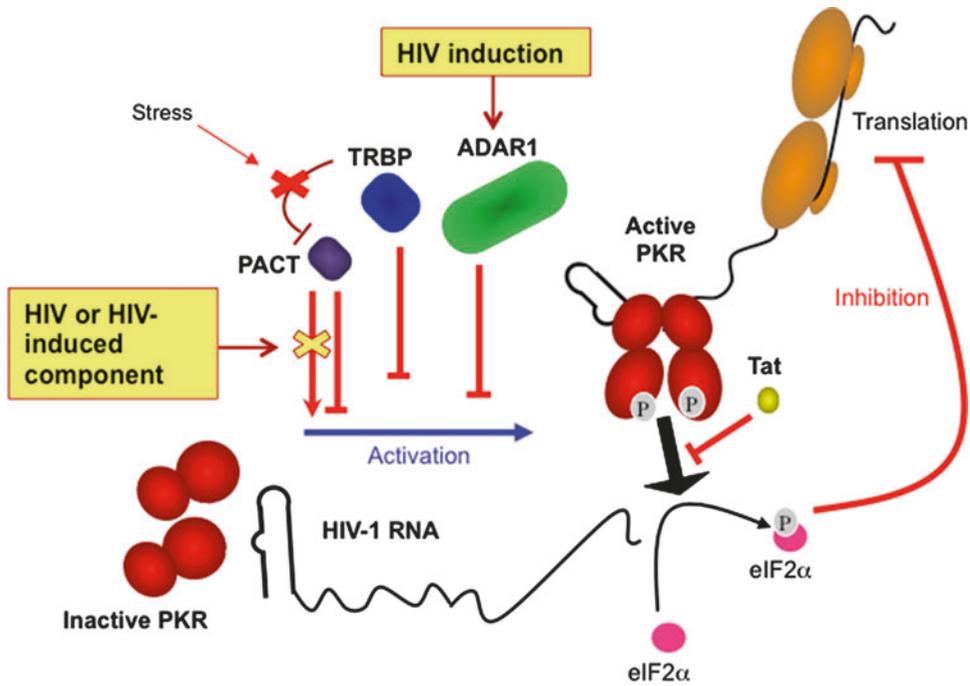
heightened PKR activation caused by the weak expression of PKR inhibitors (Ong et al. 2005). Despite PKR activation by the TAR RNA, HIV replicates efficiently in many permissive cell lines, including lymphocytes and monocytes. Various mechanisms leading to specific counteractions of PKR activation by viral and cellular proteins in permissive cells have been elucidated (Fig. 2).

Control of PKR Activation by the Viral Tat Protein

The HIV Tat protein directly binds to PKR *in vitro* and in cells and contributes to its inhibition. The Tat amino acids sequence required for PKR interaction lies in residues 40–58 encompassing the arginine-rich region important for TAR RNA binding and for Tat function. Tat inhibits the activation of the kinase by both RNA-dependent and RNA-independent mechanisms. It sequesters dsRNA, it interacts and prevents PKR autophosphorylation, and it acts as a pseudosubstrate for PKR due to a sequence homology between an eIF2 α and a Tat motif (Bannwarth and Gatignol 2005; Clerzius et al. 2011).

Control of PKR Activation by the Cellular TRBP Protein

TRBP is a cellular dsRBP, whose cDNA was isolated based on the protein's ability to bind HIV TAR RNA. TRBP also binds small interfering (si)RNAs and is an integral part of the RNA-induced silencing complex, which mediates RNA interference (Gatignol et al. 1991; Daniels



PKR and HIV Replication, Fig. 2 Mechanisms of the inhibition of PKR activity during HIV replication. PKR is inhibited by the viral Tat, which acts as a pseudo-substrate. The cellular TRBP and ADAR1 proteins directly bind to PKR and inhibit its activation. HIV replication induces an increase in ADAR1 expression. TRBP heterodimerization with PACT prevents PACT activation of

PKR, but this is reversed by stress. HIV or an HIV-induced component changes PACT function to become an inhibitor of PKR (Adapted from Daniels and Gatignol (2012) with permission of American Society for Microbiology. Copyright © American Society for Microbiology))

and Gatignol 2012). TRBP has two dsRBDs and a KR-helix motif within dsRBD2 mediates the strongest interaction with TAR RNA. TRBP also has a third domain called Medipal, which mediates protein-protein interactions identified for Merlin, Dicer, and PACT. The dsRBDs also mediate protein-protein interactions with PKR and PACT. TRBP enhances the cellular translation of HIV RNA by relieving a block due to TAR RNA and by blocking PKR activation. TRBP inhibition of PKR occurs by direct binding, by dsRNA sequestration, and by PACT binding and inhibition (Clerzius et al. 2011; Daniels and Gatignol 2012; Burugu et al. 2014). TRBP rescues HIV expression and replication in cells where the virus was suppressed by overexpressed PKR (Benkirane et al. 1997; Daher et al. 2001).

Astrocytic cells infected with HIV provide a natural model of the direct involvement of TRBP in the counteraction of PKR activation. These

cells have a major block in the translation of HIV structural proteins and an enhanced PKR activation, which blocks HIV production. Astrocytes express a low amount of TRBP, which is not sufficient to counteract PKR activation and allow HIV production. This low TRBP expression is due to a weak activity of its promoter caused by a partial lack of specific transcription factors. Overexpression of TRBP partially rescues this block in HIV replication (Bannwarth et al. 2001; Ong et al. 2005). Silencing TRBP by siRNAs or short hairpin RNAs inhibits HIV expression and replication, suggesting that the protein is required for HIV replication as a counteracting PKR factor (Christensen et al. 2007; Eekels et al. 2011). The cell type differences lead to the conclusion that HIV has evolved to replicate in cells that express a large amount of TRBP to suppress PKR activation (Clerzius et al. 2011).

Control of PKR Activation by the Cellular ADAR1 Protein

IFNs also induce the production of ADAR1. ADAR proteins convert adenosines to inosines in a process called RNA editing, which then results in A to G mutations in RNAs. RNA editing affects transcripts and noncoding RNAs (Nishikura 2010). In addition, ADAR1 also binds to and inhibits PKR activation in the context of virus infections. Full-length ADAR1 enzymes possess two N-terminal Z-DNA-binding domains (DBDs), three central dsRBDs, and a C-terminal deaminase domain. Three related isoforms of ADAR1 are found in human cells: the IFN-inducible cytoplasmic 150-kDa protein and the constitutively expressed nuclear 110-kDa and 80-kDa proteins, which lack the first Z-DBD and both Z-DBDs plus the first dsRBD, respectively. The expression of ADAR1 is induced during HIV infection of lymphocytic cell lines and primary lymphocytes, and this induction positively correlates with the expression of HIV proteins (Clerzius et al. 2009, 2013). The 150-kDa and the 110-kDa forms of ADAR1 bind to PKR, inhibit its activity, and increase the cellular susceptibility to several viral infections. ADAR1 is present in a multiprotein complex formed around PKR during HIV infection of lymphocytes. It reverses the block in HIV expression and virus production mediated by PKR in HIV-producing cells. It also increases HIV production from a transfected HIV molecular clone in astrocytic cells, which have an increased PKR response. ADAR1 mediates PKR inhibition by direct binding through its first dsRBD (Clerzius et al. 2009, 2011). Therefore, ADAR1 contributes to the inhibition of PKR activation and consequently enhances HIV expression and production. ADAR1 expression is triggered after IFN production and is further boosted by HIV replication.

Control of PKR Activation by the Cellular PACT Protein

PACT was cloned from a cDNA library based on its interaction with a catalytically inactive PKR. It

is a cellular stress-inducible PKR activator, with proapoptotic functions in the absence of dsRNA or viral infection. PACT is 313 amino acids long with two dsRBDs, and its PKR-activating domain is located in its C-terminus between amino acids 240 and 300 (Patel and Sen 1998; Patel et al. 2000). PACT is highly homologous to TRBP but has opposite functions on PKR. PACT naturally binds to TRBP in cells where TRBP is abundant, and the heterodimer acts as an inhibitor of PKR. Indeed, PACT acts as a PKR activator in cells where TRBP is in low concentration, whereas it acts as a PKR inhibitor in cells with high amounts of TRBP. TRBP-PACT interaction occurs through the two dsRBDs and the Medipal domain in both proteins. A cellular stress dissociates TRBP-PACT heterodimers and releases PACT function as an activator of PKR. In astrocytes that have low TRBP expression, PACT activates PKR with concomitant eIF2 α phosphorylation in the absence of stress. It consequently inhibits the expression of a reporter gene produced from the HIV promoter (Laraki et al. 2008; Daher et al. 2009).

During HIV replication, PACT expression is increased when HIV is expressed at high levels and the protein is pulled down with the multiprotein complex associated with PKR, ADAR1, and TRBP. Furthermore, PACT is unable to activate PKR and becomes a PKR inhibitor in HIV-expressing cells. This change of function occurs both in cells with high or low HIV production and in cells with high or low TRBP expression. Indeed, PACT induces an increased viral expression and production with concomitant PKR and eIF2 α dephosphorylation in HIV-transfected HEK293T cells and in astrocytes (Clerzius et al. 2013). The conclusion of these observations is that an HIV component or an HIV-induced cellular protein reverses PACT activity on PKR.

Conclusion

PKR is activated and acts as a restriction factor at the beginning of HIV infection. Activation is due to low levels of the viral TAR RNA, but high

levels of TAR reverse this function. The viral transactivator Tat inactivates PKR by acting as a competitive substrate. The cellular TRBP inhibits PKR activation by direct binding and by competing with dsRNA. HIV induces the production of high levels of ADAR1, which binds to and inhibits PKR activation. Finally, HIV reverses the function of PACT, which becomes a PKR inhibitor in HIV-replicating cells. To counteract a block by PKR activation, HIV has evolved to replicate in cells that express a large amount of TRBP, it induces the production of ADAR1, and it induces mechanisms, which reverse the PACT function. These mechanisms provide tools to act on these interactions to enhance PKR activation for a better control of persistent HIV replication in replicating cells or in latently infected reservoir cells (Clerzius et al. 2011; Burugu et al. 2014).

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Plasmablastic Lymphoma

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Definition

Plasmablastic lymphoma (PBL) is a rare, aggressive lymphoma that is mainly seen in the human immunodeficiency virus (HIV)-positive population. It is a mature large B-cell lymphoma exhibiting plasmablastic morphology and with terminal B-cell, namely, plasma cell-like differentiation. PBL has a tendency to occur in the oral cavity; however, extraoral involvement is not infrequently encountered. While PBL shares some overlapping morphologic and immunophenotypic features with other mature B-cell lymphomas, PBL is currently classified as a distinct entity in the 2008 *WHO Classification Tumours of Haematopoietic and Lymphoid Tissues* (Delecluse et al. 1997).

Introduction

Plasmablastic lymphoma (PBL) is a rare B-cell lymphoma that predominately arises in patients with HIV infection. PBL was originally described by Delecluse et al. in 1997 (Delecluse et al. 1997). It was documented as a variant of ► **diffuse large B-cell lymphoma (DLBCL)** with distinctive immunohistological features consistent with plasmacellular differentiation; the initial cases describing PBL cells expressed VS38c, frequently stained positive for CD79a, and demonstrated monoclonal rearrangement of the immunoglobulin (*Ig*) heavy chain gene. In these first reported cases, all presented in the oral

cavity, and a majority of patients were infected with HIV. Since the initial description of PBL, a number of case reports and series have been published further describing this unique lymphoproliferative disorder, which has now been documented to occur at many other sites other than the oral cavity. While PBL shares some overlapping morphologic and immunophenotypic features with other mature B-cell lymphomas, it is currently classified as a distinct entity in the 2008 *WHO Classification Tumours of Haematopoietic and Lymphoid Tissues* (Swerdlow et al. 2008).

PBL can be a diagnostic challenge given its atypical morphology and immunohistochemical profile similar to other lymphomas with plasmablastic differentiation such as primary effusion lymphoma (PEL) and plasmablastic plasma cell myeloma (PCM). The clinical outcome is typified by a highly aggressive course. At present, there are no established standard of care treatments for PBL in the era of highly active antiretroviral therapy (HAART). In addition to specific therapy for PBL, HAART appears to be an important component of management. With increased understanding of the pathophysiology of PBL, more targeted therapy and future treatment options may be available for this unique and aggressive B-cell lymphoproliferative disorder.

Epidemiology

The relationship between PBL and immunosuppression has been clearly established. PBL is most commonly diagnosed in the setting of HIV infection, and to date, a majority of patients with PBL have concurrent HIV. PBL can be seen in HIV-negative patients as well. However, even in HIV-negative cases, immunosuppression often plays a large role in pathogenesis as a number of HIV-negative patients have immunosuppression from solid organ transplantation, steroid therapy, or concurrent malignancy (Castillo et al. 2010; Teruya-Feldstein et al. 2004; Ustun et al. 2009). Other cases of PBL occur in the setting of decreased immune surveillance, such as in advanced age (Colomo et al. 2004).

PBL accounts for 2.6% of all HIV-associated non-Hodgkin lymphomas (NHLs) (Carbone et al. 1997). Given that the majority of patients with PBL have HIV, the disease in the United States occurs predominantly in males. The median age at diagnosis in the HIV population is 38 years (Castillo et al. 2008). Due to its rarity, it is not clear as to whether there are racial or ethnic predispositions. However, PBL has been reported in Europe, Asia, Africa, Australia, South America, and North America (Castillo et al. 2012; Castillo and Reagan 2011).

Pathogenesis

PBL is, in essence, an aggressive mature large B-cell lymphoma that exhibits plasmablastic morphology and terminal B-cell, namely, plasma cell-like differentiation. The postulated normal counterpart of PBL is the plasmablast. By analyzing mutations of *Ig* variably heavy chain (*IgVH*) for somatic hypermutation and *BCL-6* genes, Gaidano et al. divided PBL of the oral cavity into at least two subgroups: a subset that carries the molecular clues of germinal center (GC) transit (hypermutated *IgVH* with a subset of which showing antigen stimulation) and thus conceivably originates from a B-cell subset corresponding to post-GC cells and another subset that appears to originate from naïve B-cells (devoid of *IgVH* mutations) (Bose et al. 2009).

Clinical Presentation

Clinical presentation of PBL is largely heterogeneous. PBL can be the initial presentation of HIV infection. PBL has a tendency to occur in the oral cavity; however, extraoral involvement is not infrequently encountered (Castillo et al. 2008). Since its initial report in the oral cavity in patients with HIV in 1997 (Delecluse et al. 1997), PBL has been found to occur outside of the oral cavity in both HIV-infected and uninfected patients. It has been reported to arise in the retro-orbital; nasal cavity; jaw; parotid gland; larynx; bone marrow; lung; gastrointestinal tract including the

esophagus, stomach, small intestine, colon, and anorectal region; liver; retroperitoneum; testis; penis; vulva; skin; and bone. Central nervous system (CNS) involvement including the orbit, leptomeninges, and parenchyma as well as peripheral nervous system involvement have been reported to occur with systemic disease at the time of diagnosis or at the time of relapse (Colomo et al. 2004; Riedel et al. 2008).

A high percentage of cases of PBL present with masses in the oral cavity. Masses in the gingiva, hard palate, and alveolar mucosa are common. PBL also may arise from areas next to the oral cavity such as the mandible. Patients typically present with symptoms localized to the disease site(s), though others may present with systemic symptoms such as fatigue, unexplained fevers, drenching night sweats, or weight loss. With CNS involvement, symptoms such as altered mental status, headaches, or blurry vision are common.

HIV-positive cases usually present either as early stage IE (extranodal) disease or advanced stage IV disease (Ustun et al. 2009). There is a paucity of data documenting associated laboratory abnormalities at times of diagnosis; however, lactate dehydrogenase (LDH) was noted to be elevated in a majority of patients in one series (Castillo et al. 2012). The CD4+ count at diagnosis varies. In a study of 112 patients spanning from the early HAART to present HAART era, the median CD4 count at diagnosis of PBL was at 178 cells/mm³ (range, 10–498 cells/mm³) (Castillo et al. 2008). The duration of HIV infection before diagnosis was 5 years (range, 0–20 years). In a smaller study of 53 patients in the HAART era, the median CD4+ count was 206 cells/mm³ (range, 5–683 cells/mm³), and the median viral load at presentation was 261,560 copies/mL (range, from undetectable to 4.7 million copies/mL) (Castillo and Reagan 2011). In this study, the duration between HIV infection and PBL diagnoses was 8.9 years.

Diagnosis and Evaluation

Clinical features are not adequate to distinguish PBL from other malignancies that arise in the oral

cavity, such as carcinoma, melanoma, and other types of lymphomas. Given extraoral involvement can occur at many other sites, the differential diagnosis can range from carcinomas to other types of lymphoma or opportunistic infections. A biopsy of the mass lesion or enlarged lymph node is needed for diagnosis. Whenever possible, an excisional biopsy of suspected lesions is needed to further define the morphologic and immunophenotypic features of PBL and distinguish it from other types of lymphomas and myeloma.

Full staging scans are indicated at the time of diagnosis. Given the rarity of the disease, the usefulness of PET scans for diagnosis and prognostication in PBL is not specifically established. There are only few case reports documenting FDG uptake in PBL of the oral cavity and metastatic lesions of PBL affecting the skin, testis, and bone. It must be noted that FDG uptake can occur in HIV-related adenopathy, and therefore false positivity with PET should be anticipated (Makis et al. 2011). Marrow involvement is not infrequent; bone marrow biopsies are recommended as for any aggressive lymphoma. Given its aggressive nature and reported cases of CNS involvement, a lumbar puncture to assess for CNS disease is appropriate as is CNS imaging in the setting of headaches or neurologic symptoms.

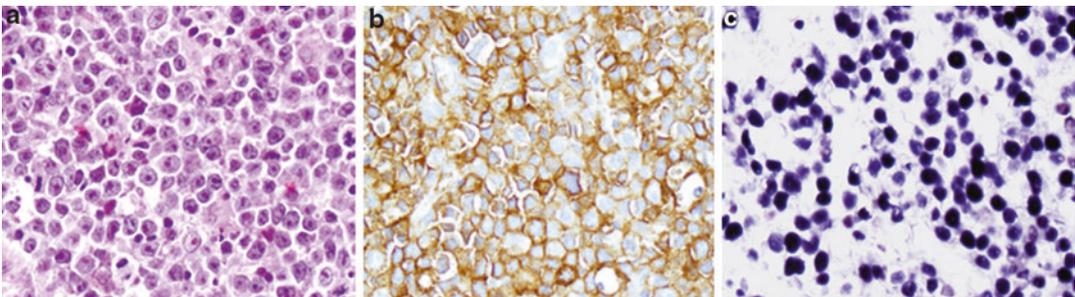
Pathologic Features

Morphology and Immunophenotype

The 2008 *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (Swerdlow et al. 2008) recognizes PBL as a distinct subtype of very aggressive mature large B-cell lymphoma (Swerdlow et al. 2008). While most of PBL occur de novo, secondary/transformed PBL from low-grade B-cell lymphoma including follicular lymphoma and plasmacytoma has been described.

Histologically, PBL tends to show a diffuse and cohesive growth pattern (Swerdlow et al. 2008). Cytologically, cells from PBL are medium-sized to large cells showing a spectrum of cytology ranging from pure immunoblastic morphology to immunoblasts or plasmablasts with more plasmacytic differentiation. Typically, the lymphoma cells show centrally or slightly eccentrically located nuclei with round to oval nuclear contours, vesicular to condensed nuclear chromatin, one prominent centrally located nucleoli with or without recognizable perinuclear clearing mimicking perinuclear hof often seen in plasma cells (Fig. 1a). In addition, brisk mitoses and numerous apoptotic bodies are easily appreciated.

Immunohistochemically, the tumor cells from PBL are typically positive for plasma cell markers including CD138 and IRF4/MUM-1 and



Plasmablastic Lymphoma, Fig. 1 (a) and (b) Representative microphotographs of a plasmablastic lymphoma (PBL) from an anal fistula in a 46-year-old male with HIV infection. H&E shows the atypical lymphoid cells which are large with oval nuclear contours, vesicular nuclear chromatin, conspicuous prominent nucleoli, and relative

abundant cytoplasm (a). The lymphoma cells are positive for CD45 (b), as well as Oct2 and CD30 (not shown). These cells are negative for CD20, CD79a, and HHV-8/KSHV (not shown). (c) PBL cells from an oral cavity of a 32-year-old male are positive for EBV by in situ hybridization (EBER). All images' original magnification is 400×

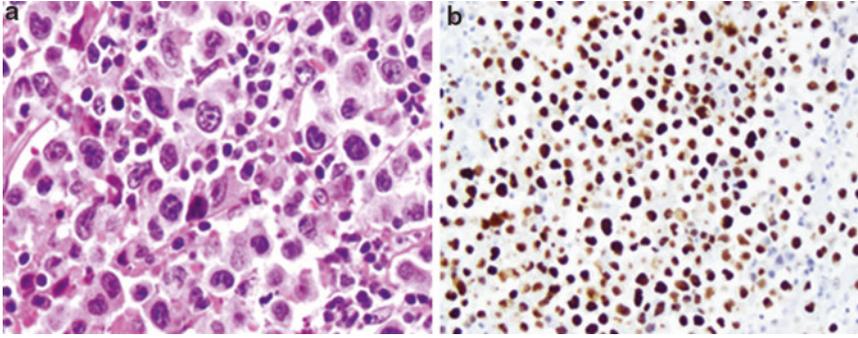
immunoglobulin light chain (kappa or lambda), activation markers such as CD30, and epithelial markers such as epithelial membrane antigen (EMA) and CD79a (Bibas et al. 2010). However, they are negative or rarely positive for mature B-cell antigens including BCL-6, CD20, CD22, CD23, and PAX-5 (Carbone et al. 1997). CD45, commonly known as leukocyte common antigen (LCA) (Fig. 1b), shows unequivocal positivity in 71% of the 14 PBL cases reported by Dong et al. (2005). The tumor cells are typically positive for ► [Epstein-Barr virus \(EBV\)](#), not by immunohistochemistry (IHC) for latent membrane protein (LMP), but by in situ hybridization to assess the expression of EBV-encoded RNA (EBER) (Fig. 1c; Dong et al. 2005).

Human herpesvirus-8 (HHV-8), also known as ► [Kaposi sarcoma herpesvirus \(KSHV\)](#), is typically absent in PBL (Carbone et al. 1997; Bibas et al. 2010), although occasional HHV-8/KSHV-positive PBL cases have been reported in the literature. For example, Dong HY et al. reported 60% (6/10) HHV-8/KSHV positivity in 10 cases of PBL in which HHV-8/KSHV status was evaluated by polymerase chain reaction (PCR); however, only 10% (1/10) showed HHV-8/KSHV positivity by IHC. Goedhals J et al. have also showed 12.5% (1/8) HHV-8/KSHV positivity in the 8 PBL cases analyzed by PCR, but none of them (0%, 0/8) was positive for HHV-8/KSHV by IHC. Therefore, it is the view of the 2008 *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* that HHV-8/KSHV is absent in PBL (Bibas et al. 2010). The current notion is that if a large B-cell lymphoma with plasmablastic morphology exhibits HHV-8/KSHV positivity especially by IHC then it could represent one of the following three entities: an extra-cavitary ► [primary effusion lymphoma \(PEL\)](#), a large B-cell lymphoma arising in HHV-8-associated multicentric Castlemans disease, or a yet to be defined HHV-8/KSHV(+) large B-cell lymphoma recently proposed by one of the authors.

Differential Diagnosis

Some of the lymphomas that must be distinguished from PBL include the following:

- (a) *Plasmablastic plasma cell myeloma (PCM)*
PBL shares overlapping cytomorphological and immunohistochemical features with those of plasmablastic PCM to the extent that Vega et al. claim these two entities have nearly identical immunophenotypic profiles except that EBV is absent in all 7 cases of plasmablastic PCM but is present in all 5 cases of PBL they studied. While BCL-6, CD4, CD10, CD79a, and PAX-5 were detected more often in PBL than in plasmablastic PCM, no statistical significance between PBL and PCM was seen in any of the aforementioned markers. Thus, separation of PBL from plasmablastic PCM has to be based on multiple parameters including, but not limited to, clinical history of PCM, presence or absence of paraprotein and the amount of paraprotein, lytic bone lesion, kidney function, morphology, immunophenotype, and genetics.
- (b) *Extra-cavitary ► primary effusion lymphoma (PEL)*
PBL can be confused with PEL, especially the extra-cavitary form, easily based on cytomorphological features alone; therefore, the differential diagnosis between PBL and extra-cavitary PEL has to be based on other factors such as extensive immunophenotyping and presence or absence of effusion in the body cavities. Generally speaking, the cells from PEL are larger and show more cytologic atypia (Fig. 2a). While PBL and extra-cavitary PEL share many overlapping immunohistochemical profiles, it is generally accepted that PBL is negative, but extra-cavitary PEL is typically positive for HHV-8/KSHV (Fig. 2b).
- (c) *Large B-cell lymphoma arising in HHV-8-associated multicentric Castlemans disease*
PBL should be differentiated from large B-cell lymphoma arising in HHV-8-associated multicentric Castlemans disease (MCD), which occurs more often in HIV(+) patients with MCD. While both entities display plasmablastic cytomorphology, the neoplastic cells from the large B-cell lymphoma



Plasmablastic Lymphoma, Fig. 2 Representative microphotographs of an extra-cavitary primary effusion lymphoma from an enlarged right inguinal lymph node from a 77-year-old male with HIV and TB infection. The tumor cells are large with irregular to indented nuclear

contours, relative abundant cytoplasm, and conspicuous nucleoli (a H&E, original magnification of 400×). The lymphoma cells are strongly positive for HHV-8/KSHV (b original magnification of 200×)

arising in HHV-8-associated MCD generally express HHV-8/KSHV latency-associated nuclear antigen (LANA), are typically positive for IgM and lambda-light chain restriction, and are negative for CD138.

(d) *Other aggressive large B-cell lymphomas*

DLBCL, not otherwise specified (DLBCL, NOS), occurring in HIV(+) patients often exhibit plasmacytoid features. Despite cytomorphological overlap between PBL and DLBCL, NOS in the setting of HIV infection, the latter can be easily distinguished by its expression of most of the B-lineage specific and associated antigens.

Genetic Aberrancies

C-MYC rearrangements have been reported to be the most commonly encountered cytogenetic abnormalities. For example, in a large study of oral PBLs, Boy et al. reported rearrangement of the *MYC* gene in 60% of cases with the *IgH* locus as a partner gene in 51% of cases. Similarly, Valera et al. reported *MYC* rearrangements in 49% of 41 PBL cases they studied. By using fluorescence in situ hybridization, additional genetic abnormalities such as increased copy number of cyclin D1; gains of *MYC*, *BCL-2*, *BCL-6*, *MALT1*, and *PAX-5*; and aneuploidy for *BCL-6* were observed in 41, 20, 31, 41, 33, 32, and 28% of cases, respectively. However, no rearrangements of *BCL-2*, *BCL-6*, *MALT1*

(mucosa-associated lymphoid tissue 1), or *PAX-5* were detected in any PBL cases they examined (Castillo et al. 2010; Ustun et al. 2009).

By employing array-based comparative genomic hybridization (cCGH) technology, Chang et al. reported frequent segmental gains (>40%) of 1p36.11–1p36.33, 1p34.1–1p36.13, 1q21.1–1q23.1, 7q11.2–7q11.23, 11q12–11q13.2, and 22q12.2–22q13.3, which correlated with segmental gains occurring in high frequency in DLBCL regardless of HIV status.

Treatment

A key aspect of the treatment of PBL is the use of combination cytotoxic chemotherapy, which has been shown to provide a significant increase in survival in HIV-positive patients with PBL (Castillo et al. 2010). Patients treated with combination chemotherapy have an overall response rate (ORR) to chemotherapy of 77%, with 46% of patients achieving a complete response (CR) and 31% a partial response (PR).

HAART in addition to chemotherapy and/or radiotherapy can improve the prognosis of PBL. Good responses with HAART alone have been reported, but such responses are not usually durable. Correspondingly, relapsed PBL has been documented after cessation or interruption of HAART and subsequent drop in CD4 counts and

rise in viral loads. These cases of recurrence after disruption of HAART substantiate the finding that HAART is an important cornerstone in the treatment of PBL.

Despite the wide use of combined chemotherapy, there is no standardized treatment for PBL. Many treatments have been employed including CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high-dose methotrexate and cytarabine), dose-adjusted EPOCH (etoposide, doxorubicin, vincristine, cyclophosphamide, prednisone), or CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, methotrexate, ifosfamide, etoposide, cytarabine) (Castillo and Reagan 2011). It is not currently known whether any particular treatment is more efficacious than others. A small study did not show any survival benefit comparing CHOP with more intense treatment, although the patients who received more intensive treatment may have had higher risk and more advanced disease, biasing the results. National Comprehensive Cancer Network (NCCN) currently suggests more aggressive regimens such as dose-adjusted EPOCH, hyper-CVAD, and CODOX-M/IVAC.

Newer drugs have been used in a few cases of PBL. Drugs used in multiple myeloma therapy, particularly the proteasome inhibitor bortezomib, have been reported to be active in cases of PBL (Bibas et al. 2010; Bose et al. 2009). In a report of a case of newly diagnosed concurrent HIV and PBL, bortezomib was administered at a dose of 1.3 mg/m² intravenously on days 1, 4, 8, and 11. Bortezomib was administered because of the patient's poor hepatic performance status and inability to tolerate cytotoxic chemotherapy. The patient had a dramatic response by PET after two doses.

Radiation alone or in combination with combined chemotherapy has been utilized (Castillo et al. 2008). However, it is unknown whether radiation alone or combination of radiation and chemotherapy is better than chemotherapy alone, particularly for stage I disease.

Due to the small number of patients with PBL, there is not a wealth of data regarding the utility of

autologous stem cell transplants in PBL. A few case reports of autologous stem cell transplants in patients with HIV-positive PBL have found varying clinical outcomes. Given the few case reports of transplantation for PBL, few conclusions can be reached for this modality of treatment.

Prognosis

Prior to widespread HAART use, the prognosis of PBL was poor. HAART in addition to chemotherapy and/or radiotherapy appears to improve the prognosis of PBL. In one retrospective study of HIV-positive patients from 2000 to 2010, the median survival was 15 months (Castillo et al. 2008). However, longer survivals have been reported in the literature. In a small retrospective study examining 19 patients with central pathology review confirmation of PBL, 1-year survival was 67% for 12 newly diagnosed patients, and 2 of 6 patients with relapsed refractory disease were alive 2 years post relapse. In another review of 6 HIV-positive patients with PBL, 4 out of 6 patients had a survival of 22 months or more. Interestingly, HIV-positive patients had a better overall survival (OS) than HIV-negative patients, possibly in part explained by immune reconstitution obtained with the addition of HAART. (Castillo et al. 2010)

In a study of 53 patients by Castillo et al., ECOG status ≥ 2 , stages III–IV, age-adjusted IPI score, and MYC rearrangement were associated with a poorer OS on a univariate analysis (Castillo et al. 2012). In another study of 157 patients, age ≥ 60 , advanced stage, bone marrow involvement, lack of chemotherapy, HIV-negativity, and Ki-67 $> 80\%$ were all prognostic factors in OS. The only independent factors in OS were advanced stage and lack of chemotherapy (Castillo et al. 2010). Patients who are not treated with chemotherapy have a median survival of only 3 months (Castillo et al. 2010). Oral versus extraoral involvement has not been shown to significantly alter OS (Castillo et al. 2010). Due to the relatively few cases of PBL with CNS involvement, it is difficult to conclude whether or not CNS involvement portends a poorer survival; of

the cases reported, median OS has ranged from months to a year or more.

Conclusions

PBL makes up an infrequent but distinct type of AIDS-related NHLs. Given its overlapping cytomorphological and immunohistochemical features, it is important to distinguish PBL diagnosis from other mature aggressive large B-cell lymphomas. More data and studies are needed to further clarify clinical prognosticators and to determine the most effective treatments of PBL. Updated survival data in the age of newer and current therapy with HAART will be important to best describe PBL in the present era.

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Positive Health, Dignity, and Prevention (PHDP)

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Definition

Positive Health, Dignity and Prevention (PHDP) is an approach to HIV prevention that focuses on people living with HIV/AIDS (PLWHA). A PHDP approach involves interventions that empower PLWHA to (1) take care of their physical and mental health and well-being, (2) adopt behaviors that reduce their risk of transmitting HIV to others, and (3) actively participate in and provide leadership in HIV prevention and advocacy activities (USAID and ProjectSEARCH 2011).

Overview and History

Previous HIV prevention approaches that have targeted PLWHA have emphasized primary prevention and the role of PLWHA in reducing HIV transmission. Prevention for PLWHA has been referred to as positive prevention, prevention for positives, prevention with positives, and prevention by positives. Founded upon a human rights framework, Positive Health, Dignity and Prevention (PHDP) can be distinguished from these previous approaches by its emphasis on promoting the dignity, well-being, empowerment, and equality of PLWHA (USAID and ProjectSEARCH 2011). PHDP encompasses a broader perspective on and multiple levels of HIV prevention because it focuses on reducing PLWHA's risk for HIV transmission (secondary prevention) as well as improving their health and well-being in living with HIV/AIDS (tertiary prevention). The PHDP approach advocates for the integration of HIV prevention, medical treatment, health promotion, and empowerment for PLWHA (USAID and AIDSTAR-One 2011). This participatory, holistic approach espouses that PHDP interventions should be designed and implemented with the meaningful involvement of PLWHA, treating them humanely and with dignity, and promoting knowledge and skills, and an array of health, human, and social services and legal support (GNP+ and UNAIDS 2011).

PHDP interventions encourage empowerment and self-determination among PLWHA, providing them with resources and support so they may be the managers of their own health and well-being, as well as valuable contributors to their communities, including their families, neighborhoods, social networks, and other affiliation-based groups (e.g., men who have sex with men [MSM] or persons who inject drugs) (GNP+ and UNAIDS 2009). A PHDP approach encourages and empowers PLWHA to: (1) take care of their physical and mental health and well-being, (2) adopt behaviors that reduce their risk of transmitting HIV to others, and (3) actively participate in and provide leadership in HIV prevention and advocacy activities (USAID and ProjectSEARCH 2011). Active participation in HIV prevention and

advocacy may include acting as informal peer educators or advocates for PLWHA in their communities or by leading or participating in HIV prevention program design, implementation, and monitoring or helping to conduct exploratory or evaluation research (GNP+ and UNAIDS 2011).

PHDP, or HIV prevention targeting PLWHA, is a relatively new approach. It is distinct from primary HIV prevention approaches focused on reducing new HIV infections by delivering messages for those presumed to be HIV-seronegative to protect themselves from HIV, which have dominated the prevention landscape since the beginning of the HIV epidemic (GNP+ and UNAID 2011). In the late 1990s, HIV prevention efforts began to be directed at PLWHA as well. PHDP has been facilitated by increased utilization of HIV testing and counseling and advances in the medical treatment of HIV. HIV-positive individuals are increasingly aware of their HIV status and are living longer, healthier, and more productive lives on antiretroviral therapy (ART) (USAID and ProjectSEARCH 2011). If PLWHA are aware of their status, adopt behaviors that reduce the risk of HIV, and access and adhere to treatment sufficient to reduce their viral load, then they will be less likely to transmit HIV to other individuals. Many PLWHA have cultivated knowledge about HIV, how it is transmitted, and the associated risk behaviors (CDC 2003). Thus, they may be uniquely qualified – and indeed in many contexts have been on the forefront of the effort – to educate others about reducing the risk of transmitting or acquiring HIV through peer-based intervention approaches (Aggleton et al. 2011).

The defining objective of PHDP interventions is to actively engage PLWHA as equal participants in potentially all aspects of HIV prevention activities (GNP+ and UNAIDS 2009). While reducing HIV transmission remains a critical objective of PHDP interventions and PLWHA are viewed as central players in reducing the spread of HIV, PHDP interventions are careful not to stigmatize or blame PLWHA for problems associated with HIV. Rather, PLWHA are seen as part of the solution, with shared responsibility in working toward curbing the pandemic and limiting its adverse impact on health and society

(USAID and ProjectSEARCH 2011). Reducing stigma and discrimination against PLWHA in the community environment is among the key objectives of PHDP interventions. In social contexts in which stigmatization of PLWHA is severe, it is challenging to implement PHDP interventions because PLWHA may not be willing to self-disclose and publicly participate in these programs (GNP+ and UNAIDS 2011). Tragically, misguided messages targeting PLWHA in some settings have had negative connotations and have further stigmatized PLWHA. Such negative messages have included “PLWHA should not have sex” or “PLWHA need to be careful,” which clearly were not intended to empower PLWHA or engender their further participation in prevention initiatives (NAM – AIDSMap 2011, p. 1). Fortunately, by now most HIV prevention programs communicating with HIV-seropositive audiences have moved away from using such alienating, stigmatizing language. Health communication messages targeting PLWHA based on the PHDP approach are positively framed and inclusive. Examples include: “We are more than patients,” “We are responsible for HIV prevention,” and “We have needs and desires to be fulfilled” (GNP+ and UNAIDS 2011, p. 10).

Current Status of PHDP Implementation

The PHDP approach has been increasingly accepted in the field of HIV prevention. Research confirms that most PLWHA express a desire to make positive contributions to their community, maintain good physical and mental health, enhance their sense of dignity, and take responsibility for preventing the transmission of HIV to others (King et al. 2009). Therefore, PLWHA are generally receptive to HIV prevention interventions embodying the PHDP approach. PHDP interventions have been implemented in many developing and more developed countries across a wide variety of social contexts and settings (PEPFAR 2011). In the past decade, various US and international agencies and groups have endorsed the use of PHDP interventions for HIV prevention, including the Centers for Disease

Control and Prevention, Health Resources and Services Administration, the National Institutes of Health, the US President’s Emergency Plan for AIDS Relief (2011), Joint United Nations Programme on HIV/AIDS, the World Health Organization, the Global Network of People Living with HIV/AIDS, and the International HIV/AIDS Alliance (GNP+ and UNAIDS 2009; International HIV/AIDS Alliance 2007).

Interventions that are illustrative of the PHDP approach to HIV prevention include the programs of the Mexican Network of People Living with HIV/AIDS and the Khmer HIV/AIDS NGO Alliance in Cambodia (International HIV/AIDS Alliance 2007). The Mexican Network of People Living with HIV/AIDS provides a wide variety of services in the setting of HIV patient care that employ PHDP strategies, including individual and group counseling to PLWHA who attend the clinic to help them address disclosure and internalized stigma; training workshops for peer educators on HIV/STI prevention; one-on-one peer counseling sessions in which peer educators employ active listening and strategic questioning while they provide information and give ongoing support for behavior change; and support groups for PLWHA to have a safe space to meet, share their experiences, and mutually provide social support. PLWHA are considered essential for effectively reaching and supporting other HIV-positive individuals and play an active role in implementing all of the HIV prevention strategies and in providing the ancillary services as well (International HIV/AIDS Alliance 2007). In Cambodia, the Khmer HIV/AIDS NGO Alliance (KHANA) offers a home care program to provide HIV prevention information and condoms for PLWHA and other community members. KHANA health workers visit the homes of PLWHA and provide HIV prevention education to the community. The home care program achieved wide reach among PLWHA, was able to earn their trust, and has improved access to HIV medical care and treatment among PLWHA in the community. The program has improved the quality of life of PLWHA, their family members, and caregivers, increased their knowledge and understanding of HIV prevention and care, reduced

stigma and discrimination toward PLWHA among their neighbors, and has offered social and economic support empowering some of the poorest and most disadvantaged individuals and families in the community (International HIV/AIDS Alliance 2007).

Research Evidence Supporting PHDP Interventions

Many studies evaluating the effectiveness of HIV prevention programs targeting PLWHA populations have been published, though most do not explicitly refer to the PHDP approach. There are now several review articles and meta-analyses that have been published on this topic (Kennedy et al. 2010; Gilliam and Straub 2009; Crepaz et al. 2006; Johnson et al. 2006). Based on these review articles, the most common PHDP interventions promote condom use and other sexual risk-reduction behaviors among HIV-seropositive individuals or serodiscordant couples. Intervention studies focused on reducing drug-related risk behaviors among HIV-seropositive persons who inject drugs are less common in the research literature (USAID and AIDSTAR-One 2011). The primary target audience of PHDP interventions is PLWHA, though some secondarily or simultaneously target their partners, family members, or the community at large. Various behavior change interventions to affect HIV risk reduction of PLWHA have been studied in both developing and more developed countries, for example, one-time individual counseling session using motivational interviewing techniques; multiple group sessions to build skills around condom negotiation and disclosing one's HIV-seropositive status; counseling following HIV testing; and educational interventions, such as videos (Kennedy et al. 2010; Gerbert et al. 2006).

There is strong scientific evidence to support the effectiveness of PHDP behavioral interventions for reducing sexual risk behaviors among PLWHA. However, there is insufficient evidence that the PHDP approach effectively reduces all HIV transmission risk behaviors or that it is equally effective across subpopulations (USAID

and AIDSTAR-One 2011). In a meta-analytic review of controlled trials with PLWHA in the United States, Crepaz and colleagues (2009) found that behavioral interventions significantly reduced the odds of PLWHA practicing unprotected sex and acquiring sexually transmitted infections (STIs). Behavioral interventions with the most documented success were theory-based; developed through formative research to appropriately frame messages; targeted specific HIV risk behaviors; delivered by healthcare providers or counselors to persons where they receive routine medical care or other services; included motivational interviewing, cognitive-behavioral counseling, and skills-building components; and addressed mental health and other challenges to medication adherence and HIV risk behavior change (Crepaz et al. 2006; Gerbert et al. 2006; Johnson et al. 2006; Gilliam and Straub 2009). Of note, the effects of these types of behavioral interventions appear to be less effective among HIV-seropositive persons who inject drugs, MSM, HIV-seronegative individuals, and HIV-serodiscordant couples (Crepaz et al. 2006; Johnson et al. 2006; Kennedy et al. 2010). The latter finding is especially relevant in sub-Saharan Africa, where heterosexual sex accounts for the vast majority of new HIV infections (Bunnell et al. 2008).

There is substantial evidence that PHDP behavioral interventions are effective for reducing sexual risk behaviors among PLWHA across a wide spectrum of cultures and contexts, particularly among HIV-seropositive heterosexual individuals. PHDP interventions have been effectively implemented in both clinic and community settings by healthcare providers, counselors, or social workers and by laypeople or peer educators, and there is evidence that these interventions are cost-effective (PEPFAR 2011).

However, evidence suggests that PHDP interventions are less effective among MSM, and there is insufficient evidence to conclude that injection drug use-related risk behaviors are reduced through PHDP approaches. Furthermore, the body of literature on PHDP-related interventions only describes the effectiveness of these

interventions for changing HIV risk behaviors; studies rarely report whether other key objectives of the PHDP approach were met by the HIV prevention interventions for PLWHA. The potential outcomes of PHDP interventions related to empowering HIV-seropositive individuals to protect their physical and mental health and well-being or engaging PLWHA in the development, implementation, and evaluation of HIV prevention programs and advocacy activities are often either not reported in the published literature or are presented only in case study format, not as part of quantitative program evaluations. More research is needed to determine whether PHDP interventions, in addition to secondary HIV prevention, are able to produce a wide array of intended outcomes. In particular, empirical evidence is needed to support the PHDP approach in: empowering PLWHA to access and optimally use healthcare and HIV treatments and other resources to improve their physical and mental health and well-being, involving PLWHA in HIV prevention program development and advocacy activities, and reducing stigma and discrimination against PLWHA.

Conclusion

The PHDP approach involves targeting PLWHA with HIV prevention interventions that emphasize the empowerment and dignity of HIV-positive individuals and may be implemented in any setting with PLWHA who are willing to actively participate in an HIV prevention program. While PHDP interventions should not be implemented instead of risk-reduction interventions targeting HIV-negative individuals or individuals whose status is unknown, they represent another critical component of a comprehensive HIV prevention strategy (CDC 2003). Although there has been increased focus and investment in prevention interventions targeting PLWHA, interventions based on a PHDP approach continue to receive less attention than they arguably deserve given their established effectiveness for reducing sexual risk behaviors (Aggleton et al. 2011).

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Post-treatment Controllers

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Definition

Post-treatment controllers (PTC) are HIV-infected patients able to maintain a durable control of viremia at undetectable levels after interruption of antiretroviral treatment. Differently from natural HIV “elite” controllers (HIC), post-treatment controllers require therapeutic intervention to first reach undetectable viral loads. Post-treatment controllers still carry infected cells and, thus, are not cured but considered to be in sustained remission of HIV infection. These patients have also been termed “secondary controllers.”

Introduction

The introduction of combination antiretroviral treatment (cART) as the standard of care for HIV-infected individuals changed the face of the

AIDS epidemics. cART has drastically reduced mortality and morbidities of HIV-infected patients (Boyd 2009) and has also proven its value as a prevention tool by decreasing the risk of transmission (Cohen et al. 2011). A few years after cART was implemented, it was hypothesized that the perfectly controlled viral replication obtained with treatment may lead to the elimination of infected cells in treated patients. However, it was soon observed that HIV persists in different cell subsets, forming reservoirs that are untouched by cART (Eisele and Siliciano 2012). Infection and homeostatic proliferation of long-lived resting T cells, establishment of sanctuaries in regions not fully accessible to antiretroviral molecules, and/or residual viral replication may contribute to the persistence of HIV reservoirs in the presence of cART. Accordingly, regardless of the duration of the antiretroviral treatment, its interruption leads to prompt resumption of viral load replication to levels that are close to those observed during primary HIV infection (PHI) (Paton 2008). However, a few individuals have shown a remarkable capacity to keep efficient suppression of viremia long time after therapy discontinuation, supporting the idea that remission of HIV infection may be achievable, at least in some patients.

Early Treatment and Control of Infection

Cases of transient control after interruption of treatment initiated close to acute infection pointed to the interest of early treatment (Rosenberg et al. 2000). Indeed, analytical treatment interruption (ATI) protocols consistently reported delayed rebound of viremia and slower CD4⁺ T cell decay in some patients in whom treatment was initiated during PHI (Steingrover et al. 2008). Early treatment has been proposed to limit the establishment of viral reservoirs, reduce viral diversity and evolution, control deleterious immune activation, and preserve innate and adaptive immune responses (O'Brien and Markowitz 2012). Nevertheless, the clinical benefits of PHI treatment observed in ATI protocols often wane several months after therapy discontinuation and just a handful of patients have

been reported to extend viral control for many years (Hocqueloux et al. 2010).

The ANRS VISCONTI (Viro-Immunological Sustained CONTROL after Treatment Interruption) study reported the case of 14 post-treatment controllers who were able to keep an extremely well-controlled viremia and stable CD4+ T cell counts for more than 7 years after interruption of antiretroviral treatment (Saez-Cirion et al. 2013). These patients were not cured as they still carried infected cells, although at very low levels, and viral blips or residual replication with ultrasensitive techniques were detected at some time point for all of them. All these patients shared the characteristic of having started antiretroviral treatment within or close to PHI (before Fiebig stage VI (Fiebig et al. 2003)). Different epidemiological studies suggest that the probability of durable Post-treatment controllers may be of 5–15% among patients who initiated treatment during the first 6 months which followed contamination and kept the treatment for more than 12 months (Saez-Cirion et al. 2013; Goujard et al. 2012; Lodi et al. 2012). The ratio between the duration of the antiretroviral treatment and the immediateness of initiation of this treatment has been proposed to impact the probability to achieve durable Post-treatment controllers (Hocqueloux et al. 2010). Along this line, for the 14 patients in the VISCONTI study, the median treatment initiation delay was of 39 days after the presumed date of contamination, and the median treatment length was of 3 years (Saez-Cirion et al. 2013). Studies on non-human primate models of SIV infection have shown that treatment initiation during the very first days following contamination drastically limits the establishment of viral reservoirs (Whitney et al. 2014). However, even extremely early treatment does not suffice to prevent the seeding of the reservoir. Nevertheless, early treatment has been proposed to particularly protect long-lasting memory T cells from infection (Ananworanich et al. 2013). Longer treatment periods initiated during primary infection have been shown to have a stronger impact on viral reservoirs than the same periods of treatment initiated during chronic infection (Hocqueloux et al. 2013; Buzon et al. 2014). The questions

about the optimal treatment duration and the existence of a window of opportunity for treatment initiation are still open and require study of much larger groups of patients.

A singular case of Post-treatment controllers was reported in an in utero infected baby who started prophylactic cART 30 h post-birth and maintained the treatment for more than 15 months (Persaud et al. 2013). The viral load in the baby decreased from 19,000 RNA copies/ml of plasma to undetectable levels within 1 month of treatment. Four consecutive positive viral loads were measured during this period, confirming that the baby was infected. Weak adherence to treatment was observed from month 15, and cART was completely discontinued sometime between months 15 and 18. Viral loads were undetectable with standard techniques for over 2 years after treatment discontinuation, and HIV antibodies were negative. Traces of virus were detected with different ultrasensitive HIV-RNA and DNA determinations at months 24 and beyond, suggesting that the virus had not been completely eradicated but controlled to extremely low levels. Unfortunately, control of infection was eventually lost in this child after 2 years and a half off therapy (<http://www.niaid.nih.gov/news/newsreleases/2014/Pages/MississippiBabyHIV.aspx>). Despite this setback, this case significantly stressed the relevance of very early treatment as a starting point to achieve remission of infection in some patients and perhaps particularly in infants (Ananworanich et al. 2014; Luzuriaga et al. 2014).

Among adults, rarity of PTC might be explained by the analyses of clinical data from patients included in the French Hospital Database on HIV showing that only 2% of all the patients diagnosed close to primary infection and included in the database (>3,000 patients) underwent the therapeutic strategy that led to control of infection in the VISCONTI study (Saez-Cirion et al. 2013). Even when treatment is initiated during primary infection, the frequency of patients losing control is steep during the first months after therapy discontinuation. The patients who are able to control viremia for 12 months are likely to keep it for longer periods of time. So far, no reliable markers allow predicting which patients are more likely to

achieve a PTC status after treatment interruption. Different reports coincide to point out that patients who will control infection have lower levels of cell-associated HIV DNA at the time of treatment interruption (Hocqueloux et al. 2010; Saez-Cirion et al. 2013; Goujard et al. 2012), although low viral reservoirs alone do not suffice to ensure control of infection.

Differences Between Natural Control and Post-treatment controllers of Infection

Early treatment onset in most PTC may mask some cases of spontaneous control of infection. However, in general, many differences have been reported between PTC and HIC. In two PTC treatment initiation was due to prophylaxis after exposure (Saez-Cirion et al. 2013; Persaud et al. 2013), but in most cases treatment initiation in PTC was prompted by symptomatic primary infection accompanied by high viral loads (Saez-Cirion et al. 2013). In contrast, most HIC seem to start controlling infection very early and have weak viral loads and no symptoms during primary infection (Saez-Cirion and Pancino 2013). Natural control of infection in HIC is often associated with efficient immune responses, in particular T cell responses, which are favored by the presence of protective HLA class I alleles (e.g., HLA-B*27 and B*57) in most of these individuals (Saez-Cirion and Pancino 2013). Accordingly with their PHI profile, most PTC do not carry protective HLA class I alleles but rather neutral or high risk of progression to AIDS alleles (such as HLA-B*35 or B*07) and have very weak CD8+ T cell responses (Saez-Cirion et al. 2013). The levels of T cell activation are also higher in HIC than in PTC.

One PTC was shown to control infection of a virus that caused AIDS in his transmitting spouse (Salgado et al. 2011). Interestingly, this PTC, who initiated cART during PHI, showed a strong viral rebound when transiently discontinued treatment ~9 months after initiation. Treatment was immediately resumed and maintained for 14 additional months after which the patient was able, off

therapy, to control infection to undetectable levels for 9 years. The transient loss of control of infection in this PTC after firstly interrupting treatment further argues that Post-treatment controllers may be favored by sustained early cART in some patients non-predisposed to naturally control the virus.

PTC After Treatment in Chronic Infection

Although most PTC initiate treatment during PHI, some patients who were treated during the chronic stage of infection have been shown to control viremia after treatment interruption (Piketty et al. 2010; Van Gulck et al. 2012). It is important to differentiate these PTC, who had detectable viral loads before cART, from HIC who started treatment while keeping undetectable (or <1,000 copies) viral loads (Boufassa et al. 2014). Control of infection after interruption of cART initiated during the chronic phase of infection seems far rarer than when treatment is initiated during PHI (Saez-Cirion et al. 2013; Piketty et al. 2010). The study of a larger number of cases of PTC with sustained undetectable viremia after treatment interruption will be needed to find similarities and/or differences between chronic and PHI-treated PTC. The available reports suggest that Post-treatment controllers in patients treated during chronic infection is also associated with low levels of infected cells at the time of treatment interruption (Piketty et al. 2010; Van Gulck et al. 2012). Early initiation of treatment (with CD4+ T cell levels >500 cells/mm³) (Piketty et al. 2010) or infection with low-fitness viruses (Van Gulck et al. 2012) may have contributed to achieve PTC status in these patients.

Conclusion

In conclusion, some patients are able to keep undetectable HIV loads for long periods of time after interrupting antiretroviral treatment. In general this treatment was initiated close to the acute infection and maintained for several years before discontinuation. These patients are considered to

be in sustained remission of HIV infection: to counteract HIV replication, they needed therapeutic intervention, which interruption years later did not provoke viral rebound or progression to disease. These patients have not eliminated the virus and carry low levels of infected cells that are controlled within this remarkable status of stable remission. Although only one case of HIV eradication has been reported until now (Hutter et al. 2009; Yukl et al. 2013), the existence of several PTC cases suggests that the search for sustained HIV remission may be a more reachable goal than HIV eradication, at least in a medium term. Because of new recommendations shifting to treat HIV patients as early as possible, it is likely that more patients with favorable conditions to control infection after treatment interruption will appear over time. In this context, the priority is to identify markers that will allow the identification of patients with good probabilities to become PTC and in whom cART could be safely interrupted.

Cross-References

- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [HIV-Associated Immune Exhaustion](#)
- ▶ [HIV-2: Lessons from the Dakar Cohort](#)
- ▶ [MHC Locus Variation](#)
- ▶ [Overview: Immunopathogenesis](#)
- ▶ [Viremic Nonprogressors](#)

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Preexposure Prophylaxis (PrEP)

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Definition and Rationale for Preexposure Prophylaxis (PrEP)

Traditionally, HIV prevention methods have focused on approaches such as education, evidence-based behavioral modifications, prevention with positives, harm reduction, and consistent condom use. Given that there are an estimated 7,000 new HIV infections globally each day, an estimated 48,000 new infections in the United States annually, and lack of success in developing an HIV vaccine thus far, these approaches have had a limited impact on stemming the HIV epidemic. Therefore, recent efforts have focused on the use of biomedical prevention methods to reduce HIV incidence.

Preexposure prophylaxis for HIV or “PrEP” is a biomedical prevention method that involves HIV-negative persons using oral or topical antiretroviral medications prior to sexual or parenteral exposure to prevent HIV. PrEP is seen as an intervention that should complement existing HIV prevention strategies. It is one of several biomedical prevention methods, including treatment as prevention, male circumcision, and HIV vaccine development, being used to prevent HIV transmission.

Use of PrEP

There are various routes of administration of PrEP including oral and topical PrEP. Oral PrEP involves taking a pill by mouth, whereas topical PrEP can be administered either vaginally or rectally through the use of gels containing antiretrovirals that are inserted prior to sex (precoitally) or after sex (postcoitally). These gels are often referred to as microbicides. PrEP should not be used alone but rather is intended for use in combination with other behavioral interventions including condoms, risk reduction counseling, and sexually transmitted disease (STD) testing. Preliminary guidelines for the use of PrEP include the need for baseline assessment of a person’s HIV status, frequent monitoring for side effects, frequent follow-up inclusive of routine HIV testing, and education on the importance of adherence (CDC 2011, 2012).

Biological Plausibility

The concept of using PrEP as a method for HIV prevention stems from research conducted in both animals and humans. Preexposure prophylaxis is commonly used to prevent infections with other illnesses. For example, preexposure prophylaxis with antimalarial medications is used to prevent malaria infections in people traveling and living in endemic areas. Furthermore, the use of pre- and postexposure prophylaxis in the prevention of mother-to-child transmission of HIV has been shown to dramatically reduce the risk of perinatal

transmission to newborn infants to as little as 1% (Connor et al. 1994). Similarly, the use of post-exposure prophylaxis (PEP) to reduce the risk of infection after occupational exposures and high-risk behavioral exposures has been shown to be effective with an 81% reduction in transmission among persons taking PEP (Cardo et al. 1997).

Tenofovir disoproxil fumarate (TDF, Gilead brand name Viread[®])-based regimens have been the most widely studied for use in preexposure prophylaxis for HIV. TDF, a nucleoside reverse transcriptase inhibitor (NRTI), is a well-tolerated and safe antiretroviral with minimal side effects. In recent PrEP animal studies and clinical trials, TDF with emtricitabine (FTC/TDF, Gilead brand name Truvada[®]) has shown the potential to be an even more effective medication for HIV prevention (García-Lerma et al. 2008).

Animal studies provided early evidence in support of PrEP as an HIV prevention method (García-Lerma et al. 2008; Parikh et al. 2009; García-Lerma et al. 2010; Cong et al. 2011). Studies conducted in monkeys have found high levels of protection and significant reductions in transmission when TDF was given orally, rectally, and intravenously. These unequivocal findings in animal models led to further trials in human beings. Additional animal studies are currently being conducted to identify new antiretroviral candidates for PrEP (Veazey et al. 2010; Dobard et al. 2011).

PrEP Trials Among Humans

Multiple studies among human beings, mostly in the form of phase I, II, and, III clinical trials, have been conducted on PrEP. These studies were conducted in various geographic locations and among specific high-risk populations. Descriptions of key studies and their major findings follow.

Phase I/II Clinical Trials

FHI TDF West African Trial: From June 2004 to March 2005, 936 HIV-negative heterosexual women in Ghana, Cameroon, and Nigeria were recruited into the Family Health International

(FHI) TDF West African trial (Peterson et al. 2007). This study was the first to examine the safety and effectiveness of taking once-daily oral dose of TDF to prevent HIV-1/HIV-2. The study found no significant safety outcome differences between the intervention and control arms. Insufficient follow-up time due to the premature closing of two study sites resulted in the study not being able to achieve an adequate sample size. Therefore, the investigators were not able to adequately assess the effectiveness of once-daily oral TDF as preexposure prophylaxis for HIV.

CDC 4323: Another phase II study, CDC 4323, examined the clinical and behavioral safety of daily oral TDF among men who had sex with men (MSM) in three US cities: Atlanta, Boston, and San Francisco (Grohskopf et al. 2010; CDC 2010). The 400 participants enrolled were randomized to receive either the active or placebo drug. Additionally, receipt of TDF or placebo was delayed for half of the participants in each arm in order to assess the potential behavioral effects of taking a daily medication. No significant biomedical safety concerns were discovered nor were there any significant behavioral differences identified between participants who immediately started the study drug and those with delayed initiation. CDC 4323 was not designed to examine drug effectiveness in the prevention of HIV.

IAVI E001: The International AIDS Vaccine Initiative (IAVI) conducted a phase I/II placebo-controlled trial in Kenya to evaluate the safety, acceptability, and adherence of daily versus intermittent oral FTC/TDF (IAVI E001) (Mutua et al. 2012). From October to December 2009, the study enrolled and randomized 72 MSM and female sex workers. Investigators discovered no safety concerns in either PrEP regimen compared to placebo and found few differences between the two regimens in terms of safety and acceptability. Eighty-three percent of the study participants indicated they would be willing to take PrEP if it was safe, effective, and not prohibitively expensive. The IAVI E001 study found that participants randomized to receive PrEP intermittently (twice weekly with a postcoital dose) were slightly less adherent to study medication, and from follow-up focus groups and interviews, they identified

potential adherence obstacles with the postcoital dosage, in particular (Mutua et al. 2012).

Phase IIB/III Clinical Trials

The following Table 1 presents a brief summary of major phase IIB/III PrEP clinical trials listed in chronological order of the release of study findings.

CAPRISA 004: The Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 trial assessed the safety and efficacy of a 1% vaginal gel formulation of tenofovir as PrEP among a population of 889 heterosexual South

African women (Abdool Karim et al. 2010). Participants were instructed to use one dose of the gel within 12 h prior to vaginal sex and to use a second dose within 12 h of having sex. After 30 months of follow-up, investigators found that the tenofovir gel reduced the risk of HIV-1/HIV-2 by 39% compared to those using the placebo gel. Among women with high gel adherence (i.e., taking >80% of doses) that efficacy increased to 54%. No significant safety concerns emerged and investigators concluded that tenofovir gel could prove to be an important HIV prevention tool for women who have difficulty negotiating

Preexposure Prophylaxis (PrEP), Table 1 Summary of major preexposure prophylaxis phase IIB/III clinical trials and their results

Study name (clinical trial number)	Active drug regimen	Phase	Sample size ^a	Recruitment site(s)	Study population ^b	Efficacy % (95% CI)
CAPRISA 004 (MTN 004)	Pre- and postcoital TFV	III	889	South Africa	Heterosexual women	39 (6–60)
iPrEx	Daily oral FTC/TDF	III	2,499	Peru, Ecuador, South Africa, Brazil, Thailand, the United States	MSM and transgender women	44 (15–63)
FEM-PrEP	Daily oral FTC/TDF	III	2,120	Kenya, South Africa, Tanzania	Heterosexual women	6 (–52–41) ^c
TDF2 (CDC 4940)	Daily oral FTC/TDF	III	1,219	Botswana	Heterosexual men and women	62 (22–83)
Partners PrEP	(1) Daily oral TDF (2) Daily oral FTC/TDF	III	4,758	Kenya, Uganda	Heterosexual discordant couples	TDF, 67 (44–81); FTC/TDF, 75 (55–87)
VOICE study (MTN 003)	(1) Daily TFV (2) Daily oral TDF (3) Daily oral FTC/TDF	IIB	5,029	Uganda, South Africa, Zimbabwe	Heterosexual women	TFV, 15 (–20–40) ^{c,d} ; TDF, –49 (–129–3) ^{c,d} ; FTC/TDF, –4 (–49–27) ^c
Bangkok Tenofovir Study (CDC 4370)	Daily oral TDF	III	2,413	Thailand	Injection drug users (IDU)	74 (17–94)

MTN Microbicide Trials Network, CDC Centers for Disease Control and Prevention, N/A not available, TFV 1% tenofovir vaginal gel, TDF tenofovir disoproxil fumarate, FTC/TDF TDF with emtricitabine

^aNumber of participants enrolled and randomized

^bUnless otherwise noted, all participants were HIV uninfected and “at risk” due to potential sexual or injection exposure

^cResults were not significant

^dPrematurely discontinued

monogamy or the use of condoms with their sexual partners.

iPrEx: Some of the first oral PrEP efficacy results, released November 2010, came from the Preexposure Prophylaxis Initiative (iPrEx) trial. This phase III study evaluated the effect of once-daily FTC/TDF compared to placebo among a population of 2,499 men and transgender women who have sex with men, recruited from multiple countries in the Americas, Africa, and Southeast Asia (Grant et al. 2010). A 44% relative reduction in new HIV infections was found among study participants taking daily oral FTC/TDF compared to the placebo group. Further analysis showed that strict adherence to the daily medication (i.e., taking pills on $\geq 90\%$ of days) significantly improved protection, increasing the efficacy to 73%.

FEM-PrEP: Not all phase III PrEP studies have shown a significant increase in HIV prevention, however. In the FEM-PrEP study, another investigation intended to evaluate the safety and effectiveness of daily oral FTC/TDF in the prevention of HIV acquisition; 2,120 heterosexual women from Kenya, South Africa, and Tanzania were randomized to receive either FTC/TDF or placebo drug (Van Damme et al. 2012). Preliminary analyses showed similar rates of HIV in both arms of the study (4.7 per 100 person-years in the FTC/TDF group and 5.0 per 100 person-years in the placebo group), with slightly elevated occurrences of adverse events among study participants receiving the active drug. As a result of these findings, the study was terminated early in April 2011. The study investigators proposed that the lack of significant HIV protection seen in the FTC/TDF group may have been due to poor medication adherence. Although self-reported adherence rates were high (95%) and pill count data (88%) seemed to corroborate high adherence in the study population, they found low levels of TDF in the plasma of women who became HIV positive during the study period. This implied that true adherence may have been significantly lower than suggested from either self-reports or pill count data, although a biological reason for this difference also needs to be examined.

TDF1 and TDF2: In 2005, the US Centers for Disease Control and Prevention (CDC) in cooperation with the Botswana government launched TDF1, a phase III study examining the safety and efficacy of once-daily TDF compared to placebo drug in the prevention of HIV-1/HIV-2 (Thigpen et al. 2012). After the results from animal studies were released which showed a clear advantage in using FTC/TDF over the single-drug TDF, study investigators changed the active study drug to FTC/TDF and offered TDF1 participants the opportunity to enroll in the new TDF2 study. In the TDF2 study, 1,219 heterosexual men and women were enrolled from February 2007 through October 2009 and randomized to receive either daily oral combined FTC/TDF or placebo drug. Investigators found that daily FTC/TDF reduced the likelihood of HIV by 62%. This efficacy improved to 78% when they limited analyses to only those participants who were confirmed to have received a supply of the study drug in the 30 days prior to their last visit. While minor side effects such as nausea, vomiting, and dizziness were more commonly reported among participants receiving the active study drug, the risk of serious adverse effects did not increase with FTC/TDF use. Drug resistance in one undiagnosed HIV-positive TDF2 participant who received FTC/TDF highlighted the need to closely monitor HIV seroconversion among persons taking PrEP.

Partners PrEP: From July 2008 to November 2010, investigators with the Partners PrEP study enrolled 4,758 HIV-serodiscordant heterosexual couples from Uganda and Kenya (Baeten et al. 2012). A serodiscordant couple is one in which one of the partners is HIV positive, while the other is HIV negative. One-third of the study population was randomly assigned to receive oral TDF, one-third to receive oral FTC/TDF, and one-third to receive placebo drug. All study medication was taken once daily. In July 2011, preliminary analysis showed such a significant reduction in HIV acquisition among both active study drug arms that investigators discontinued the placebo arm of the study. The final Partners

PrEP results found that daily oral TDF reduced the risk of HIV by 67% and that daily oral FTC/TDF reduced the risk even further by 75%. TDF and FTC/TDF proved to be safe and efficacious in both male and female participants.

VOICE Study: Another study with mixed results was the Vaginal and Oral Interventions to Control the Epidemic (VOICE) study, which began enrolling participants from Uganda, South Africa, and Zimbabwe in September 2009 (MTN 2011). In this phase IIB study, 5,029 young heterosexual women were randomized into one of five arms: daily 1% tenofovir vaginal gel, daily placebo vaginal gel, daily oral TDF, daily oral FTC/TDF, and daily oral placebo drug. In September 2011, the daily oral TDF arm was discontinued as preliminary analysis found that, while safe, it did not significantly reduce the risk of HIV. Similarly, the daily vaginal gel arms of the study were discontinued in November 2011 because the tenofovir gel was not shown to significantly reduce the risk of HIV. Follow-up for the daily oral FTC/TDF arm of the study was completed in August 2012 and was not shown to significantly reduce the risk of HIV compared to placebo (Marrazzo et al. 2013).

Bangkok Tenofovir Study: Launched in June 2005, the Bangkok Tenofovir Study aimed to evaluate PrEP safety and effectiveness in a population of injection drug users recruited from Thailand (Choopanya et al. 2013). Enrollment concluded in December 2010 with 2,413 participants successfully randomized to receive either daily oral TDF or placebo in a double-blinded fashion. Results from this study, released in June 2013, showed significantly lower HIV incidence rates among injection drug users who receive daily oral TDF (0.35 per 100 person-years) compared to those randomized to the placebo group (0.68 per 100 person-years). After examining tenofovir concentration levels in the plasma of seroconverted participants, investigators presented a modified efficacy estimate of 73.5%, reinforcing the findings of previous studies that medication adherence has critical impact on PrEP's ability to prevent HIV infection.

Future and Ongoing Studies

Several additional PrEP studies are ongoing or planned. These include studies that will address the use of PrEP in different populations, the long-term use of PrEP, and the effectiveness of different HIV medications and modalities for delivering these medications (Cohen et al. 2012; Underhill et al. 2010). For participants who were enrolled in some of the abovementioned trials, those who received placebo will be offered the medications, and participants will continue to be monitored to assess the long-term effectiveness and impact of PrEP on behaviors, safety, tolerability, and adherence to these medications (Cohen et al. 2012; Grant et al. 2010).

Public Health Implementation of PrEP

The results of the various PrEP clinical trials provided proof of concept that PrEP for HIV prevention could be an effective way to reduce HIV and onward transmissions. In January 2011, after the results of the iPrEx study were published, the CDC issued interim guidance on the use of PrEP among MSM (CDC 2011). In July 2012, the US Food and Drug Administration (FDA) approved the licensure of oral daily Truvada® (FTC/TDF) for use as preexposure prophylaxis in adults at high risk for HIV or who engage in sexual activity with HIV-positive partners (U.S. FDA 2012). In August 2012, the CDC issued guidance on the use of PrEP among heterosexuals at high risk for HIV (CDC 2012). Following the receipt of results from several additional PrEP clinical trials and FDA approval, final clinical practice guidance from the US Public Health Service has been published (2014).

Challenges of PrEP Use

Despite the success of many of the PrEP trials, the scalability, feasibility, and acceptability of PrEP at a population level are under much debate

(Underhill et al. 2010; Cohen et al. 2012). Many questions continue to linger regarding how best to implement, scale up, and provide PrEP in a real-world setting. Additionally, many potential issues and challenges related to PrEP use remain, including the safety and tolerability of taking antiretroviral medications on a regular basis, the optimal frequency and duration of PrEP use, the need for optimal adherence, the risk of developing drug resistance, the risk of behavioral disinhibition, the potential for PrEP use among certain high-risk populations, cost-effectiveness, and ethical concerns. These issues are discussed below.

Safety and tolerability: Research has shown that the side effects of using FTC/TDF as a prophylaxis are minimal. Those who did have side effects experienced reductions in bone mineral density, decreased renal function, and gastrointestinal upset including diarrhea and nausea. However, these side effects were not severe enough to preclude its use. Toxicities from using TDF are less likely in HIV-negative persons who are on fewer medications and are generally healthier individuals. Current guidelines for PrEP use include the routine monitoring of side effects. In order to clinically monitor for these side effects, both in short- and long-term use, it is important for persons taking PrEP to have a consistent and reliable source of medical care.

Frequency and duration of use: As taking any daily medication as prescribed can often prove difficult, the optimal frequency, dosing, and duration of PrEP use is currently under investigation. Several clinical trials will be assessing the use of both pre-coital or “on-demand” PrEP in which FTC/TDF is taken both a few hours prior to and after sexual exposure (Lorente et al. 2012), as well as intermittent dosing strategies for PrEP or “iPrEP” which might, for example, entail weekly dosing. Intermittent dosing strategies and coitally dependent dosing regimens may provide viable and effective alternatives to daily dosing.

Furthermore, unlike traditional prevention approaches, the time frame for using PrEP has not been well established. PrEP use may be continuous but the long-term effects of using antiretrovirals for HIV prevention among HIV-negative persons are not well described to

date. An alternative scenario may be that people will take PrEP for a discrete period of time (for example, during a high-risk period in their lives) and then discontinue it when they perceive their risk of HIV to be lower (for example, when they enter into a relationship with a stable partner).

Adherence: Adherence to PrEP is essential in order to achieve the maximum effectiveness and benefits of risk reduction. Studies have shown that PrEP efficacy for HIV prevention is directly affected by level of medication adherence, with significantly lower efficacy rates among those who are not fully adherent compared to those who are fully adherent (Grant et al. 2010; Thigpen et al. 2012; Choopanya et al. 2013). As previously mentioned, further research is needed to determine alternative dosing methods that may prove to be easier to follow. Additional research on adherence to PrEP and potential barriers to being fully adherent is also needed (Mutua et al. 2012).

Drug resistance: Individuals with undiagnosed HIV who are taking PrEP are at risk for developing drug resistance. For example, in the iPrEx study, two HIV-positive individuals developed resistance to emtricitabine (Grant et al. 2010). Without careful and frequent monitoring of one’s HIV status among persons on PrEP, the risk of transmitting drug-resistant virus could increase. This could potentially lead to a loss of efficacy of FTC/TDF, resulting in the need for more second-line antiretroviral therapies among these newly diagnosed individuals (Hurt et al. 2011). High levels of adherence, regular HIV testing, and the ability to detect acute infections may help to limit the development of drug resistance.

Behavioral disinhibition: One common concern regarding the use of PrEP is that individuals taking PrEP may believe that they are sufficiently protected from acquiring HIV and may start to engage more frequently in high-risk behaviors. This concept, known as behavioral disinhibition, can manifest itself in an increase of behaviors such as irregular or no condom use or increasing the number of sexual partners or risky sexual acts. Several PrEP trial studies have attempted to examine this possibility, including the iPrEx trial which reported no significant increase in sexual risk behaviors among its participants (Liu

et al. 2013). These investigations continue, and adequate and ongoing counseling regarding risk reduction will be necessary among persons taking PrEP (Cohen et al. 2012).

PrEP among other high-risk populations: Due to the conflicting results of PrEP use among high-risk women and the ongoing research among injection drug users, PrEP efficacy and use among these populations are relatively unknown. Additionally, the potential use of PrEP among serodiscordant couples is also being examined in more depth, and studies focusing on particular subpopulations such as young MSM are underway. As all of these populations are considered at high risk for HIV, this research is needed to increase understanding of the potential for PrEP as an effective prevention strategy among each of these populations.

Cost-effectiveness: To date several cost-effectiveness studies have been conducted to model the potential impact of PrEP use in different regions of the world under a variety of circumstances and assumptions. In general, these studies have concluded that PrEP use would be cost-effective and has the potential to significantly reduce HIV incidence among high-risk individuals (Paltiel et al. 2009; Walensky et al. 2012).

Ethical issues: Ethical issues regarding PrEP have also been raised, particularly in areas where there are limited resources to treat persons who are already HIV positive (Leibowitz et al. 2011). For example, in resource-limited countries, there are millions of HIV-positive persons who either cannot access and/or afford antiretroviral treatment. Similarly, in some areas of the United States, there are waiting lists for persons who need antiretroviral medications. Thus, proposals to conduct widespread implementation of PrEP in areas where medication access is already limited for HIV-positive persons present an ethical dilemma regarding the equitable allocation of antiretrovirals for treatment versus prevention.

Implementation Challenges in the United States

Several challenges have arisen as broader PrEP implementation begins. Studies are underway to

assess how best to implement PrEP in the United States in the form of demonstration projects and extensions of ongoing clinical trials. One challenge is that PrEP differs from traditional HIV prevention strategies as it requires access to a clinical setting for prescription receipt, regular HIV testing, and clinical monitoring. In addition, HIV-negative persons may not routinely seek care from infectious disease clinicians who are most familiar with prescribing antiretroviral medications. Thus, providers who are not familiar with or who do not routinely provide antiretrovirals, such as most primary care providers, will need to be trained on how to manage or appropriately refer people who are interested in taking PrEP. Lack of insurance coverage may also present an obstacle as those persons who may be at highest risk for HIV may be more likely to be un- or underinsured. Furthermore, those persons at highest risk for HIV may have limited access to care as well as awareness of PrEP as an HIV prevention method (Underhill et al. 2010).

Conclusion

Despite these challenges, preexposure prophylaxis for HIV prevention presents a novel approach to reducing HIV incidence and adds another tool to the HIV prevention toolkit. Because it is a relatively new prevention approach, there remain many unknowns regarding its prolonged use, potential side effects both biologically and behaviorally, the best methods for optimal implementation, and its cost-effectiveness in real-life settings. Despite these gaps, with additional research, careful monitoring, and education at both the patient and provider level, PrEP has the potential to play an important role in the overall strategy to reduce HIV globally.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Behavioral Aspects of HIV Mother-to-Child Transmission](#)

- ▶ Behavioral Aspects of HIV Treatment as Prevention
- ▶ Behavioral Science Highlights of Evidence and Research
- ▶ Circumcision and AIDS
- ▶ Combination Approaches to HIV Prevention
- ▶ Female, Male and Transgender Sex Workers, Epidemiology of HIV/AIDS
- ▶ Harm Reduction for Injection Drug Users
- ▶ HIV Counseling and Testing, Prevention of HIV
- ▶ HIV Prevention and Women
- ▶ HIV Prevention for MSM
- ▶ HIV Prevention for Serodiscordant Couples
- ▶ HIV Prevention for Stimulant Using Men Who have Sex with Men
- ▶ HIV Testing and Counseling
- ▶ HIV Transmission in Female Commercial Sex Workers and Host Protective Factors
- ▶ HIV-1 Transmission Blocking Microbicides
- ▶ Male Condoms
- ▶ Positive Health, Dignity, and Prevention (PHDP)
- ▶ Preexposure Prophylaxis (PrEP)
- ▶ Preventing HIV-1 Transmission through Vaccine-Induced Immune Responses
- ▶ Prevention Clinical Trials: Highlights of Evidence and Research
- ▶ Prevention for People Living with HIV

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Pregnant Women: Care and Treatment

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Epidemiology of HIV/AIDS in Women

HIV/AIDS continues to be a major global cause of morbidity and mortality in women. In 2011, more than 50% of the 34 million people infected with HIV were women (UNAIDS/WHO 2012). HIV/AIDS is the major cause of death in women of childbearing age (ages 15–49) in the developing world. The primary mode of HIV transmission in women in the United States and globally is heterosexual contact. In 2013, there were 24.7 million people living with HIV/AIDS in sub-Saharan Africa, the global region that accounts for approximately 70% of HIV/AIDS cases, and 58% of those were women. In the United States, women accounted for approximately 24% of new diagnoses of AIDS in 2012 (Centers for Disease Control 2014), and there were over 270,000 women among the 1.1 million

people living with HIV infection and AIDS in the United States as of 2013. With the increase in access to potent antiretroviral therapy (ART) in developing countries, the rates of perinatal infection have decreased from over 570,000 in 2003 to an estimated 260,000 children in 2012.

Preconception Counseling and Care for HIV-Infected Women of Childbearing Age

Surveys performed in the United States suggest that HIV-infected women have similar desires to have children as HIV-uninfected women. Pre-conceptual counseling is imperative and should include a discussion about the woman's HIV status and optimal treatment strategies for her health and strategies to decrease perinatal transmission. Discordant couples may present a particular challenge. If the potential father is HIV negative, the safest approach to conception is intravaginal or intrauterine insemination. If the potential father is HIV positive and the woman is HIV negative, the use of assisted reproductive technologies including sperm washing and in vitro fertilization techniques appears to have a low risk of horizontal transmission to the woman or vertical transmission to the developing fetus, but of course these techniques are not completely risk-free. Treatment with ART for prevention of transmission and pre-exposure prophylaxis are also strategies to permit conception without also transmitting HIV.

The goals of preconception care for women living with HIV are to prevent unintended pregnancy, optimize maternal health before pregnancy, improve maternal and fetal outcomes in pregnancy, prevent perinatal transmission, and prevent possible HIV transmission to an HIV-uninfected partner during attempts at conception (Cohn and Clark 2015). In addition to addressing fertility and reproduction, women should be counseled on safe sexual practices to prevent HIV transmission to sexual partners and to prevent sexually transmitted diseases. Because of concerns of teratogenicity of efavirenz, non-pregnant women with childbearing potential should undergo pregnancy testing before starting

efavirenz and should be counseled on risks to the developing fetus if she were to become pregnant while taking efavirenz (see below: "[Use of Antiretroviral Therapy in Pregnancy](#)").

Large studies in the developed world have failed to show worse HIV outcomes or increased rates of progression to AIDS in pregnant women. Some studies in developing countries have indicated that pregnant women with HIV progress to AIDS at a faster rate compared to nonpregnant women, but these studies could be confounded by such factors as poor nutrition, presence of other infectious diseases, and more advanced HIV disease at the time of diagnosis.

HIV infection appears to increase the risk of pregnancy-associated adverse outcomes such as preterm birth, stillbirths, small for gestational age, and neonatal death. Data on whether risk of these adverse events is higher in women on ART are conflicting (see below: "[Use of Antiretroviral Therapy in Pregnancy](#)"). The pathogenesis of premature birth is unknown for all women, and the etiology of the potential increased risk among HIV-infected women is not clear.

Perinatal Transmission

Antiretroviral medications have had a major impact on perinatal transmission of HIV. Prior to the use of antiretroviral prevention, the frequency of perinatal transmission was estimated between 15% and 45%. Effective ART for the prevention of mother to child transmission (PMTCT) reduces HIV perinatal transmission to <2% in the absence of breastfeeding and to <5% among breastfeeding infants (Ciaranello et al. 2013; WHO 2012). However, only 57% of HIV-infected pregnant women in developing countries received preventive antiretroviral medications in 2011. ART decreases maternal viral load in the blood and in genital secretions, thereby decreasing perinatal transmission. However, ART has also been shown to reduce the risk of transmission in women whose HIV RNA levels are less than 1000 copies/mL. An additional mechanism of transmission prevention may be preexposure infant prophylaxis provided by antiretroviral therapies that

cross the placenta, especially during labor when the infant is exposed to high levels of HIV in the mother's genital tract secretions.

The most important risk factor for perinatal transmission is maternal plasma or breast milk viral load. There is no "safe" level of HIV viremia as women with undetectable viral load have transmitted HIV to their infants (Mayaux et al. 1997; Sperling et al. 1996). Other associated maternal factors include CD4+ cell count, anemia, advanced WHO clinical disease stage, reproductive tract infections, mastitis, and acute maternal HIV infection during pregnancy or breastfeeding. HIV can be transmitted from mother to child during intrauterine gestation, at delivery, or in the postpartum period through breastfeeding. Infants infected in late pregnancy or at birth have a slower progression to AIDS and prolonged survival compared to infants infected in utero. Most cases of perinatal transmission are thought to occur near or during delivery. Studies performed prior to the availability of combination ART demonstrated that elective cesarean section before the onset of labor and the rupture of membranes decreased the risk for perinatal transmission of HIV-1 by 50% (The International Perinatal HIV Group 1999). The degree of protection afforded by elective cesarean in the era of combination ART is unknown. HIV-positive women may be at increased risk for postoperative complications such as infection. Current US guidelines recommend discussion of elective cesarean section at 38 weeks gestation with women whose HIV viral load is greater than 1000 copies/mL (Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission 2014). Conversely, the WHO does not recommend cesarean section in resource-limited settings specifically for HIV infection; rather it is recommended for obstetric and other medical indications (WHO guidelines 2013).

Care of Pregnant HIV-Infected Women

Antenatal care of the HIV-infected pregnant woman requires coordination between multiple providers including her primary care provider,

obstetrician, and HIV specialty care providers. An initial evaluation should include thorough examination of her HIV history, including past and current CD4 counts and HIV viral loads, and determination of appropriate ART treatment, whether she is ART-naïve or whether her current regimen needs to be altered. The history and physical should also assess risk factors for increased perinatal transmission, including the use of tobacco, alcohol, or drugs, a history of sexually transmitted diseases, and whether she engages in unprotected sexual intercourse. The CD4 count should be assessed at least every trimester in most pregnant women. Consideration can be given to check the CD4 count every 6 months in women who have been virally suppressed for two or three years and are clinically stable. Because of hemodilution, the CD4 percentage may be a more stable parameter to follow during pregnancy compared to the absolute CD4 count. Viral loads should be monitored closely (at the initial visit, 2–6 weeks after starting/changing ART, monthly until undetectable, and then every 3 months) because of the importance of decreasing the pregnant woman's viral load as soon as possible to decrease the risk of perinatal transmission. Viral load should also be measured at 34–36 weeks gestation to inform decisions about the mode of delivery. Gestational age should also be assessed via ultrasound in the first trimester or as soon as the woman presents for care because if a cesarean section is deemed appropriate, this is usually scheduled at 38 weeks gestation. Pregnant women receiving NRTI therapy should also have their hepatic enzymes and electrolytes monitored because of an increased risk of lactic acidosis with these therapies. HIV-infected women on ART should receive a standard glucose tolerance test at 24–28 weeks although some experts would recommend earlier screening in women taking protease inhibitors prior to becoming pregnant as they may be at increased risk of glucose intolerance. The risk of transmission during amniocentesis or during other invasive procedures such as chorionic villus sampling in women on effective ART resulting in viral suppression does not appear to be increased.

The ultimate decision for initiation of ART is the responsibility of the woman. A detailed discussion of the risks and benefits of ART during pregnancy should be undertaken with a strong emphasis placed on the health benefits of HIV treatment for both the mother and the infant. Adherence to ART should also be strongly emphasized and social support resources should be employed if possible to increase chances of successful ART in promoting her health and reducing perinatal transmission.

Use of Antiretroviral Therapy in Pregnancy

The goal of ART use in pregnancy is to improve maternal health, reduce transmission of HIV to the newborn, prevent horizontal HIV transmission to sexual partners, and avoid potential maternal or fetal toxicity associated with ART. The first major breakthrough in PMTCT was the Pediatric AIDS Clinical Trial Group Protocol 076 in which pregnant women were randomized to receive pre- and postpartum zidovudine (ZDV) monotherapy, including an intravenous bolus during labor vs. placebo during pregnancy and intrapartum. Newborns were given ZDV for the first 6 weeks of life. The trial was completed in 1994, and the rate of perinatal transmission was 8.3% in the ZDV group compared to 25.5% in the placebo group, a reduction in risk of perinatal transmission of almost 70% (Connor et al. 1994). The three-part regimen proved logistically difficult to carry out in resource-limited settings, and later trials evaluated shorter regimens, including single-dose nevirapine given to the mother at the onset of labor and to the newborn within 72 h of birth. Single-dose nevirapine reduced perinatal transmission by 50–60% (Guay et al. 1999). Subsequent clinical trials and observational studies have demonstrated that combination ART given to the mother both pre- and postpartum was associated with further declines in perinatal transmission to less than 2%. Antenatal combination drug regimens are now considered the standard of care for preventing perinatal transmission, and the goal of global

health agencies is to supply suppressive ART for all pregnant and breastfeeding women.

PMTCT has evolved significantly since the initial use of zidovudine monotherapy in the mother and infant. The 2010 WHO PMTCT ART guidelines stratified the need for lifelong HIV treatment versus the need for treatment only during pregnancy and the breastfeeding period based on the mother's CD4 count (WHO 2010). The two available options, Option A and B, also differed in the recommended ART regimen for healthier women – monotherapy with zidovudine versus triple drug ART, respectively. Compliance with Option A was difficult for many programs, because of logistical difficulties in providing different ART during different periods of pregnancy and the need for a timely CD4 count result in order to stratify women to the correct treatment arm. The updated WHO guidelines published in 2013 represented a significant paradigm shift and reflected the emphasis on the global community's commitment to the elimination of new HIV infections in children by 2015 (UNAIDS 2011). Option A was no longer recommended, and all pregnant women were to start triple ART during pregnancy. Further, it was recommended that all pregnant and breastfeeding women with HIV continue lifelong ART (Option B+). Countries could choose to adopt a policy wherein consideration may be given to stopping ART after the breastfeeding period if the mothers are not eligible for ART for their own health (Option B). The selection of Option B or Option B+ is conditional, based on the epidemic setting and the country program. It is estimated that with the new treatment eligibility threshold of CD4+ cell count ≤ 500 cells/mm³, approximately 60% of HIV-infected pregnant women will meet treatment eligibility criteria for their own health. Options B and B+ are also more efficient because the same ART regimen can be used for the duration of pregnancy and into the postpartum period. Option B+ has several additional advantages including (1) no need for CD4 count testing prior to initiation of treatment; (2) extended protection for future pregnancies, starting at conception; (3) potential for prevention of transmission to an HIV-negative partner; (4) benefit to the woman's health and avoidance

Pregnant Women: Care and Treatment, Table 1 Summary of first-line antiretroviral regimens recommended by the World Health Organization for adults, including pregnant and breastfeeding women

Preferred first-line regimen	Alternative first-line regimens
Tenofovir (TDF) + lamivudine (3TC) or emtricitabine (FTC) + efavirenz (EFV)	1. Zidovudine (ZDV) + 3TC + EFV 2. ZDV + 3TC + nevirapine (NVP) 3. TDF + 3TC or FTC + NVP

of risk of stopping/starting ART; and (5) extending the public health message in communities that once ART is started, it is taken for life. While the cost of lifelong ART continues to be an issue in low-resource settings, the average cost of ART is continuing to decrease, and a study from the WHO in 2014 found that from a cost perspective, switching from Option B to Option B+ was feasible (O’Brien et al. 2014). Similarly, a cost-effectiveness study performed in Malawi, one of the first countries to adopt Option B+, found that although Option B+ would cost more initially, it would save societal resources in the long term (Fasawe et al. 2013).

Transplacental drug passage is an important mechanism for infant preexposure prophylaxis and as such NRTIs with high placental transfer should be included (i.e., zidovudine, lamivudine, emtricitabine, tenofovir, or abacavir). The preferred first-line regimen recommended by the WHO for HIV-positive pregnant or breastfeeding women is tenofovir (TDF) plus lamivudine (3TC) or TDF plus emtricitabine (FTC) in combination with efavirenz (EFV) (Table 1). Alternative regimens are also recommended (Table 1). The advantage of TDF/FTC/EFV as a first-line regimen is that it is available as a fixed-dose combination that allows once-daily dosing, has a more favorable toxicity profile, and has an enhanced activity against hepatitis B.

Guidelines for the use of ART in HIV-infected pregnant women differ in resource-rich settings, but there is significant overlap with the WHO guidelines. Guidance on care of HIV-infected pregnant women in the United States is provided by the Department of Health and Human Services

Pregnant Women: Care and Treatment, Table 2 Initial combination antiretroviral therapy regimens recommended by the Department of Health and Human Services (USA) for antiretroviral-naïve pregnant women

Preferred two-NRTI backbone	ABC/3TC, TDF/FTC or 3TC, ZDV/3TC
Preferred PI regimen	ATV/r ^a + two-NRTI backbone LPV/r ^a + two-NRTI backbone
Preferred NNRTI regimen	EFV + two-NRTI backbone
Alternative PI regimen	DRV/r + two-NRTI backbone SQV/r + two-NRTI backbone
Alternative NNRTI regimen	NVP + two-NRTI backbone
Integrase inhibitor regimen	RAL + two-NRTI backbone

NRTI nucleoside reverse transcriptase inhibitor, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *ABC* abacavir, *3TC* lamivudine, *TDF* tenofovir, *FTC* emtricitabine, *ZDV* zidovudine, *ATV/r* ritonavir-boosted atazanavir, *LPV/r* ritonavir-boosted lopinavir, *EFV* efavirenz, *DRV/r* ritonavir-boosted darunavir, *SQV/r* ritonavir-boosted saquinavir, *NVP* nevirapine, *RAL* raltegravir

^aAll ritonavir-boosted PI regimens are low dose

(DHHS Guidelines 2014). As in the WHO guidelines, a combined approach of antepartum and intrapartum ART administered to the mother and postpartum infant prophylaxis is recommended to reduce perinatal transmission of HIV. Similarly, a regimen that is the same in HIV-infected pregnant women and nonpregnant individuals is ideal. In resource-rich settings, ARV treatment resistance testing should be undertaken in all women prior to initiating an ART regimen. Preferred NRTI regimens in ART-naïve pregnant women include TDF plus either 3TC or FTC, abacavir (ABC) plus 3TC, or ZDV plus 3TC (Table 2). ZDV may be contraindicated if a woman has significant anemia. In addition to the dual NRTI backbone, either a low-dose ritonavir-boosted protease inhibitor (PI) (lopinavir or atazanavir) or an NNRTI (i.e., EFV) is recommended. Data are limited on newer-generation PIs such as darunavir or tipranavir or entry inhibitors enfuvirtide or maraviroc. Experience with the integrase inhibitor, raltegravir, is increasing, especially as an

addition to an existing regimen to rapidly decrease viral load in late pregnancy.

Physiologic changes during pregnancy such as changes in body water and fat and changes in renal and liver blood flow can affect pharmacokinetics of antiretroviral medications. In general, the pharmacokinetics of NRTIs and NNRTIs are largely unaffected during pregnancy. Protease inhibitor pharmacokinetics is more variable during pregnancy, and levels of several PIs such as lopinavir and ritonavir are reduced in the second and third trimesters.

While concern for genotypic resistance exists when ARVs are used only during pregnancy and then discontinued, treatment failure is rare in high-income settings and also is not common in Africa when reinitiating combination ART after prophylactic use of ARVs during pregnancy. While the DHHS guidelines recommend genotyping for drug resistance prior to the initiation of ART, genotyping is often not accurate after 4 weeks have passed between testing and discontinuation of the prior regimen because the virus will often revert to wild type. Therefore, when selecting a new regimen, all information about a woman's previous HIV history, including previous regimens and adherence and tolerability, should be obtained. If a woman presents late in pregnancy, ART should be initiated without waiting for genotype results. Genotyping is also recommended in pregnant women that do not achieve optimal viral suppression. Adherence to an ART regimen is the best way to prevent development of HIV drug resistance. Another strategy is to continue the NRTI components of an NNRTI-based regimen for 7–30 days after discontinuation of the NNRTI to minimize the risk of resistance given the prolonged half-life of NNRTI medications.

Any antiretroviral drug use during pregnancy is not without risk to the fetus, but the benefit of preventing HIV transmission and the benefit of ART to the mother's health are generally felt to outweigh the risk. Early data about neural tube defects among primates exposed to EFV led to concern about using EFV in the first trimester of pregnancy or in women with childbearing potential. More recent data are reassuring; an updated

systematic review and meta-analysis reported no increase in overall birth defects in infants born to mothers receiving EFV in the first trimester (Ford et al. 2011). While EFV remains FDA pregnancy category D, both the WHO and DHHS guideline panels felt comfortable recommending EFV as part of first-line therapy in HIV-infected pregnant women given the available data.

Efavirenz is preferred over nevirapine (NVP) because of the potential for hepatotoxicity in women with CD4 counts >250 cells/mm³. While there are continued concerns about an even higher risk of severe hepatic and skin reactions in pregnant women using NVP at higher CD4 counts, a systematic review suggested that the frequency of adverse events is elevated but no higher than that observed in the general HIV-infected adult population (Ford et al. 2013).

A previous systematic review did not report an increased prevalence of overall birth defects, and a number of studies have showed no difference in fetal growth between infants exposed to TDF and those not exposed. TDF also has limited penetration into breast milk which limits potential toxicity to breastfeeding infants.

Some studies have suggested that women taking combination ART, with or without protease inhibitors, have an increased risk of adverse pregnancy outcomes such as preterm delivery, stillbirth, or low birth weight compared to pregnant women with HIV infection on no medications or on mono- or dual-NRTI prophylaxis. However, data are conflicting as several variables can confound these observational studies, such as overall maternal health and severity of HIV disease. While clinicians should be aware of the possibility of an association between combination ART and preterm delivery, the benefits of combined ART to maternal health and to decreasing perinatal transmission are clear, and these medications should not be withheld out of concern for preterm birth.

If a woman is already on ART at the time that she becomes pregnant, treatment should be continued for the duration of the pregnancy. Current evidence does not suggest that teratogenic effects are caused by most antiretroviral medications. Certain drugs are of greater concern than others, specifically EFV, which should be avoided in the

first trimester due to nonhuman animal data of concern. However, an HIV-infected woman should continue EFV if it is already part of her existing regimen. This is because the greatest risk of neural tube defects is in the first 5–6 weeks gestation and pregnancy is rarely recognized before 4–6 weeks. Stopping a regimen abruptly is associated with HIV viral rebound which can have serious consequences on HIV transmission and progression of disease.

Intrapartum Management of HIV-Infected Pregnant Women

Intravenous ZDV should be administered intrapartum for pregnant women with HIV RNA greater than or equal to 400 copies/mL (WHO) or 1000 copies/mL (DHHS), poor adherence, or unknown HIV RNA levels near delivery, regardless of mode of delivery or antepartum ART regimen. Intrapartum prophylaxis with ZDV in women who are virally suppressed has not been associated with reduced risk of perinatal transmission. Women that are already receiving ZDV as part of their antepartum ART regimen should be administered IV ZDV while the other antiretrovirals can be administered orally.

It is recommended in resource-rich countries that women who have been on antepartum ART but have not achieved HIV viral suppression (i.e., HIV RNA >1000 copies/mL) should undergo scheduled cesarean section at 38 weeks gestation to minimize the risk of transmission of HIV to the neonate. It is not clear whether cesarean delivery after rupture of membranes or onset of labor provides benefit in preventing perinatal transmission. Management of women originally scheduled for cesarean delivery who present with ruptured membranes or in labor must be individualized at the time of presentation.

In women that experience postpartum hemorrhage due to uterine atony, oral or parenteral methylergonovine or another ergot alkaloid is often administered as a first-line agent. These medications should not be coadministered with protease inhibitors or EFV as the latter agents are CYP3A4 inhibitors. Concomitant use of these

Pregnant Women: Care and Treatment, Table 3 WHO guidelines for infant postexposure prophylaxis

Breastfeeding infants	Infant age	Nevirapine
	Birth to 6 weeks	10 mg once daily
	Birth weight 2000–2499 g	15 mg once daily
	Birth weight ≥ 2500 g	
	>6 weeks to 6 months	20 mg once daily
	>6 months to 9 months	30 mg once daily
	>9 months until breastfeeding ends	40 mg once daily
	Replacement feeding	
	Infant age: birth to 6 weeks	Zidovudine
	Birth weight 2000–2499 g	10 mg twice daily
	Birth weight ≥ 2500 g	15 mg twice daily

medications leads to increased serum levels of ergotamines and subsequent severe vasoconstriction. If there is no alternative therapy for postpartum hemorrhage, ergotamines should be used at the lowest dose and the shortest duration possible.

The goal of prophylaxis in breastfeeding infants is prevention of transmission from HIV exposure during labor and during the early breastfeeding period. The WHO recommends 6 weeks of NVP for infants born to HIV-infected mothers for breastfeeding infants and 4–6 weeks of NVP or ZDV for formula-fed infants (Table 3; see below: “Breastfeeding”). A longer duration of infant prophylaxis may be indicated in breastfeeding infants whose mothers did not receive antiretroviral agents prior to labor. The goal of prophylaxis in formula-fed infants is prevention of transmission from HIV exposure during labor. The DHHS guidelines differ from the WHO guidelines in that breastfeeding is not recommended and 6 weeks of ZDV is recommended for all HIV-exposed neonates; a 4-week regimen can be considered if the mother has received combination ART during pregnancy with consistent viral suppression; and there are no concerns about adherence (Table 4). If mothers

Pregnant Women: Care and Treatment, Table 4 Recommended neonatal dosing for prevention of perinatal transmission of HIV from the Department of Health and Human Services (USA)

All HIV-exposed infants (initiated as soon as possible after delivery, preferably within 6–12 h)		
Zidovudine (ZDV)	Dosing	Duration
ZDV	≥35 weeks' gestation at birth: 4 mg/kg/dose PO twice daily. If unable to tolerate PO agents, 3 mg/kg/dose IV twice daily	Birth through 4–6 weeks ^a
ZDV	≥30 to <35 weeks' gestation at birth: 2 mg/kg/dose PO twice daily, advanced to 3 mg/kg/dose PO (or 2.3 mg/kg/dose IV) twice daily at age 15 days	Birth through 6 weeks
ZDV	<30 weeks' gestation at birth: 2 mg/kg PO or 1.5 mg/kg/dose IV twice daily, advanced to 3 mg/kg/dose PO (or 2.3 mg/kg/dose IV) twice daily after age 4 weeks	Birth through 6 weeks
Additional antiretroviral prophylaxis agents for HIV-exposed infants of women who received no antepartum antiretroviral prophylaxis (initiated as soon after delivery as possible)		
In addition to ZDV as shown above, administer nevirapine (NVP)	Birth weight 1.5–2 kg: 8 mg/dose PO Birth weight >2 kg: 12 mg/dose PO	3 doses in the first week of life 1st dose within 48 h of birth (birth to 48 h) 2nd dose 48 h after 1st 3rd dose 96 h after 2nd

^aA 6-week course of neonatal zidovudine is generally recommended. A 4-week neonatal zidovudine chemoprophylaxis regimen may be considered when the mother has received standard ART during pregnancy with consistent viral suppression and there are no concerns related to maternal adherence

have not received combination ART, exposed neonates should receive 6 weeks of ZDV combined with three doses of nevirapine in the first week of life. For infants born to mothers with unknown HIV status, prophylaxis should be initiated immediately and discontinued if confirmatory testing is negative.

Care of the HIV-Positive Woman in the Postpartum Period

The immediate postpartum period can pose unique challenges for adherence to ART. For women that were diagnosed when they presented in labor, comprehensive follow-up and counseling are required. The decision to continue or stop ART should take into account current recommendations for initiation of ART, CD4 count and HIV viral load, adherence issues, and patient preference. As noted above, the WHO has recommended Option B+, where a woman initiates combination ART during pregnancy and continues lifelong treatment.

Contraceptive counseling is also a critical aspect of postpartum care, and this time represents an opportunity to optimize a woman's health, including cervical cancer screening, mental health evaluation and substance abuse evaluation, and treatment as needed.

Breastfeeding

HIV-1 can be detected in the cellular and acellular compartments of breast milk. There have been several reports of breastfed infants becoming infected with HIV in the postnatal period. A meta-analysis of studies published before 1992 suggested an attributable risk for transmission through breast milk by women who were infected before pregnancy of 14% and by postnatally infected women of 26% (Van de Perre et al. 1991; Dunn et al. 1992). Not surprisingly, the higher the maternal serum viral load and the lower the CD4 count, the higher the breast milk viral load. The risk of transmission via breast

milk is highest early after delivery as the HIV-1 viral load has been observed to be higher in colostrum/early milk compared to milk produced later. The Centers for Disease Control and Prevention recommends that HIV-positive women living in the United States should not breastfeed because safe alternative replacement feeding is available in the developing world. However, safe and sanitary options for alternative feeding are often not available in low-income nations, and the decision to withhold breastfeeding because of concern for HIV transmission to the newborn can lead to increased rates of infant mortality from diarrhea, dehydration, and malnutrition. In fact, exclusive breastfeeding for 6 months is associated with lower rates of HIV transmission compared to mixed feeding (combination of breastfeeding with other food or liquid such as water, non-human milk, or formula). This is because the combination of breast milk with other food and liquid can disrupt the infant's fragile gut mucosa and lead to increased permeability to HIV.

The current goals as reflected in the latest WHO and UNICEF guidelines are to increase HIV-free survival of the newborn (WHO/UNICEF 2010). Several studies have demonstrated that providing ART to the infant and/or the mother is effective in preventing perinatal transmission of HIV. Based on this evidence, national programs have begun recommending breastfeeding for 12 months and exclusively for 6 months. For programmatic reasons, the WHO has focused on the use of maternal ART to prevent transmission during breastfeeding. Pregnant women should receive ART antepartum and continue for the duration of breastfeeding. Infant prophylaxis is determined by method of feeding and the mother's use of ART. All infants that are breastfed should be given 6 weeks of nevirapine prophylaxis postpartum if their mothers were initiated on ART prior to or during pregnancy. Infants whose mothers started ART during labor or immediately postpartum should receive a minimum of 6 weeks of NVP and 12 weeks is preferable. Infants who are born to mothers who interrupt ART during breastfeeding should continue NVP for 6 weeks after restarting ART or for 1 week after cessation of breastfeeding. ART should be provided to the mother and/or the

infant for at least 1 week after the cessation of breastfeeding. Because of the clear benefits of breastfeeding, mothers in developing countries should still be encouraged to breastfeed even if ART is not available unless environmental conditions are safe for alternative feeding, such as with formula. An unrestricted duration of breastfeeding is not endorsed by the WHO in the latest 2013 guidelines, even when lifelong ART is being employed for mothers, because of concerns of nonadherence to ART throughout the duration of breastfeeding, therefore increasing the risk of HIV transmission to infants. After 12 months, the benefits of breastfeeding in preventing diarrhea, malnutrition, and pneumonia decrease, at which point the risk of breastfeeding by HIV-positive mothers may outweigh the benefits of breastfeeding.

In conclusion, all pregnant women should be screened for HIV infection, and abundant, safe interventions can help reduce her disease progression and can prevent transmission to her infant. Challenges include community education, health systems strengthening, overcoming stigma, engaged men and families, and offering universal ART to women and children who need it.

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Presentation and Pathogenesis of B-Cell Lymphoid Cancers Associated with HIV Infection

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Definition

Lymphoid neoplastic manifestations of HIV infection include a spectrum of diseases each with specific clinical, epidemiologic, and biological features. This chapter reviews the important lymphoid tumors occurring in HIV infection with an emphasis on the important distinctions relevant to presentation, diagnosis, and clinical management of the various entities.

Introduction and Overview

During the late 1970s and early 1980s, marked changes in the typical occurrence and clinical

behavior of aggressive B-cell lymphomas were among the initial manifestation of the new epidemic disease that ultimately became known as the acquired immunodeficiency syndrome, AIDS. The original Centers for Disease Control and Prevention case definition expanding the definition in 1982 of AIDS to include lymphoma specified that a patient with HIV and an aggressive B-cell lymphoma would carry an AIDS diagnosis. Several years later, this definition was expanded to include intermediate-grade B-cell lymphomas. The study and treatment of AIDS-related lymphoma (ARL) consequently suffered from a poorly structured case definition terminology that failed to classify distinct lymphoma types. Instead, ARL was almost thought of as a single disease. ARL was designated as AIDS indicator conditions, meaning that ARL conferred an AIDS diagnosis when it was the initial manifestation of the syndrome. As the case definition of AIDS evolved over time, the percentage of lymphoma indicator cases shifted downward somewhat as other indicator conditions were more likely to occur before ARL. For example, if another antecedent AIDS indicator condition, such as CD4 cell count falling below $200/\text{mm}^3$, had already been documented, ARL would likely go unreported. The total burden of lymphoma cases in those who already have an AIDS diagnoses is estimated to be up to 25%. About 3% of those with HIV come by their AIDS diagnosis due to ARL.

Early in the AIDS epidemic, prior to the discovery of HIV, immunologic derangement was recognized as the common pathophysiologic event leading to both infectious and neoplastic disease complications. In the ensuing thirty-plus years, the epidemiology of the common AIDS complications has evolved dynamically, owing largely to the availability of effective combination antiretroviral therapy (cART). At the same time, the approaches to lymphoid tumor biology and lymphoma classification have been revolutionized as the science has advanced. The epidemiologic patterns of ARL in the pre-cART and cART eras and the improved lymphoid tumor classification schema provide important clues to ARL presentation and pathology. This chapter will review the

highlights of HIV-related lymphoid tumor epidemiology and pathophysiology that have been described in the context of the still evolving AIDS epidemic. Although the formal AIDS case definition does not include all the lymphoma types discussed in this chapter, the broader range of lymphoid tumors occurring in those with HIV infection will be presented under the designation of AIDS-related lymphomas (ARLs).

A critical concept to appreciate is that ARL is not a clinically or biologically specific term. There are specific ARL entities, each of which requires clinical management approaches specific to the ARL type. Expert hematopathology consultation is thus required to ensure accurate diagnosis and to thus plan optimal therapy. Owing to substantially improved prospects for near-normal life span with effective management of HIV infection, curative treatment for ARL should be considered in most cases.

Mounting evidence suggests the immune status plays a substantial role in the type of ARL that develops. This evidence is comprised of studies of the epidemiology, pathobiology, and clinical studies in ARL. As immunodepletion advances, the risk of ARL increases. Moreover, the type of ARL likely to manifest is related to the degree and duration of HIV replication and immunosuppression. Highlighting this is the marked shift in the occurrence of certain lymphoid tumor types that has been observed in populations where cART is widely used. The ARL epidemiology in such populations diverges markedly from that of pre-cART historic controls and from those populations without widespread access to cART. Cataloging the specific ARL entities thus becomes important not only to individual patient management but also for understanding these tumors in AIDS. Table 1 summarizes the clinicopathological HIV-lymphoma types according to the WHO classification of lymphoid tumors and also incorporates other ARL-specific data (Little et al. 2003; Swerdlow et al. 2008).

ARLs are nearly all B-cell lymphomas. B-cell neoplasms to some extent resemble normal B cells at a particular stage of differentiation. This similarity between the neoplasm and the normal or postulated normal counterpart forms in large part

Presentation and Pathogenesis of B-Cell Lymphoid Cancers Associated with HIV Infection, Table 1 Human immunodeficiency virus (HIV)-associated lymphomas: viral, genetic, and clinical feature

Histological subtype	Percent association										CD4 cells	Prospects for chemosensitivity	Prognosis in cART era
	EBV	KSHV	BCL-2	BCL-6	TP53	MYC	CD4 cells	Prospects for chemosensitivity	Prognosis in cART era				
Burkitt	<50	0	0	100	40–60	100	Usually relatively well preserved	Excellent	Excellent	Excellent			
DLBCL-GC	<30	0	0	>75	Rare	0–50	Variable but rarely depleted	Favorable	Excellent				
DLBCL-ABC	>50	Rare	30	0	0	0–20	Usually low	Intermediate	Intermediate				
PCNSL	100	0	90	<50	0	0	<50	Good (limited data)	Intermediate/good if CD4 recovery can be achieved				
Hodgkin lymphoma	>70	0					Generally preserved	Good	Intermediate (relapse common)				
Primary effusion lymphoma ^a	>80	100	0	0	0	0	Variable	Poor	Poor				
Plasmablastic lymphoma ^a	50–90	0	20	<10		40	Variable	Intermediate	Poor				
Large B-cell lymphoma arising in HHV-8 multicentric Castleman disease ^a	0	100					Variable	Intermediate	Poor				

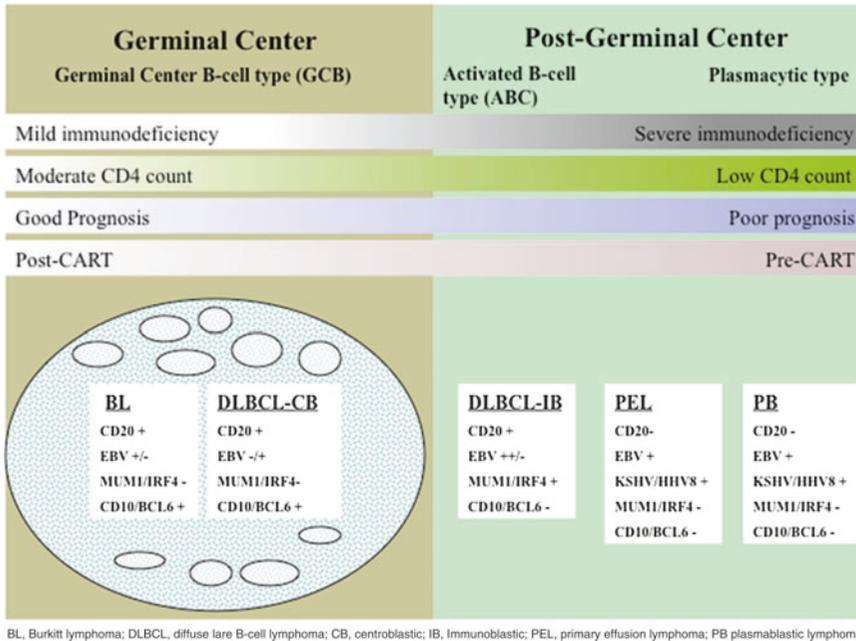
^aOccurs more specifically in HIV

the classification and nomenclature of lymphoid tumors. An advantage to this organizational system is that it aids in correlating the tumor cell morphology, its immunophenotype, and provides insights into clinical behavior by relating tumors back to the cells from which they seem to derive. It is beyond the scope of this chapter to review normal B-cell development, but there are several important aspects in relation to ARL that should be mentioned.

Normal B cells begin from common lymphoid progenitor cells in the bone marrow and develop into mature B cells expressing surface immunoglobulins. During this process, immunoglobulin heavy chain (IgH) and light chain (IgL) genes are formed through a process where the V, D, and J gene segments are recombined to create a vast immunologic repertoire. Until the B cells encounter an antigen with a specific fit for their surface immunoglobulin receptor, they are considered mature naïve B cells. When they encounter antigen, the B cell is activated to proliferate, and this begins a process wherein the cells differentiate into centroblasts or plasma cells. This is accomplished via interactions within peripheral lymphoid tissues. A process of immunoglobulin somatic hypermutation (SHM) of the immunoglobulin heavy chain variable region (IGHV) genes occurs, and class-switch recombination is the end result. In HIV disease, the lymph node's normal architecture, including a derangement in the proportion of follicular T-helper cells involved in regulating B-cell responses, is disrupted (Lindqvist et al. 2012). The degree of lymph node derangement is correlated with the HIV disease status and may influence the processes of normal B-cell differentiation. It may also influence both the risk and type of B-cell neoplastic disease that occurs. Consistent with this hypothesis is the differential risk of the specific lymphoma types that occur in association with the duration and magnitude of HIV replication and the degree of T-cell depletion in HIV disease (Little et al. 2003; Shiels and Engels 2012).

Aggressive B-cell neoplasms, such as those typically found in association with AIDS, broadly correspond to later stages of B-lymphocyte development (beyond the stem cell and lymphoblast

stages). If naïve to antigen, such a cell will undergo a blast transformation when it is exposed to antigen. These are large proliferating cells that give rise to progeny cells that have activity against the specific inciting antigen. HIV-related antigenic stimulation results in this type of activation and is thought to be an important initiator of lymphomagenesis. The effect is related to the degree and duration of immunodepletion, which can be modified by effective cART. In addition to the CD4 cell counts and HIV viral loads, several other biomarkers appear to be related to the lymphomagenic immunologic interface. For example, HIV induces cytokine dysregulation and polyclonal B-cell expansion (Breen et al. 2006). Biomarkers that correlate with B-cell expansion, such as elevated serum CD30 and CD23 and serum free light chains, appear to be associated with increased risk of ARL. The gammaherpesviruses EBV and HHV-8 are associated with ARL that occur at low CD4 cells and could reflect derangements in immune control of the viruses in association with chronic antigenic stimulation. These viruses, along with other infections, can perturb the cytokine profile in patients, potentially making them useful as diagnostic or risk biomarkers. However, dysregulation of cytokines such as IL-6, IL-7, IL-8, IL-10, and IL-15 is found to be perturbed in HIV cases without lymphoid neoplastic disease. These cytokines are not at present reliable markers for lymphoma, either diagnostically or prognostically, most likely owing to a variety of features including host immune genetics such as HLA type. Yet, these features suggest that early therapeutic intervention for HIV could have a beneficial effect on lymphoma prevention. Indeed, preservation of higher CD4 cells has reduced the incidence of ARL overall by around 50% (Besson et al. 2001). Although overall ARL risk is reduced with cART, there is not absolute risk normalization. In addition to reduced overall ARL risk, cART appears to shift the ARL type that does occur toward those having more favorable biology and prognosis (Fig. 1) (Dunleavy and Wilson 2012). The positive effects of cART on lymph node preservation, reduction in antigenic stimulation, and gammaherpesvirus control are features



Presentation and Pathogenesis of B-Cell Lymphoid Cancers Associated with HIV Infection, Fig. 1 A modal for the histogenesis of human immunodeficiency

virus (HIV)-associated lymphomas showing molecular and viral pathogenesis and diffuse large B-cell lymphoma taxonomy (From Dunleavy and Wilson 2012)

that underlie the changing ARL epidemiology in the cART era.

background population, and so the possibility of lymphoid malignancy should always be a consideration in the HIV-infected patient.

The clinical presentation of lymphomas can be understood in terms of these immunologic and cytokine effects as well. The systemic symptoms that are associated with frank lymphoma, such as fever, fatigue, and weight loss, are partly related to cytokine abnormal production. Likewise, cytopenias, and even psychiatric symptoms, may be explained in part by cytokine derangements, though these can also be due to the presence of tumor in the affected organ systems. However, some cases present with no symptoms at all, other than adenopathy. The mass effect from enlarged nodes can mechanically compress nerves and other organs with specific related pain or vague discomfort in some cases. Thus, given the elevated risk of NHL in HIV-infected persons, vigilance and recognition of such symptoms merit consideration for ARL in the differential diagnosis for patients presenting with any of these symptoms. Even with well-preserved CD4 cell counts in patients on cART, lymphoid tumors have a markedly excess incidence relative to the

Specific AIDS-Related Lymphomas

ARL can broadly be viewed as (a) those lymphoid tumors that also occur in immunocompetent hosts and (b) those that occur more specifically in persons with HIV infection (Table 1). The following section reviews the specific lymphoma types in that context. A common theme among the histologic types is that the more advanced the immune dysregulation, the greater the propensity for an oncogenic virus to be associated with the tumor and for other biological features that may confer relative treatment resistance to be present.

Burkitt Lymphoma

Burkitt lymphoma accounts for approximately 30% of lymphomas in HIV-infected persons.

Burkitt typically occurs early in the course of HIV disease before the CD4+ cells are greatly depleted, and this is true for patients treated with cART as well as those who are not. This likely accounts for the relatively little change in Burkitt incidence comparing pre-cART and cART treatment eras. It is not yet clear whether these tumors continue to occur at a constant risk over the duration of HIV infection or whether with time, the lymphoma risk largely shifts to another histologic type, even though the CD4 cell counts are preserved. After all, non-AIDS-Burkitt occurs mainly in children and younger adults, so as those with HIV infection age, the age pattern of NHL may parallel that observed in the HIV-unrelated setting. If this turns out to be the case, over time, a person's risk of Burkitt lymphoma may decline, and the risk for diffuse large B-cell lymphoma may increase, similar to the background population epidemiologic patterns. However, in the developing world, Burkitt incidence may have increased to some extent since the introduction of cART (Abayomi et al. 2011). As yet these findings are not fully understood, but may reflect differences in guidelines for cART including timing of treatment initiation and definitions for viral suppression. The role of EBV in African Burkitt may also influence the emerging epidemiologic patterns there.

There are three epidemiologic forms of BL: sporadic (sBL), seen in developed Western countries; EBV-associated endemic BL (eBL), seen in Africa; and HIV-associated BL (HIV-BL). The three forms are clinically and biologically distinct, but share major similarities. Burkitt lymphoma is an aggressive mature B-cell neoplasm that histologically appears as sheets of medium-sized cells with a growth fraction of essentially 100%, and this in part accounts for the treatment failure seen with standard CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) therapy. The proliferation rate is essentially 100%, and the cells rapidly undergo apoptosis and are phagocytosed by macrophages, leading to an appearance commonly called the "starry sky" pattern. Burkitt express CD10 and BCL6, along with mutated immunoglobulin genes suggesting a germinal center origin. CD20 and CD79a (both B-cell

markers) are essentially always present. The cells are generally negative for BCL2. Constitutive MYC protein expression is characteristic owing to the translocation of the MYC gene to one of the immunoglobulin gene loci, causing deregulated cell cycle control. The most common translocation is t(8;14) (q24;q32), which occurs in 70–80% of patients. MYC activation can also be caused by variant translocations, t(2;8) (p12;q24) and t(8;22) (q24;q11), which occur in 10–15% of patients. MYC overexpression induces apoptosis through a p53-dependent pathway in normal B cells. TP53 mutations occur in Burkitt and could override this effect. However, this may be negated by BIM (Bcl-2-interacting mediator of cell death) downregulation through a variety of mechanisms including EBV, thus in balance promoting cell survival.

Clinically, eBL, which is nearly always EBV associated, occurs mainly in children and involves the abdomen and jaw most frequently. Its geographic distribution overlaps areas where malaria and parvovirus infections are high, and these appear to be not only epidemiological features but also possibly biological cofactors. The incidence of eBL is up to 200 times greater than other BL forms. Indeed, there appear to be molecular interactions between malaria and EBV resulting in viral reactivation that may in part explain the epidemiologic findings.

The sBL form typically occurs in a wide range of ages, but the median is in the young adult years. EBV is seen in 30% or less of cases. HIV-BL is similar to sBL in terms of EBV association and is often the first AIDS complication and initial manifestation of HIV disease. However, in areas of Africa where HIV and eBL overlap, it is not as yet fully clear what the epidemiological dynamics are. This may be in part due to patterns of diagnosis and treatment. The increase in HIV-BL is only partially explained by the various cofactors for sBL and eBL. As mentioned above, immunosuppression is generally relatively mild. However, there is ongoing immune activation in the presence of HIV, even when well treated. This can result in deregulated cytokine profiles affecting B-cell proliferation and survival involving activation-induced cytidine deaminase (AID),

which can lead to c-MYC translocation. Duration of HIV viremia, independent of CD4 cell counts, appears to be associated with risk of NHL development. If HIV viremia is key, then it would seem a potent effect at low replication levels. Apparently, early cART initiation after HIV diagnosis has no effect on Burkitt incidence, but it will be important to see whether this observation holds over time.

An emerging appreciation for the biological differences in the three BL forms suggests that the eBL and sBL have distinct molecular characteristics and that the HIV-BL shares some characteristics of both (Schmitz et al. 2012). The eBL and HIV-BL have higher rates of immunoglobulin heavy chain mutations than sBL, and they show signs of antigen selection, in contrast to the sBL form. When the tumors are segregated on the basis of EBV association (regardless of which epidemiologic form), the differences segregate more pronouncedly by EBV status. Overall, the molecular findings suggest that EBV-negative BL derives from early centroblasts, whereas the EBV-positive BL might derive from late GC B cells that have already started to differentiate into memory B cells. However, it has been suggested that activated C-MYC gene can influence cellular phenotype to appear similar to GC cells making definite assignment of histogenic derivation easier said than done.

The clinical presentation for AIDS-related Burkitt lymphoma is variable. Unexplained fever and night sweats may be the initial symptoms leading to the suspicion of an infection. Bone marrow infiltration can lead to bone pain and cytopenias with consequent easy fatigability, bruising, and bleeding. Abdominal pain or distention, nausea, vomiting, or gastrointestinal bleeding is relatively common owing to a predilection for intra-abdominal involvement by BL. Head and neck presentation is also common, with involvement of the sinuses, oropharynx, and tonsils accounting for symptoms referable to those sites. Involvement of the CNS is common, and the CSF of patients with Burkitt lymphoma should be checked by cytology and flow cytometry for the presence of leptomeningeal disease. Owing to the rapid proliferation rate, Burkitt lymphoma is

highly aggressive and often presents with rapid clinical decline. Elevated lactate dehydrogenase, renal and hepatic abnormalities, urate elevations, and electrolyte abnormalities are common. Coagulation proteins may also be abnormal with frank bleeding. It is essential to recognize that these derangements are due to the lymphoma, and prompt lymphoma therapy is mandatory. Delays in lymphoma therapy can adversely affect survival prospects in this disease, and as such, the cancer therapy and supportive care for the lymphoma complications should take priority in clinical management over any concerns for HIV therapy. The hepatic and renal abnormalities may render cART infeasible to administer until improvement is achieved from lymphoma therapy. It is important to recognize that CHOP is not the appropriate therapy for Burkitt, although earlier in the AIDS epidemic it was often utilized for HIV-BL. The current standard of care is to utilize standard Burkitt lymphoma regimens, such as CODOX-M/IVAC in the treatment of AIDS-Burkitt lymphoma. In the USA, the National Cancer Institute-sponsored nationwide clinical trial of rituximab with infusional dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) chemotherapy is under way. The infusional regimen appears to overcome resistance related to high proliferation providing evidence for a rational and less toxic effective therapy for Burkitt lymphoma relative to other regimens.

Diffuse Large B-Cell Lymphoma (DLBCL)

The clinical presentation for DLBCL has been reviewed in the introductory comments. Where there are special considerations, these will be mentioned for each of the following DLBCL types.

DLBCL, Not Otherwise Specified (NOS)

Patients often present with advanced stage disease, including extranodal involvement. The bone marrow should always be examined. The

cerebrospinal fluid (CSF) should be sampled for cytological and flow cytometric evidence of leptomeningeal involvement in all cases with extranodal or marrow involvement. Some experts suggest sampling the CSF regardless. Constitutional symptoms include unexplained fevers, drenching night sweats, fatigue, and unexplained weight loss.

DLBCL NOS is a diagnosis of exclusion, not corresponding to other more specific diagnostic categories within the DLBCL category. It is termed “not otherwise specified” because there are no clear and accepted criteria for further subdivision, although it is clear that within this category there are biologically heterogeneous entities. DLBCL NOS is the most commonly occurring NHL among persons with HIV infection. Within this DLBCL NOS group, there are three common morphological variants, two of which account for the bulk of HIV-DLBCL: the centroblastic and the immunoblastic variants. These also occur in the immunocompetent host. In the immunocompetent setting, DLBCL NOS comprises about 30% of adult NHL, and the median age is 70 years. In the setting of HIV, they comprise over 70%, and the median age is reported to be less than 40 years.

The immunoblastic variant of DLBCL shows a preponderance of cells with pronounced basophilic cytoplasm and plasmacytoid differentiation seen in some cells. Up to 90% are EBV associated. These tend to occur with more advanced immunodepletion. In contrast, the centroblastic variant occurs at higher CD4 cell counts and histologically appears as medium to large lymphoid cells with oval to round vesicular nuclei. There is a monomorphic cellular appearance with scanty cytoplasm that stains with either basic or acidic dyes. EBV association is found in less than 30% of cases. Some cases do include immunoblasts admixed among the predominant centroblasts.

Gene expression profiling (GEP) of DLBCL of HIV-unrelated cases has identified distinct molecular subtypes that have marked differences in survival probability. Two major types identified are those with GEP similar to germinal center B cells (GCBs) and those that are similar to activated B cells (ABCs), and this established the

concept that DLBCL biology was based on the histogenic cell of origin in lymphomagenesis (Alizadeh et al. 2000; Rosenwald et al. 2002). The GCB subtype has marked survival advantage compared to the ABC type, controlled for other clinical features. A third unclassifiable GEP type has survival intermediate between the other two. Although initially conceived as markers of cellular origin, increasingly it is recognized that the molecular subtypes also have differing signaling pathways for cellular transformation and oncogenesis. This may have therapeutic implications if the pathways can be successfully exploited. For example, in the ABC type, the chronically stimulated B-cell receptor (BCR), either through subunit mutations or CARD11, leads to constitutive NF- κ B activation. Recruitment of Bruton’s tyrosine kinase (BTK) is required as part of this signaling, and thus ABC viability involves CARD11-dependent constitutive NF- κ B activation. Also involved in NF- κ B activation is MYD88. These findings suggest potential roles in the ABC subtype for drugs such as BTK inhibitors, bortezomib (to inhibit NF- κ B activation), and lenalidomide (to downregulate MYD88 and other pathways) (Yang et al. 2012). Although the GEP-defined molecular subtyping and prognostic validity have not been confirmed in HIV-DLBCL, a number of features suggest there are parallels in the biology and clinical outcomes (Dunleavy et al. 2010). Precise determination of histogenic and molecular subtypes of HIV-DLBCL will be essential if biologically based advances in HIV lymphomas are to be successfully realized.

Attempts to use immunohistochemical (IHC) algorithms to replicate GEP results and/or stratify patients according to survival have been pursued, in part because they are technologically more straightforward. However, while IHC can accurately identify the majority of cases as GCB, the various algorithms have not been universally successful in maintaining the prognostic value of GEP (Chadburn et al. 2009; Castillo et al. 2012). For ARL cases treated across a spectrum of backbone regimens, IHC designation of GCB and non-GCB-DLBCL subtypes did not segregate the subtypes by overall survival (Chadburn et al. 2009). Also, CD4 count at cancer

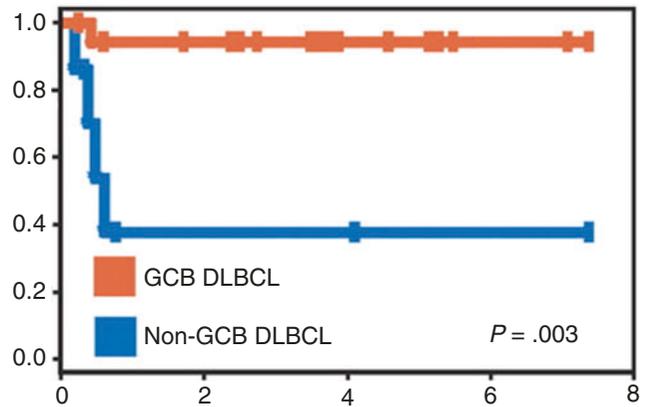
presentation did not segregate according to DLBCL subtype. A drawback to the various immunohistochemical algorithms is that while they identify the GCB type fairly well, they poorly separate the unclassifiable and the ABC type, and this blurs the outcome measures (when unclassifiable and ABC type are combined, the grouped outcome is less bad). In addition, if the treatment administered is not optimal, any potential outcome differences may be further obscured. Since ARL-DLBCL tend to be highly proliferative, they may have relative chemoresistance to standard CHOP chemotherapy, thus making outcome differences by histogenic subtype less obvious. Additionally, if there is death due to nonlymphoma causes, it is difficult to ascribe outcomes according to lymphoma subtype. There is nevertheless data supporting the parallel in HIV and non-HIV-DLBCL outcomes by histogenic subtype. A number of studies have suggested that dose-adjusted infusional EPOCH-R overcomes the resistance due to high tumor proliferation. In a phase II study using IHC to identify the DLBCL subtypes, there was marked segregation in outcome by subtype, again highlighting the critical interrelationship with tumor biology and specific treatment (Dunleavy et al. 2010). Because ARL is a rare disease, each case should be approached as a vital opportunity to better define the tumor biology. Utilization of gold standard biomarker technology should be used in order to avoid pitfalls of conflating inadequate biomarker technology with conclusions regarding biological differences and clinical behavior in ARL. RNA extraction from formalin fixed, paraffin embedded provides accurate GEP-based classification of DLBCL and has potential for use in stratified trial designs.

Epidemiological dynamics over the period of the AIDS epidemic provide important clues toward integrating the knowledge gained from HIV-unrelated cases to the AIDS lymphomas. The incidence of AIDS-defining cases of DLBCL peaked in the early 1990s and then substantially declined in areas where widespread use of effective combination antiretroviral therapy (cART) became available. The risk of DLBCL in HIV is inversely associated with CD4 cell count.

Since cART increases and/or maintains higher CD4 cell counts, the prevalence of those low-CD4 AIDS cases has decreased by over 50%, and consequently a 50% decrease in DLBCL incidence has been observed (Besson et al. 2001). This is due to cART keeping the majority of patients in a CD4 cell range where the lymphoma risk is somewhat reduced compared to the higher lymphoma risk seen when the CD4 cells are low. It is instructive to note that within any given CD4 strata, the risk on ARL is unchanged when comparing pre-cART and cART treatment eras. Also, high CD4 cell levels do not fully normalize the risk of HIV-DLBCL, which remains elevated over the background population by over 50-fold.

In addition to the CD4-related overall risk of ARL, there appears to be an association between the CD4 cell count and the specific biologic and clinical characteristics of the ARL type most likely to occur (Dunleavy et al. 2010; Dunleavy and Wilson 2012) (Fig. 1). Patients with advanced immunodepletion are more likely to develop EVB-associated immunoblastic tumors with ABC characteristics, known to have a poor outcome in the background population because of the inferior curative potential of this subtype. In contrast, those who develop DLBCL at relatively high CD4 cell counts are more likely to develop GCB-DLBCL. Thus, the observation that survival has improved for ARL in the cART era is likely to be explained in part by the improved curability of the GCB-DLBCL subtype and its rise as the predominant DLBCL type owing to less advanced immunosuppression (Little et al. 2003). Further advances in ARL outcomes will depend on accurate biologic identification of cases and will most likely involve relevant targeting of molecular pathways with rational novel therapeutic agents (Wilson et al. 2010). Moreover, it is essential to recognize the differences in prognosis on the basis of tumor biology. The GCB-DLBCL in HIV may already have excellent outcome for which further improvement will be difficult to measure. However, ABC-DLBCL clearly has a poor prognosis, and outcome improvements can be meaningfully targeted in reasonably sized clinical trial designs (Fig. 2).

Presentation and Pathogenesis of B-Cell Lymphoid Cancers Associated with HIV Infection, Fig. 2 PFS for GCB and non-GCB-DLBCL (From Dunleavy et al. 2010)



Primary Diffuse Large B-Cell Lymphoma of the Central Nervous System

AIDS-related primary central nervous system lymphoma (AR-PCNSL) is a CD20+ immunoblastic diffuse large B-cell lymphoma. It is almost always restricted to patients with severe T-cell depletion of less than 50 CD4+ cells/mm³ and is essentially always associated with EBV in the malignant cells. The risk of AR-PCNSL is several thousandfold greater than in the background population. Thus, while PCNSL also occurs in the immunocompetent population, in those with HIV infection, its singular relationship with severe immunodepletion and the fact that each cell harbors latent EBV infection suggest substantial pathobiological differences relative to PCNSL occurring in the immunocompetent host. AR-PCNSL is truly an opportunistic cancer. This is highlighted by the marked decrease in incidence of AR-PCNSL in areas where there is widespread use of cART. Occasionally PCNSL develops in an HIV-infected person with no prior AIDS complications and with CD4 cells counts in the normal range. Such cases may be EBV unrelated and likely represent a qualitatively separate process more akin to those PCNSL occurring in the background population. It is questionable whether these cases are truly AR-PCNSL, and it is more likely that they represent primary brain lymphoma coincidentally occurring in a substantially non-immunosuppressed HIV-infected person. It is worth recognizing that HIV-infected persons get cancers seen in the background population

with similar presenting features and risks. Discussed here are those PCNSL occurring at very low CD4 cell counts.

The pathogenesis of AR-PCNSL remains unclear. The finding of sentinel white matter lesions in the brain suggests that normal B-lymphocytes may undergo clonal proliferation as the basis for lymphomagenesis. The EBV latent membrane protein LMP1 likely contributes to malignant transformation through upregulation of the antiapoptotic protein BCL2. Alternative explanations involve the trafficking of peripheral lymphocytes into the brain. Toll-like receptor (TLR) activation from HIV-nef and other proteins may lead to immune activation and dysregulation of cytokines (Sasakawa et al. 2012). TLR polymorphisms (e.g., TLR9) may predispose to AR-PCNSL through effects of EBV. Preclinical models of PCNSL suggest higher expression of certain adhesion molecules (such as LFA-1 and ICAM-1) mediated by dysregulated cytokines such as IL-8 in HIV compared to non-HIV-derived specimens and that these can be interrupted with agents such as zidovudine. Interestingly, AR-PCNSL responses have been reported with the use of high-dose zidovudine (Raez et al. 1999). In patients with advanced HIV infection, poor immunosurveillance enables EBV-related lymphoma cells to escape detection and then to disseminate and lodge through interactions between chemokine receptors on lymphocytes and brain vascular endothelium owing to HIV-induced aberrant cell adhesion and chemokine receptor expression. These factors likely

create a unique microenvironment for the development and growth of opportunistic tumors. Though no studies have performed molecular profiling on AR-PCNSL, the few studies that have done so in HIV-negative PCNSL suggest that these tumors are of late or post-germinal center origin and may be susceptible to strategies that target NF-kappa B.

The clinical presentation for AR-PCNSL varies considerably. Patients can be symptomatic in a very subtle sense, showing only vague personality changes such as poor attentiveness or memory loss or mild lethargy. Neurological deficits such as cranial nerve paralysis, visual impairment, aphasia, and seizures can be seen. Headache is less common than one might suspect, but can be the only presenting feature. Rapid clinical deterioration over days or weeks is typical.

Radiological findings are nonspecific but very sensitive. There can be considerable overlap with toxoplasmosis or other CNS abscess. On brain imaging, there can be unifocal or multifocal lesions that are variably radiodense on non-contrast CT scan. MRI typically shows a hypointense or isointense T1-weighted image. Gadolinium-enhanced MRI can appear as ring enhancing or a variety of other forms and even non-enhancing at all.

The near-universal association with EBV in AR-PCNSL and the fluorodeoxyglucose (^{18}F) positron emission tomography (FDG-PET) avidity of the tumor provide the basis for an important diagnostic biomarker. In cases where CSF is collected and found to be positive for EBV by polymerase chain reaction (PCR), and where the FDG-PET is also positive, the positive predictive value for AR-PCNSL approaches 100%. If both tests are negative, the negative predictive value approaches 100%. Thus, in cases where definitive biopsy diagnosis is not feasible, definitive therapy for lymphoma can be commenced without the traditional delays in waiting for evidence that antibiotic intervention is failing. The standard approach in the era prior to cART was to first treat for presumptive infectious toxoplasmosis; if the patient did not respond, it was then thought likely that the diagnosis was lymphoma. This approach is no longer justifiable, at least for many cases. Understanding the rationale that

distinguishes past and current practice is critical. Prior to cART, patients with AR-PCNSL had a dismal prognosis based on both the brain tumor and the advanced irreversible HIV disease. In contrast, a patient in the current era may have highly reversible HIV disease, particularly if cART naïve. Thus, a patient who presents with AR-PCNSL may have a rapid immune reconstitution (including specific EBV immunosurveillance) and rapid tumor resolution if therapy is promptly initiated for both the HIV and the brain tumor. Therefore, urgent initiation and completion of definitive diagnostic work-up and treatment initiation for the identified pathology should be the standard of care in the current era. In the HIV-unrelated setting, a trial of antibiotics for undiagnosed intracranial processes would be an egregious and indefensible approach. In the cART era, if the HIV is highly treatable, the approach to those with AIDS should be just as urgent as in the HIV-unrelated setting.

Unfortunately, use of a biomarker-driven diagnostic approach and its potential lifesaving application is difficult to study well owing to the rarity of the disease. The irony is that with improved therapy and improved diagnostics available, the condition has become even more rare, and producing a high level of evidence to inform practice is increasingly elusive. Nevertheless, there is little justification for adhering to the clinical approach established in the early 1980s. Administering a trial of anti-toxoplasmosis therapy and waiting for that to fail before commencing with definitive lymphoma only increase the permanent morbidity and lethality of the tumor. Though the evidence is limited, AR-PCNSL may be particularly responsive to therapy, and the approach to care should consider the prospects for long-term control of the underlying HIV.

It is important to reiterate that more typical PCNSL may occur in those with well-treated HIV disease without advanced immunosuppression. These cases are likely to be like those in the elderly background population. The diagnostic approach should be the same as in the background population, as the association with EBV is likely to be much less than then AR-PCNSL as categorized here.

Hodgkin Lymphoma

Hodgkin lymphoma clinical presentation is similar to that for DLBCL, with the exception that the central nervous system is not involved. Patients may present with minimal symptoms or with the constellation of systemic symptoms mentioned as in the DLBCL section above.

Although not designated as an AIDS-defining condition, the risk of classical Hodgkin (CHL) lymphoma is elevated eightfold or more in HIV-infected persons relative to the background population. CHL is distinguished from nodular lymphocyte predominant Hodgkin lymphoma (NLPDHL) owing to distinct clinical, epidemiological, and biological features. NLPDHL is not elevated in HIV. There are four histological subtypes of CHL: lymphocyte-rich CHL, nodular sclerosis CHL (NSCHL), mixed cellularity CHL (MCCHL), and lymphocyte-depleted CHL (LDCHL). In HIV, MCCHL and LDCHL are the predominant forms seen. In the background population, NSCHL is the most common form.

The risk of CHL appears to have increased during the cART era, though whether this is mainly due to changes in diagnostic accuracy, the increasing prevalence of persons with HIV in the cART era, or to aging over the period of the AIDS epidemic is somewhat unsettled. Also, the risk of CHL appears greatest during the first few months after initiation of cART and then decreases or stabilizes with chronic therapy, and this may contribute to the apparent increase in CHL in the cART era. Patients with 50–99 CD4 cells/mm³ appear to be most affected by cART initiation, suggesting that CHL could be related to immune recovery. Most cases present with advanced stage, and nearly 50% have extranodal sites or marrow involvement. This may reflect in part that MCCHL has a propensity for disseminated presentation with abdominal and splenic involvement, in contrast to NSCHL, which typically spreads in an orderly fashion among contiguous lymph node groups. Also, in HIV infection, 80% of cases are EBV associated. Interestingly MCCHL is more likely to EBV associated than NSCHL regardless of HIV status. The EBV-associated cases express the latent membrane protein 1 (LMP1) and are

EBER positive. An interesting finding in CHL is that while the immunophenotype of the tumor cells is similar among the subtypes, there are marked difference in other features including the sites of involvement, clinical features, growth pattern, presence of fibrosis, and the composition of the cellular background. It has been postulated that cART enables the cellular background to thrive and promote CHL (Biggar et al. 2006).

CHL is characterized by the presence of Reed-Sternberg (HRS) cells that are multinucleated monoclonal B cells residing in a background of nonneoplastic reactive cellular infiltrate (Swerdlow et al. 2008). It is the appearance of this infiltrative process that defines the CHL subtype. HRS variants that have only one nucleus are called Hodgkin cells. The HRS or variant usually is the minority of cells comprising the affected tumor tissue, making up less than 10% of the total cellularity. They are CD30+ in nearly all cases, and this feature is being exploited in clinical trials of the conjugated monoclonal antibody brentuximab vedotin in HIV-CHL. CD15 is found in about 85% of cases. If CD20 expression is detected, it is generally variable in its intensity on the neoplastic cells, with many of them not expressing it at all. Other markers of B-cell specificity include PAX5/BSAP seen in activated B cells, though its comparatively weaker intensity in HRS cells distinguishes them from the former. There is overexpression of cytokines and chemokines and/or their receptors in CHL, and this feature is consistent with the inflammatory CHL tumor appearance. Clonal immunoglobulin gene rearrangement is found in the HRS cells in nearly all cases. Along with the finding that these show somatic hypermutation and other features, HRS cells appear to be derived from a germinal center B cell. In cases associated with HIV, this is consistent with the finding that many cases occur when the CD4 cells are relatively intact or improving during cART initiation as mentioned above. Nevertheless, HRS appears to have lost much of the typical B-cell gene expression pattern. Deregulated NKκB, JAK/STAT, and other transcription factors are found by gene expression in HIV-unrelated cases. These features require further study in HIV-related cases.

Therapeutically, classical HL in the setting of HIV should be approached in the same way as HIV-negative HL, and regimens such as ABVD should be the standard approach. Recent data shows HIV status does not influence outcome in patients with cHL treated with ABVD chemotherapy in the cART era (Montoto et al. 2012).

Primary Effusion Lymphoma

Primary effusion lymphoma (PEL) is a large B-cell lymphoma with variable morphology (Swerdlow et al. 2008). It can appear as plasmablastic, immunoblastic, or frankly anaplastic. It almost always presents as a serous effusion, hence its synonym body cavity lymphoma. Generally it is asymptomatic until the effusion has accumulated sufficiently to cause mechanical effect, such as pain or a feeling of fullness. If the effusion is in the pleural space, shortness of breath may occur. If the effusion is in the pericardial space, fatigue, dizziness, and signs of heart failure can occur as cardiac function becomes compromised. Patients presenting with PEL may have concomitant Kaposi sarcoma and/or multicentric Castleman disease (MCD) or a history of these.

Diagnosis is confirmed by examination of the cellular component of the effusion, which can sometimes be aspirated in large quantities. PEL is always positive for the human herpes virus-8 (HHV-8, also known as the Kaposi sarcoma-associated herpes virus, KSHV). In upwards of 70% of cases, the tumor cells are dually coinfecting with HHV-8 and EBV. If HHV-8 is not present, but EBV is found in a lymphomatous effusion, the likely diagnosis is pyothorax-associated DLBCL, not PEL.

B-cell immunophenotypic markers can be lost in PEL, though some are weakly CD20 positive. Nonlineage antigens including CD30 can be present, possibly suggesting a role for the use of brentuximab vedotin therapeutically. B-cell histology can be confirmed by molecular analysis and the finding that immunoglobulin genes are clonally rearranged and hypermutated. GEP shows a distinct profile with features of both

plasma cells and EBV-transformed lymphoblastoid cell lines (Fan et al. 2005).

It is reported that PEL occurs mainly with depleted CD4 cell counts, though cases clearly do occur when the CD4 cell count is near normal. The HHV-8 gene expression program includes a number of human gene analogues that can affect immunosurveillance and immune evasion. The prognosis is generally not favorable, and the response to chemotherapy is poor. Targeted therapies based on the virology and tumor pathobiology will likely be critical to improving the therapeutic outcomes.

Plasmablastic Lymphoma

Plasmablastic lymphoma (PBL) is an uncommon B-cell tumor occurring predominantly in HIV-positive middle-aged males (Swerdlow et al. 2008). It can also occur in other immunodeficiency states. Among the elderly, it can occur without evident immunodeficiency. In nearly all cases, the tumor is EBV infected. PBL frequently involves the oral cavity and originally was termed PBL of the oral cavity prior to the realization that it does involve other anatomic sites as well. Most patients have stage III–IV disease at presentation and a high-risk international prognostics index score, a clinical rating system of lymphoma prognosis.

There is a spectrum of morphologic appearances in PBL. PBL can resemble immunoblasts or have a more plasmacytic differentiation appearing similar to plasmablastic plasma cell myeloma. They often have a high proliferation fraction with a monomorphic plasmablastic cytology, particularly when presentation involves the oral, nasal, and paranasal areas in those with HIV infection. The immunophenotype shows expression of CD138, CD38, Vs38c, and IRF4/MUM1, with weak or no expression of CD45 and CD20. CD79a (a B-cell marker) is present in the majority of cases. Cytoplasmic immunoglobulins are expressed and most are IgG with restriction to either kappa or lambda light chain. Clonal immunoglobulin heavy chain rearrangement is found even when immunoglobulin expression is not detectable. CD30 is frequently expressed and

may suggest a role for brentuximab vedotin. Ki67 index is usually over 90%, so the tumor is highly proliferative. EBV EBER in situ hybridization is positive in most cases, but LMP1 is rarely expressed. HHV-8 is absent. The clinical course is very aggressive with median survival less than 1 year. Cases with limited involvement may respond well to chemotherapy followed by involved field radiotherapy.

HHV-8 Multicentric Castleman Disease

HHV-8 multicentric Castleman disease (MCD) is a syndrome of constitutional symptoms with variable organ and hematopoietic dyspoiesis. Histopathologically it is characterized by an involution and hyalinization of germinal centers of B-cell follicles in the lymph nodes and spleen. The mantle zone can be prominent and intrude into the germinal center. In the mantle zone are found larger plasmablastic cells that are positive by immunohistochemistry for HHV-8 latent nuclear antigen-1 (LANA). The syndrome is mediated in part by aberrant interleukin-6 (IL-6) production (Uldrick et al. 2011). HHV-8 encodes for a viral form of IL-6 and can also serve to promote human IL-6. Immunoglobulin M lambda is expressed without evidence of monoclonality. Consideration for HHV-8 MCD should be raised in a patient with intermittent constitutional symptoms, fevers, and cytopenias. HHV-8 serology and viral loads can be determined in support of the diagnosis. Though splenomegaly may be impressive, there may be only moderate adenopathy. FDG-PET may be useful to identify metabolically active nodes and help to identify the most likely lymph node to biopsy in order to confirm the histopathologic diagnosis. Needle aspiration yields inadequate architecturally intact material for diagnostic utility.

Conclusions

The availability of cART has significantly changed the landscape of HIV-associated lymphoma. Both BL and DLBCL, in the setting of HIV infection, are now highly curable with current strategies. For

these lymphomas, future directions should focus on identifying driver pathways and novel targets and improving strategies particularly for the treatment of non-germinal center tumors.

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Preventing HIV-1 Transmission Through Vaccine-Induced Immune Responses

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Definition

According to UNAIDS, globally 35.0 million people were living with HIV at the end of 2013. Sub-Saharan Africa remains most severely

affected, accounting for 71% of the people living with HIV-1 worldwide. The number of people newly infected or who died from AIDS-related causes declined worldwide. Despite genuine, although unequal and fragile, successes in prevention programs attributed to strengthening and scaling up prevention strategies and antiretroviral treatment, the development of a preventive HIV-1 vaccine as part of a comprehensive prevention package remains among the best hopes for controlling the HIV-1 pandemic. By inducing appropriate immune responses directed against a specific pathogen, vaccines remain the most powerful public health tool to prevent infectious diseases. They may either prevent infection by the pathogen or the development of the disease due to this pathogen.

Goals of an HIV-1 Vaccine

The most promising approach to lead an HIV-1 vaccine to licensure remains to prevent HIV infection and provide sterilizing immunity. A second goal would be to reduce HIV-1 viral load (VL) by controlling viral replication to stop progression to disease, and reduce subsequent transmissibility, in vaccine recipients who became HIV infected. These two goals are complementary. However, from a regulatory and licensure perspective, the acceptance of a vaccine that would only reduce VL seems unlikely, as the validation of immune markers that counter viral replication would need to be supported by tangible long-term clinical benefits. This might not be feasible and is likely unethical to demonstrate, as HIV-infected individuals should benefit from early antiviral treatment. High community VL may be a central driver of the HIV-1 epidemic in Africa. Thus, by decreasing community VL over time as the portion of the infected community members with reduced viral load, thanks to vaccination, increases, a reduction in overall may be achieved. This rationale has been at the basis of treatment-for-prevention strategies now developed for HIV-infected people. However, demonstrating the same impact from vaccines would take a very large study over an extended

period and is likely not feasible. Thus the primary goal of an HIV vaccine is prevention.

HIV-1 Vaccine Immunological Concepts

Natural immunity to HIV-1 does not protect against infection and progression to disease. Theoretically then, an effective HIV-1 vaccine needs to generate qualitatively or quantitatively different immune responses, perhaps responses not identified in natural infection, sufficient to block or modify infection. Until recently, the nature of a vaccine-induced protective immune response remained elusive and the development of an HIV-1 vaccine included a substantial empiric approach. The understanding of which immune responses should be induced for protection (immune correlates of protection) in humans and nonhuman primates studies (NHP) was, and still is, limited.

One of the major difficulties for an HIV-1 vaccine is the induction of immune responses that overcome HIV-1 variability. The HIV-1 envelope is particularly variable among HIV-1 subtypes and circulating recombinant forms. Ideally, antibodies induced by the HIV-1 envelope immunogens should neutralize free virus and/or by linking the envelope expressed at the surface of HIV-infected cells to killer cells will eliminate infected cells through antibody-dependent cell-mediated cytotoxicity (ADCC). In contrast, core proteins (Gag, Pol, Nef, Tat, Vif) expressed at the surface of HIV-infected cells are more conserved among HIV-1 subtypes. By targeting HIV-1 proteins expressed at the surface of HIV-infected cells, HIV-specific cytotoxic T lymphocyte (CTL) will contribute to eliminate these HIV-infected cells.

It has therefore been postulated that both vaccine-induced humoral and cell-mediated immune responses would be needed to counter HIV-1 in the peripheral compartment (blood, lymph nodes) and mucosal tissues (cervicovaginal, gut), the entry point of the virus for sexual transmission. These considerations have led to vaccine and regimen designs aiming at inducing both types of responses (Walker et al.

2011). Antibodies are better induced by vaccination with HIV-1 soluble envelope subunit proteins. CTL are classically induced by vaccine components such as DNA and recombinant vectors either alone or in combination and expressing HIV-1 core proteins presented to the immune system via the HLA-I pathway. To that effect, the “prime-boost” regimen consisting of priming the immune system with a vaccine component (e.g., vector or DNA) and boosting the immune response with another vaccine component (e.g., an Env subunit protein or vector) is now common.

Vaccine Efficacy and Immunogenicity

Table 1 shows the various vaccine approaches tested in humans so far. The main findings of vaccine-induced immune responses in early clinical development (phase I and II trials) are summarized in Table 2. Table 3 shows vaccine regimens that have reached advanced development and efficacy clinical trials in humans.

Preventing HIV-1 Transmission Through Vaccine-Induced Immune Responses, Table 1 Generic HIV-1 vaccine products tested in humans

Soluble subunits
Envelope (Env) proteins, Env-derived peptides, Tat, Env-Nef-Tat fusion protein
Recombinant vectors
Pox vectors
Canarypox (ALVAC), fowlpox, attenuated replication-incompetent vaccinia: modified vaccinia Ankara (MVA), NYVAC
Replication-defective adenovirus vectors
Human (Ad5, Ad35, Ad26) and chimpanzee (ChAdV63) subtypes
Replicating vectors
Vaccinia Tiantan, paramyxoviruses (measles, Sendai), vesicular stomatitis virus
Other vectors
Adeno-associated virus vector type 2, alphavirus replicon Venezuelan equine encephalitis
DNA
Vaccine combinations (prime-boost)
DNA + Env subunit protein, DNA + recombinant vector, Vector + Env or non-Env fusion subunit protein, Vector + vector

Preventing HIV-1 Transmission Through Vaccine-Induced Immune Responses, Table 2

Main immunogenicity findings of phase I/II trials

Antibody responses

No broadly neutralizing antibodies to HIV-1 are induced by current vaccines

Binding antibodies, ADCC, and neutralizing antibodies against easy-to-neutralize and some against more difficult-to-neutralize HIV-1 isolates are induced by Env subunit proteins formulated with potent adjuvants

Env antibody titers increase after Env protein booster doses but wane rapidly to lower levels

An Env subunit protein boost seems necessary to induce higher levels of serum antibodies

Mucosal and systemic immunizations are able to induce both IgG and IgA in plasma and mucosal secretions

T-cell-mediated responses

Polyfunctional CD4⁺ and CD8⁺ T-cell immune responses are detected in a majority of vaccine recipients immunized by vectors alone and to some extent by DNA alone. Generally of low to moderate magnitude, these responses are generally significantly augmented by immunization with vaccine combinations

CD8-mediated inhibition of *in vitro* viral replication can be detected after vector vaccination

Cell-mediated responses to DNA administered by electroporation are significantly augmented compared to intramuscular needle injection

Specific gene activation immune signatures can be identified as predictive of the immune responses

Natural preexisting immunity to vectors may modulate vaccine-induced immune responses

Protection of HIV Acquisition by Antibodies

Three efficacy trials (RV144, Vax003, and Vax004) tested the hypothesis that antibodies directed against the HIV-1 envelope could protect against HIV acquisition.

RV144

RV144, a seminal community-based efficacy trial conducted in Thailand, tested a recombinant canarypox vector (ALVAC-HIV) prime and gp120 Env protein boost regimen and provided the first evidence that protection against HIV-1 acquisition could be achieved. These findings have generated considerable interest and opened new and unprecedented avenues to accelerate the development of a licensed HIV vaccine.

The vaccine regimen conferred an estimated vaccine efficacy of 31.2% against HIV-1 acquisition after 42 months of follow-up (Rerks-Ngarm et al. 2009). The interaction of risk status and acquisition efficacy was significant with greater benefit in low-risk individuals. Vaccine efficacy appeared to be higher (60%) at 12 months post vaccination, suggesting an early, but nondurable, vaccine effect (Robb et al. 2012). The vaccine had no effect on early postinfection HIV-1 RNA VL or CD4⁺ T-cell count, nor did it affect the clinical course of HIV disease after infection. A further analysis showed evidence of lower seminal VL in vaccine recipients who acquired HIV infection (Rerks-Ngarm et al. 2013).

HIV-specific T-lymphocyte responses detected were predominantly polyfunctional effector memory CD4⁺ T cells and recognized peptides derived from the Env V2 loop region (de Souza et al. 2012). The V2 loop region includes the $\alpha 4\beta 7$ integrin-binding site, a mucosal homing marker.

Plasma binding antibodies against Env was nearly uniformly present including antibodies to V2 synthetic peptides. ADCC activity was also detected (Bonsignori et al. 2012). In the TZM-bl system, neutralizing antibodies (NAb) to easy-to-neutralize (tier-1) viruses were detected but not against difficult-to-neutralize (tier-2) viruses (Montefiori et al. 2012).

Vax003

Vax003 was a phase III trial testing a gp120 vaccine (subtype B and CRF01_AE gp120 proteins) in injecting drug users (IDU) in Bangkok, Thailand. The vaccine did not prevent HIV acquisition or disease progression (Pitisuttithum et al. 2006). The reasons why Vax003 regimen failed to protect whereas the RV144 regimen conferred some protection despite using similar envelope immunogens are unclear. NAb to tier-1 viruses were higher in Vax003 than in RV144 and sporadic tier-2 NAb were detected. The quality of the antibody response, in particular the Env-specific IgG subclasses, may explain such difference. In previous trials, gp120 only induced high levels of Env-specific IgG4 antibodies while ALVAC prime and gp120 boost

Preventing HIV-1 Transmission Through Vaccine-Induced Immune Responses, Table 3 HIV-1 vaccine efficacy trials in humans

Study protocol	Vaccines	Population enrolled	Location	Result
HVTN 505	DNA + Ad5	Circumcised MSM and TG who are Ad5 Ab negative	USA	Stopped for futility. No efficacy; nonsignificant increase of HIV infection rate in vaccinees
RV144	ALVAC-HIV and AIDSVAX B/E gp120	Community mostly at low risk for HIV infection	Thailand	31.2% efficacy against HIV acquisition. No effect on plasma viral load
HVTN 502 Step trial	MRKAd5 HIV-1 gag/pol/nef B	MSM, high-risk heterosexual men and women	North and South America, Australia, Caribbean	No efficacy; transiently increased HIV infection risk in vaccinees
HVTN 503 Phambili trial	MRKAd5 HIV-1 gag/pol/nef B	Heterosexual men and women	South Africa	No efficacy; nonsignificant increase of HIV infection rate in vaccinees
Vax003	AIDSVAX B/E gp120	Injecting drug users	Bangkok, Thailand	No efficacy
Vax004	AIDSVAX B/B gp120	MSM, high-risk women	USA	No efficacy

ALVAC-HIV: recombinant canarypox vector (vCP1521) encoding HIV-1 Gag and protease subtype B and Env gp120 CRF01_AE linked to the transmembrane-anchoring portion of subtype B gp41 protein

Ad5 Ab: Ad5-specific neutralizing antibody

DNA plasmids expressing HIV-1 Gag, Pol, and Nef subtype B and HIV-1 Env subtypes A, B, and C

Ad5: mixture of four Ad5 vectors encoding the HIV-1 Gag-Pol polyprotein subtype B and HIV-1 Env A, B, and C matching the DNA Env components

MSM: men who have sex with men; TG: male-to-female transgender persons who have sex with men

elicited lower IgG4 relative to IgG1 and IgG3 antibodies. Antigen-specific IgG3 antibodies have been associated with control of the pathogen and clinical protection in several infectious diseases. Despite lower antibody responses in RV144, specific qualities of these responses involving IgG3 antibodies and functions were higher in RV144 than in Vax003 and may have played a role in protection. Other plausible explanations include the role of ALVAC priming and the low-risk mostly heterosexual study population in RV144 compared to Vax003 conducted in high-risk IDU with a radically different, nonsexual mode of transmission.

Vax004

Vax004 tested a similar bivalent gp120 vaccine (subtype B) in US men who have sex with men (MSM) and women at high risk for heterosexual transmission of HIV-1. The vaccine did not prevent HIV-1 acquisition or disease progression. Peak antibody levels inversely correlated with

HIV-1 incidence. The levels of antibodies either caused an increased risk among low responders or a decreased risk among high responders for HIV-1 acquisition. Most vaccine recipients mounted potent NAb against tier-1 viruses and occasional weak tier-2 NAb. Vaccination was associated with the presence of gp120-binding IgG in mucosal secretions and plasma and IgA in plasma. HIV acquisition correlated inversely with serum antibody-dependent cell-mediated virus inhibition (ADCVI) activity but poorly with neutralizing or CD4-gp120-blocking antibody activity against laboratory strains.

Immune Correlates of Risk for HIV-1 Acquisition

A correlate of risk is defined as an immunological measurement that correlates with the rate or level of a study endpoint used to measure vaccine efficacy in a define population. A correlate of protection is an immune marker statistically correlated with vaccine efficacy (equivalently, predictive of

vaccine efficacy) that may or may not be a mechanistic causal agent of protection (Plotkin and Gilbert 2012).

RV144 provided the first opportunity to study the correlations between vaccine-induced immune responses with vaccine efficacy (Haynes et al. 2012). A complex set of analyses identified two immune variables that correlated significantly with HIV-1 infection risk: plasma IgG binding antibody to scaffolded gp70 V1V2 envelope proteins correlated inversely with risk, while Env plasma IgA correlated directly with risk, raising the hypothesis that IgA responses against Env and IgG responses directed against V1V2 may be mechanistically associated with protection. Neither low levels of V1V2 antibodies nor high levels of Env-specific IgA antibodies were associated with higher rates of infection than in the placebo group, suggesting there was no evidence for enhancement of infection risk. IgG avidity, ADCC, NAb, and Env-specific CD4+ T cells were inversely correlated with risk of infection in vaccinees with low levels of Env-specific IgA antibodies. These V2-specific antibodies can mediate ADCC, neutralization, and low-level virus capture.

Most of RV144 incident HIV-1 infections were CRF01_AE, the HIV-1 strain predominantly circulating in Thailand and much of South East Asia. An analysis looking for a difference in the sequences of viruses infecting vaccine versus placebo recipients, also called “sieve” analysis, supported the hypothesis that immune responses directed against two specific amino-acid positions in the V2 loop were associated with protection (Rolland et al. 2012).

The reasons for negative association between high levels of plasma IgA and protection are unclear. IgA may block the action of IgG, in particular ADCC and phagocytosis. The gp120 C1 region contains a target epitope on the surface of virus-infected cells for antibodies that mediate ADCC. Vaccinees with IgA antibodies to the gp120 C1 region had a higher risk of infection than vaccinees without these antibodies. IgA antibodies elicited by RV144 can block C1 region-specific IgG-mediated ADCC.

Hypothetical Mechanisms of Action

There is no clear correlate of protection for several successful vaccines where antibody appears to play a major role, suggesting that protection may not be conferred exclusively by NAb. RV144 generated robust Env-binding antibodies that showed poor neutralizing activity suggesting that non-neutralizing, but functional, binding antibodies may play a role in protection from HIV-1 acquisition.

Cervical mucus and cervicovaginal mucus naturally hinder the diffusion of HIV-1 in an envelope-independent manner. One hypothesis is that binding antibodies may potentially inhibit HIV transmission at the genital mucosa by mechanisms such as formation of large complexes that cannot penetrate superficial epithelial barriers. In mucosal tissues, a subset of CD4+ T cells express the integrin $\alpha 4\beta 7$, the gut homing receptor. These T cells are highly susceptible to HIV infection. Env gp120 binding to $\alpha 4\beta 7$ is mediated by an Env region located in V1V2. It is therefore conceivable that gp120 antibodies may block the gp120- $\alpha 4\beta 7$ interaction and contribute to the protective effect against HIV sexual transmission.

Mediation of antigen-specific targeting and killing of infected cells through ADCC almost necessarily occurs after the virus had traversed the epithelial boundary. Following vaccination with rgp120 in Vax003, ADCVI activity was higher among those with a lower rate of sexually acquired HIV infection.

Nonhuman Primate Challenge Studies Supporting RV144 Findings

Following the release of the RV144 results, the outcome of various nonhuman primate (NHP) challenge studies was revisited. ALVAC-SIV prime and gp120 boost regimen recapitulating the RV144 regimen conferred protection against acquisition following a repeated low-dose mucosal challenge of SIVmac251 in 30% of the vaccinated animals, a proportion similar to that observed in RV144. Protected animals had higher avidity antibodies to gp120, recognized the V2 variable envelope region, and reduced

SIVmac251 infectivity in cells expressing high level of $\alpha 4\beta 7$, suggesting a functional role of V2 antibodies. Similarly, in macaques vaccinated with various combinations of DNA and vectors, protection against SIV acquisition correlated with Env and V2-specific binding antibodies and tier-1 NAb (Barouch et al. 2012). A sieve analysis of breakthrough infections in vaccinated animals supported the possible role of protective responses directed against the Env-V2 region.

HIV Vaccines that Primarily Induce T-Cell-Mediated Immune Responses

The failure of antibody-inducing vaccines in Vax003 and Vax004 and the lack of vaccines able to induce bNAbs led to the concept of vaccines whose principal mechanism of action was the induction of cell-mediated (largely cytotoxic) immune responses.

Step (HVTN502) and Phambili (HVTN503) Vaccine Efficacy Trials

The Step (Buchbinder et al. 2008) and Phambili vaccine trials (Table 3) were the first clinical trials assessing the efficacy of a T-cell-based vaccine. The Merck vaccine (MRKAd5 HIV-1) was a mixture of replication-defective Ad5 vectors expressing HIV-1 *gag*, *pol*, and *nef* subtype B genes; no *env* gene was present. The Step trial was unexpectedly halted. In subjects with preexisting Ad5-specific neutralizing antibody titers, a greater number of HIV infections occurred in vaccine than in placebo recipients. Post hoc analysis suggested that the greatest increased risk was in uncircumcised men with preexisting Ad5-specific NAb. The vaccine-associated risk seen at interim analysis was confirmed but waned with time from vaccination. The hypothesis that anamnestic Ad5-specific CD4+ T lymphocytes after vaccination in Ad5-seropositive subjects may have served as increased numbers of targets susceptible to HIV-1 infection was not supported by further analysis. A follow-up analysis of Phambili participants suggests a nonsignificant increased rate of

HIV infection in the vaccine recipients compared to placebo recipients.

MRKAd5 HIV-1 vaccine induced HIV-specific CD8+ and CD4+ T-cell-mediated responses in a majority of vaccinees, although of low magnitude. A sieve analysis of breakthrough infections revealed that HIV strains from vaccinees were more likely to encode epitopes that differed from those present in the vaccine. With a median frequency of four CD8 epitopes per vaccinee, highly conserved epitopes were detected at a lower frequency, similar to the frequency in natural infection, suggesting potential epitope masking of these responses. These results suggest that breadth alone may not be sufficient and that stronger T-cell responses toward conserved regions of the genome in particular are needed to rapidly eliminate and limit escape mutations and confer some protective effect.

A ► **systems biology** analysis revealed immune signatures in humans following vaccination that predicted subsequent induction and magnitude of HIV-specific T-cell responses. Responses of vaccinees with preexisting Ad5 NAb were strongly attenuated, suggesting that enhanced HIV acquisition in Ad5-seropositive subgroups in the Step Study may relate to the lack of appropriate innate activation rather than to increased systemic immune activation (Zak et al. 2012).

Ad5 vector-based vaccines did not protect macaques from infection after SHIV challenge but did reduce VL and preserve CD4+ T-cell counts after infection, findings that were not reproduced in the human trials. The outcome of the Step trial was recapitulated in a macaque study where animals vaccinated with a regimen similar to that employed in the Step trial were not protected against a SIVsmE660 challenge. In a separate study, macaques chronically infected with a host-range mutant Ad5 and then immunized with Ad5 SIVmac239 *gag/pol/nef* vaccine were not protected against challenge with escalating dose penile exposures to SIVmac251, despite SIV vaccine-induced CD8+ T-cell responses in 70% of the monkeys, a proportion similar among responders to that observed in the Step trial.

HVTN 505

A vaccine regimen with DNA-HIV vaccine prime expressing *gag*, *pol*, and *nef* subtype B genes and *env* genes from subtypes A, B, and C and replication-defective Ad5-HIV vaccine boost encoding the same genes as the DNA components was recently tested in phase I and IIA trials. The vaccine regimen induced polyfunctional CD4+ and CD8+ T cells and multi-clade anti-Env-binding antibodies, mostly tier-1 and limited tier-2 NAb.

Although the regimen failed to protect immunized macaques against SIVmac251 infection, half of vaccinated monkeys were protected against heterologous SIVsmE660 challenge. Among SIVsmE660-infected animals, a one-log reduction in peak plasma RNA VL was observed in Mamu-A*01 monkeys, suggesting that CTL were involved in the control of SIV replication. In Mamu-A*01-negative monkeys, no CD8+ T-cell response or innate immune response was associated with protection against virus acquisition. However, low levels of NAb and an Env-specific CD4+ T-cell response were associated with vaccine protection. SIV-specific CD8+ T cells showed strong virus-inhibitory activity (VIA) and displayed an effector memory (EM) phenotype. VIA correlated with high levels of CD107a and perforin expression in SIV-specific CD8+ T cells. Both the frequency and the number of Gag-specific responses were strongly correlated with VIA mediated by CD8+ T cells.

The results of the preliminary clinical studies combined with the findings of the NHP challenge studies led to the initiation of a phase IIB study of the DNA-HIV prime, Ad5-HIV boost regimen (HVTN 505) in a US population of circumcised, Ad5-seronegative men who have sex with men (MSM). HVTN 505 was stopped for futility, showing no efficacy and no significant effect on viral load. There were nominally more HIV infections in the vaccine group compared to the placebo group but the result was not significant. Studying how immune responses correlate with infection risk after the DNA primes versus after the Ad5 boost might give clues to whether certain immune responses are or aren't important for protection.

Protective Role of Cell-Mediated Immune Responses

The role of CD8+ T cells in preventing HIV infection and disease by controlling HIV replication is now well established. Several macaque studies support this hypothesis, but results differ depending on the virus challenge and immunological endpoints used and predict inconsistently the results in humans.

A relatively small number of immune effectors at the mucosal site of entry might be at the right place at the right time to be “enough and soon enough” to clear infection (Haase 2010). Consistent with this hypothesis, vaccine-induced CTL were able to delay SHIV dissemination from mucosa following rectal challenge. Similarly, prior infection of rhesus macaques with an attenuated SHIV conferred protection against vaginal challenge associated with SIV-specific CTL in cervical vaginal tissues, suggesting that a modest vaccine-induced CD8+ T-cell response in the context of immunoregulatory suppression of T-cell activation may protect against vaginal HIV transmission. Supporting this hypothesis, macaques immunized with an oral vaccine comprised of *Lactobacillus plantarum*, a commensal bacterium that favors immune tolerance, and inactivated SIVmac239-induced CD8+ regulatory T cells that conferred complete protection in most of the animals challenged intrarectally with SIVmac239 or heterologous strain SIVB670 despite the absence of SIV-specific antibodies or CTL.

Whether systemic vaccination can induce potent and durable cellular responses at the mucosal sites deserves further exploration. Different prime-boost vaccination regimens generate SIV-specific CD8+ T-cell responses as clonally diverse as those induced by SIV infection. Systemic vaccination with non-replicating vectors induced rapid and clonally diverse mucosal cellular immune responses that are sustained over time. Vaccine-induced CD8+ T cells migrate into the intestinal mucosa, differentiate into effector memory CD8+ T cells in situ, and remain resident there without recirculating.

A rhesus cytomegalovirus expressing SIV antigens elicited and maintained virus-specific effector memory CD4+ and CD8+ T cells at extra-

lymphoid sites and conferred partial protection against repeated intrarectal challenge (Hansen et al. 2009). A vaccine would need to induce and maintain a population of effectors at the portal of entry to resist the challenge, in particular for rectal transmission where infection is more quickly disseminated. Such rapid first-line intervention force would likely be achieved by functional antibodies rather than by cell-mediated responses, although both could complement each other, the first “neutralizing” the majority of the viruses and the second “mopping up” the remaining infected cells.

New Vaccine Designs

Although considerable efforts are deployed to better understand the mechanisms of neutralization and to design immunogens capable to induce broadly neutralizing antibodies, these antigens are not yet available for testing. Current efforts in multiple laboratory groups are now focusing on Env subunit protein designs aiming at improving the RV144 results, in particular to improve antibody responses directed against the V2 loop.

The induction of immune responses capable of overcoming HIV-1 variability remains one of the main hurdles for HIV-1 vaccines. The RV144 V2-specific antibodies cross-react with multiple HIV-1 subtypes. The original reagent used to identify a V2 serologic correlate of risk was a gp70V1V2 scaffold protein derived from HIV-1 subtype B. Interestingly, unpublished data show binding to scaffold proteins derived from HIV-1 subtype C and CRF01_AE is also highly correlated with reduced HIV acquisition risk. It is therefore conceivable that Env immunogens whose sequences are mismatched to those of the circulating HIV-1 strains be able to induce high cross-reactive V2 antibody titers and be effective vaccines. The potentiating effect of adjuvants in terms of magnitude, durability, and IgG subclass of the V2 antibody response compared to the classical alum adjuvant (used in RV144, Vax003, and Vax004) needs further study.

Immunization with soluble Env was reported to induce short-lived antibody responses with robust peak followed by rapid contraction of

circulating antibody and memory B cells. Increased deposition and retention of antigen drives B-cell responses locally in the tissue, enhances antigen presentation by dendritic cells, and contributes to the stimulation of the formation of germinal centers in lymph nodes with enhanced development of CD4⁺ T follicular helper cells required for the formation of memory B cells (Nutt and Tarlington 2011). Such vaccine approaches deserve to be explored for HIV-1 vaccines aiming at inducing sustained antibody responses.

None of the vaccines tested so far for efficacy in humans were able to reduce VL postinfection. Several immunogen design strategies able to increase breadth and depth of the CD8⁺ T-cell responses are being pursued such as use of HIV-1 conserved sequences (core and envelope proteins) and mosaic of HIV-1 antigens.

Conclusion

RV144 and the study of immune correlates of risk have led to improved vaccine designs and accelerating vaccine development to licensure for the most exciting time of HIV-1 vaccine development. The use of an HIV-1 envelope protein immunogen seems to be key for inducing protective immune responses against HIV acquisition by sexual transmission. The still uncertain predictive value of animal models and biomarkers of immune protection against HIV-1 necessitate however that vaccines be tested in clinical trials coordinated with animal model studies to better understand their predictive value. It remains critical to assess vaccine efficacy and correlates of risk in populations with different intensity of risk and mode of HIV-1 transmission. A long-term strategy to ultimately end the AIDS pandemic must include both scale-up of existing HIV combination prevention, treatment, and care programming and sustained investment in research and development for a preventive HIV-1 vaccine.

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Preventing Mother-to-Child Transmission (PMTCT): Prevention of HIV

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Definition

Without any intervention, 14–48% of HIV-infected women will transmit the infection to their newborn infant. The greatest risk of transmission occurs around the time of delivery, likely due to direct exposure to HIV, accounting for two-thirds of perinatal infections. Significant transmission also occurs in the antenatal period (25%) and the postpartum period (15%) if the mother breastfeeds (Simonon et al. 1994). Prevention of mother-to-child transmission (PMTCT) of HIV encompasses the range of biomedical and behavioral interventions that – used in combination and implemented fully – can reduce this rate of infant HIV infection to less than 2% (European 2005). While vertical transmission of HIV has decreased dramatically in North America and Europe, progress has lagged in many resource-constrained settings for a number of contextual reasons. Over the past decade, however, numerous scientific, policy, and program advances have dramatically changed the landscape for PMTCT and renewed hope for the virtual elimination of pediatric AIDS worldwide (UNAIDS).

Framework for PMTCT

The World Health Organization (WHO) and others have endorsed a holistic approach to reducing pediatric AIDS globally. This framework articulates four “prongs” that consider both direct and indirect contributors: (1) primary prevention of HIV among women of childbearing age, (2) prevention of unintended pregnancies among HIV-infected women, (3) prevention of mother-to-child transmission among HIV-infected women, and (4) linkage of HIV-infected women and children to long-term HIV care and treatment. Comprehensive programs for HIV prevention, care, and treatment must address each of these areas in order reach the ambitious goal of eliminating pediatric HIV globally (Mahy et al. 2010). In this section, we focus primarily on prong three: interventions that directly reduce transmission from HIV-infected mothers to their infants. Other prongs are considered elsewhere in this volume.

PMTCT in Resource-Rich Settings

Dramatic reductions in the vertical transmission of HIV have been observed in North America and Europe. In the USA, the Centers for Disease Control and Prevention reported a peak in new pediatric HIV in 1991, with sharp declines in the subsequent decade. In 2013, only 107 cases of perinatal HIV transmission were reported nationwide (Centers for Disease Control and Prevention. HIV surveillance report 2013). Similarly low rates were observed in a national surveillance study in the UK (2000–2011), where overall mother-to-child transmission declined to 0.5% following the introduction of comprehensive PMTCT guidelines (Townsend et al. 2014). These reports are also supported by other large European cohorts, including the European Collaborative Study and the French Perinatal Cohort.

Several clinical and public health interventions have played a critical role in these declines. Foremost has been the availability of effective antiretroviral drug regimens for PMTCT. In 1994, the landmark PACTG 076 trial established the

efficacy of antiretrovirals for PMTCT, showing a 67% reduction in new infant HIV infections when zidovudine was administered to mothers during pregnancy and labor and to infants during the first 6 weeks of life (Connor et al. 1994). In the decade that followed, a number of studies demonstrated that three-drug combination regimens – already recommended for HIV treatment – could drive down transmission even further, to rates consistently below 2%. The optimal combination regimen has changed over time, refined out of concerns about drug toxicity and adverse pregnancy outcomes. Nevertheless, this strategy was adopted as the standard of care in the US National Guidelines by 2007 – and continues to this date – with similar practices mirrored in other resource-rich countries.

The effectiveness of antiretroviral regimens for PMTCT is highly associated with maternal virologic suppression. As such, close virologic monitoring during pregnancy has become the standard of care in many resource-rich settings. According to the US Perinatal Guidelines, for example, viral load testing is recommended on a monthly basis until virologic suppression is achieved and then quarterly thereafter. Maternal viral load is again tested at 34–36 weeks gestation to guide counseling about mode of delivery (see below). With the growing availability of HIV genotyping, drug resistance testing is also recommended early in pregnancy and in cases of suspected treatment failure, to help to optimize antiretroviral drug regimens ([Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission](#)).

Elective cesarean delivery is associated with a 50% reduction HIV transmission rates (The International Perinatal HIV Group 1999); however, the procedure may be associated with surgical complications for the mother as well as risks of neonatal respiratory morbidity. Current evidence suggests that, for HIV-infected pregnant women with viral loads <1,000 copies/mL, infant transmission rates are similar between vaginal and elective cesarean delivery. Viral load testing in the third trimester is typically used to triage patients and to guide mode of delivery counseling. In many resource-rich settings, elective cesarean

delivery remains an important PMTCT strategy for high-risk pregnancies, including those with elevated or unknown maternal viremia. Because the PMTCT benefits of cesarean delivery appear to diminish following the onset of labor, elective cesareans are typically scheduled at (or soon after) 38 weeks gestation. To minimize the risks of an iatrogenic premature delivery, obstetrical guidelines emphasize the need for early engagement into care and reliable gestational age dating of the pregnancy ([Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission](#)).

Alternatives for infant feeding have also played an important role in limiting HIV transmission from mother to child. Unlike in many resource-constrained settings, where breastfeeding is often a necessity, the availability of replacement feeding options has eliminated continued exposure to HIV following delivery. While infant formula has traditionally been recommended, there is a growing literature as to the immunological and nutritional benefits of breastfeeding for the newborn. Harm reduction strategies for breastfeeding, including options for flash heating and banked milk, appear safe and effective ([Levison et al. 2014](#)).

PMTCT in Resource-Constrained Settings

Progress in PMTCT has lagged in comparison in the resource-constrained settings of Africa and Asia. Barriers have included some – if not all – of the following: generalized HIV epidemics with large numbers of HIV-infected women; limited infrastructure for health services, particularly in rural areas; traditions of antenatal care and delivery outside of the health system; deep-seeded misconceptions and stigma about HIV infection; and lack of safe, affordable, and socially acceptable alternatives to breastfeeding. Important advances in clinical research, international policy, and program implementation, however, have begun to address these issues in a systematic fashion, providing renewed hope for an AIDS-free generation ([UNAIDS](#)).

Antiretroviral regimens for PMTCT in resource-constrained settings have undergone considerable evolution. Early studies sought to simplify antiretroviral regimens by reducing the duration and frequency of dosing. Trials in Thailand and Cote d'Ivoire evaluated modified versions of the original PACTG 076 zidovudine regimen, including the addition of lamivudine during pregnancy. The HIVNET 012 study in Uganda demonstrated that two-dose peripartum nevirapine – one dose to the mother in labor and one dose to the newborn within 72 h of birth – could reduce the relative risk of HIV transmission at 18 months of life by 41% ([Jackson et al. 2003](#)). Although enthusiasm for this regimen eventually waned because of its moderate efficacy and its selection for drug-resistant variants, two-dose peripartum nevirapine nevertheless played a critical role in catalyzing PMTCT program expansion globally. Following the PHPT-2 results from Thailand, a combined approach of antenatal and intrapartum zidovudine coupled with peripartum nevirapine became the global standard of care for PMTCT in 2004. The antenatal and intrapartum HIV transmission rates in the intervention arms of the Thai trial were 2–3% ([Lallemant et al. 2004](#)) – i.e., comparable to that of three-drug combination regimens in the USA – for women were not yet eligible for antiretroviral therapy for HIV treatment. Despite its high efficacy, this regimen did not address continued HIV transmission risk during the postpartum period for women who breastfed their infants.

Several important studies have since demonstrated the efficacy of antiretroviral-based strategies to reduce mother-to-child HIV transmission during the breastfeeding period. Two approaches have been shown to be highly effective in preventing postpartum HIV transmission: daily nevirapine prophylaxis administered to the infant and three-drug combination regimens provided to the mother. To date, there have been no head-to-head comparisons; however, because of the low transmission rates observed in randomized studies, both strategies were included in the WHO 2010 PMTCT guidelines. While the WHO initially recommended that countries choose between one of two regimens for PMTCT ([Table 1](#)), the

Preventing Mother-to-Child Transmission (PMTCT): Prevention of HIV, Table 1 Summary of WHO guidelines for the prevention of mother-to-child HIV transmission 2004, 2006, 2010, and 2013

Year	Woman receives		Infant receives
	<i>Treatment</i>	<i>Prophylaxis</i>	
2004	<p><i>WHO stages 1 and 2:</i> Triple ARVs for CD4 count <200 cell/uL</p> <p><i>WHO stage 3:</i> Triple ARVs for CD4 count <350 cell/uL</p> <p><i>WHO stage 4:</i> Triple ARVs regardless of CD4 count</p>	<p><i>Antepartum:</i> ZDV from 28 weeks</p> <p><i>Intrapartum:</i> sd-NPV</p> <p><i>Postpartum:</i> N/A</p> <p><i>Alternative regimens</i> ZDV alone; short-course ZDV/3TC; or sd-NPV</p>	sd-NPV + ZDV for 1 week
2006	Unchanged from 2004	<p><i>Antepartum:</i> ZDV from 28 weeks</p> <p><i>Intrapartum:</i> sd-NVP + ZDV/3TC</p> <p><i>Postpartum:</i> ZDV/3TC for 1 week</p>	sd-NVP + ZDV for 1 week
2010	Option A		
	<p>Triple ARVs for CD4 count ≤ 350 cell/uL</p> <p>All women with WHO Stage 3 or 4, irrespective of CD4 count</p>	<p><i>Antepartum:</i> ZDV from 14 weeks</p> <p><i>Intrapartum:</i> sd-NVP + ZDV/3TC</p> <p><i>Postpartum:</i> ZDV/3TC for 1 week</p>	<p>NVP until 1 week after cessation of breastfeeding</p> <p><i>or</i></p> <p>NVP for 4–6 weeks if not breastfeeding</p>
	Option B		
	<p>Triple ARVs for CD4 count ≤ 350 cell/uL</p> <p>All women with WHO stage 3 or 4, irrespective of CD4 count</p>	<p><i>Antepartum:</i> Triple ARVs from 14 weeks</p> <p><i>Intrapartum:</i> Triple ARVs</p> <p><i>Postpartum:</i> Triple ARVs continued through childbirth <i>or</i> until cessation of breastfeeding</p>	NVP or ZDV for 4–6 weeks regardless of feeding method
2013	Use lifelong ART for all pregnant and breastfeeding women (Option B+)		
	<p>Triple ARVs started as soon as diagnosed and continued for life, regardless of CD4 count</p> <p>For certain settings, guidelines support the use of lifelong ART only for pregnant and breastfeeding women eligible for treatment (Option B)</p>		<p>NVP for 6 weeks if breastfeeding</p> <p><i>or</i></p> <p>NVP or ZDV for 4–6 weeks if not breastfeeding</p>

Notes: *ART* antiretroviral therapy, *ARVs* antiretrovirals, *NVP* nevirapine, *sd-NVP* single-dose nevirapine, *WHO* World Health Organization, *ZDV* zidovudine, *3TC* lamivudine

“Option B” strategy – i.e., provision of maternal three-drug regimens during pregnancy, delivery, and breastfeeding – was later endorsed as the preferred approach for operational and programmatic reasons. As part of its 2013 consolidated guidelines for HIV, the WHO went a step further to support the universal and lifelong treatment of HIV-infected pregnant and breastfeeding women, regardless of CD4 status (also known as “Option B+”). Although scientific evidence behind this

approach at the time was sparse, this innovative policy decision sought to better align PMTCT and HIV treatment strategies, improve maternal health, reduce horizontal transmission to serodiscordant partners, and minimize mother-to-child transmission in future pregnancies. Because of the shortened duration between breastfeeding cessation and subsequent pregnancies in many target countries, the rationale for Option B+ was that this approach would limit

interruptions in antiretroviral therapy and promote long-term medication adherence and retention in care over time.

Alongside these effective clinical interventions have been successful public health strategies to increase the uptake of PMTCT services by HIV-infected pregnant women. The implementation of “opt-out” antenatal HIV screening, for example, has greatly aided the identification of HIV-infected women during pregnancy (Tudor Car et al. 2013). Provision of combination antiretroviral therapy in the same location as PMTCT services and male partner involvement in care may also improve the uptake of PMTCT services and decrease attrition from care (Wettstein et al. 2012).

The evolving practices for antiretroviral treatment and prophylaxis have influenced policies on infant feeding. There has been strong evidence supporting exclusive breastfeeding over the first 6 months of life. Given the efficacy of PMTCT regimens during the postpartum period, however, the WHO extended its recommendation for breastfeeding out to 12 months, with gradual weaning afterwards. In many settings, infant formula remains a viable option, provided it is acceptable, feasible, affordable, sustainable, and safe. While programs for replacement feeding have largely been successful, there have been cases where top-down policies have led to unintended public health consequences. In Botswana, for example, extensive flooding in 2005–2006 led to an outbreak of diarrheal disease and malnutrition among children. A key factor in the high morbidity and mortality observed was the reliance on formula feeding despite the lack of reliably safe drinking water. This episode led the Government of Botswana to review its national guidelines regarding infant feeding and to ultimately align them with international recommendations from the WHO.

When fully implemented, these strategies for PMTCT hold great promise. Despite considerable investment thus far, however, coverage of services remains low in many settings. The “PMTCT cascade” (i.e., the steps that an HIV-infected woman and her HIV-exposed infant must navigate to minimize the risk of HIV transmission) has identified

multiple points of attrition – and of potential intervention – over the course of antenatal, intrapartum, and postpartum care. Program innovations are needed to increase uptake of and maximize retention within PMTCT services, including engagement of women and their partners at the community level, integration of PMTCT into existing health services, incorporation of trained lay workers to support PMTCT programs, and use of innovative technological and structural interventions to promote uptake. New models for monitoring, evaluation, and program improvement also hold potential, with important and broad collateral benefits for health systems in general.

Future Directions

Between 2009 and 2013, a 43% reduction in the number of new HIV infections among children was reported for 21 Global Plan priority countries (UNAIDS 2014), demonstrating the recent success of PMTCT programs in low- and middle-income countries (LMICs) in Africa and Asia. Despite this success, there remains much work to be done to end the epidemic and to reverse its devastating impact on health outcomes, survival, and economic development. The post-2015 global health agenda is now focused on women’s and children’s health, with new and renewed international commitments in HIV prevention and treatment, family planning, maternal health, and child survival. Much of groundwork for the so-called UNAIDS “90-90-90” target for 2020, through which 90% of HIV-infected individuals will be diagnosed, 90% receiving antiretroviral therapy, and 90% virologically suppressed, has been achieved through commitments set out in the UNAIDS *Getting to Zero: 2011–2015 Strategy*, the Global Plan towards the elimination of new HIV infections among children by 2015 and keeping their mothers alive, and the WHO *Treatment 2.0 Framework for Action*. These initiatives – and their accompanied funding – have the potential to be truly transformative and to virtually eliminate pediatric HIV infections.

Implementation of these ambitious prevention and treatment strategies will undoubtedly present challenges for many African and Asian health systems facing a growing burden of non-communicable diseases, new and reemerging infectious epidemics, and severely limited human resources for health. Future investments in implementation science research – a field that generates evidence and best practice for “real-world” program implementation – will therefore be critical to achieving the ambitious global targets set forth for eliminating pediatric HIV.

In both resource-rich and resource-constrained settings, efforts to engage vulnerable and hard-to-reach populations such as adolescents, those with substance abuse and mental health problems, incarcerated individuals, and LGBT and other marginalized communities will need to be expanded. In resource-rich settings, assisted reproductive technologies, such as sperm washing and, more recently, preexposure chemoprophylaxis (PrEP) (Savasi et al. 2013), are increasingly being integrated into individualized treatment planning for HIV-infected individuals, the majority of whom are in their reproductive years. Similar counseling and reproductive assistance for HIV serodiscordant couples wishing to conceive in resource-constrained settings should also be considered a PMTCT priority. Finally, early and multidrug ART regimens aimed at prevention for high-risk infants (Nielsen-Saines et al. 2012) and sustained remission for infants infected at birth (Rainwater-Lovett et al. 2015) will likely continue to be the focus of future pediatric trials.

Conclusion

Over the past two decades, public health policy and clinical guidelines in both resource-rich and resource-constrained settings have evolved and converged to emphasize universal testing during pregnancy and early antiretroviral treatment to improve women’s own health and to prevent mother-to-child HIV transmission. The result has been a dramatic decline in pediatric AIDS, with

perinatal HIV transmission rates now well below 5% in most countries, ever-decreasing numbers of HIV-infected children under the age of 14, and rapid expansion of HIV treatment services for women and their families. However, numerous disparities remain. Women continue to face a disproportionate burden of new HIV infections in sub-Saharan Africa and Asia. Between a third and a quarter of women in LMIC settings still do not have access to antiretroviral drugs for PMTCT. In addition, in many resource-constrained settings, the treatment gap between adults and children continues to widen. Innovations in basic, translational, and implementation science, strong and evidence-based global guidance, and sustained local and international funding commitments for women’s and children’s health are all needed to address these ongoing disparities and to achieve virtual elimination of pediatric HIV.

Cross-References

- ▶ [Mother-to-Child Transmission of HIV-1: Role of Receptor Usage and Target Cells](#)

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Preventing Mother-to-Child Transmission of HIV-1

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Definition

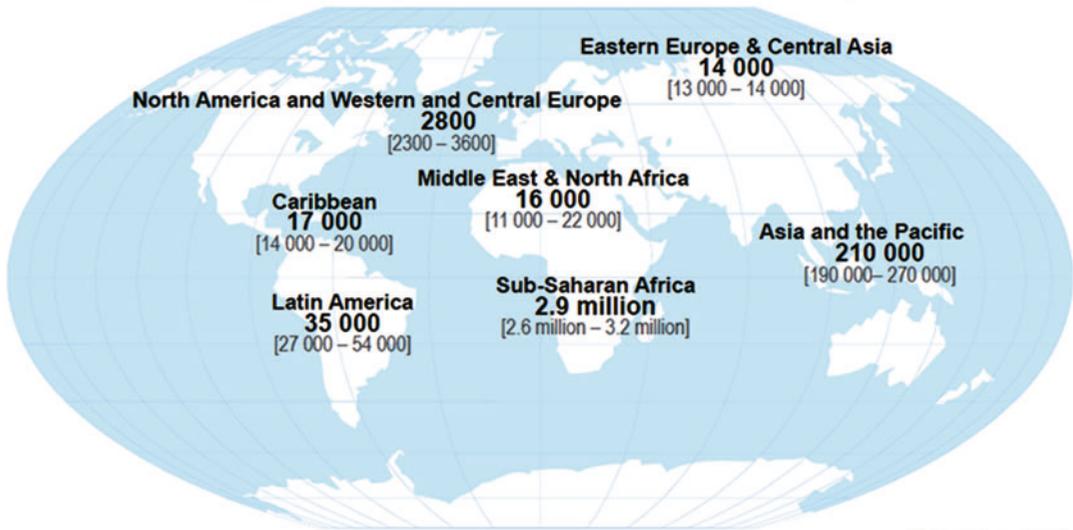
Mother-to-child transmission of HIV-1 (MTCT) occurs in utero, during delivery, and through breastfeeding. MTCT transmission risk ranges from ~30% to 45% in untreated breastfeeding HIV-1-infected mothers. With combination antiretroviral therapy (ART), transmission risk can be lowered to ~1–5%. There is a recent concerted effort toward “virtual elimination” of pediatric HIV by 2015. This will require enhanced implementation strategies both to diagnose women and promptly deliver ART.

Epidemiology and Transmission Rates

In 2013, an estimated 3.2 million children <15 were living with HIV, most of whom acquired HIV from their mother (UNAIDS 2014, Fig. 1a). An estimated 240,000 children acquired HIV in 2013, the majority (>90%) in sub-Saharan Africa (Fig. 1b). Infant HIV-1 incidence mirrors maternal HIV-1 prevalence. Thus, regions in South and East Africa, with the highest maternal HIV-1 prevalence have typically had the highest numbers of HIV-1-infected infants. However, the global number of new infant HIV-1 infections continues to decrease from its peak in the previous years (>500,000 per year), reflecting expansion of programs to prevent MTCT (PMTCT); some countries with high maternal HIV-1 prevalence have had exceptionally high PMTCT program coverage, leading to marked reductions in infant HIV-1 incidence.

MTCT includes ~5–10% risk in utero, ~10–20% risk at delivery, and ~10–20% risk

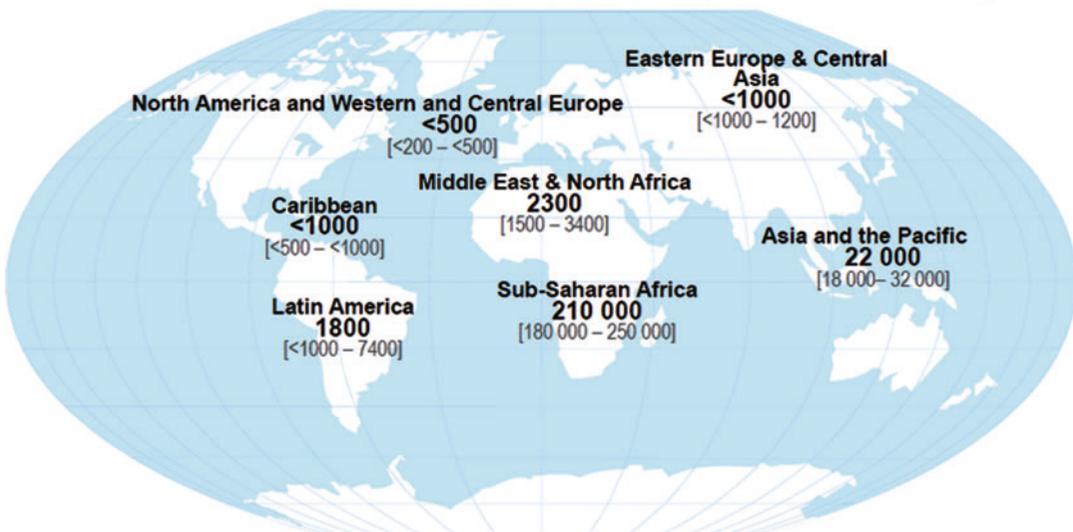
a Children (<15 years) estimated to be living with HIV | 2013



Total: 3.2 million [2.9 million – 3.5 million]



b Estimated number of children (<15 years) newly infected with HIV | 2013



Total: 240 000 [210 000 – 280 000]



Preventing Mother-to-Child Transmission of HIV-1, Fig. 1 Global Estimates of Children Living with HIV and New Infant Infections UNAIDS 2014 report (UNAIDS epidemiology slides 2012)

through breastfeeding (De Cock et al. 2000). Vertical HIV-1 transmission (or MTCT) has several features that differ from sexual HIV-1 transmission: a higher risk of transmission, three types of transmission (in utero, intrapartum,

breastfeeding), and an easily recognized time-limited period of HIV-1 exposure. In utero HIV-1 transmission predominantly occurs during the last trimester of gestation and intrapartum transmission usually occurs within a <24-h

period. Breast milk HIV-1 transmission occurs throughout breastfeeding with cumulative exposure to hundreds of liters of HIV-1-infected breast milk. Breast-milk HIV-1 infectivity has been modeled with estimated transmission risk of 0.00064 per liter or 0.00028 per day. The risk per liter of breast milk or per day of breastfeeding is similar to the risk per coital event in sexual transmission (Richardson et al. 2003). Cumulative exposure and duration of breastfeeding contribute incremental transmission risk. There is some evidence to suggest that the early postpartum period has a higher breast-milk HIV-1 transmission probability than the later postpartum period (De Cock et al. 2000).

Mechanisms and Cofactors of MTCT

As in most infectious diseases, the *transmitter pathogen burden* strongly influences transmission efficiency in MTCT (Table 1). *Host susceptibility* also influences transmission risk. Finally, the *interface between mother and child* may vary, contributing to a likelihood of transmission.

Transmitter pathogen burden: Maternal HIV-1 viral load is the most important predictor of MTCT (John and Kreiss 1996). Maternal systemic plasma HIV-1 RNA is highly associated with MTCT. Infants encounter HIV-1 transplacentally while in utero, during passage through the infected birth canal, and via breastfeeding. HIV-1 is detectable in cervical and vaginal secretions and in breast milk. While the levels of HIV-1 in these compartments are correlated with systemic HIV-1

levels, there can be compartmental differences in HIV-1 levels. HIV-1 levels in genital secretions and breast milk have each been associated with MTCT, *independent* of systemic HIV-1 burden, demonstrating the importance of HIV-1 load at the mucosal site to which the infant is exposed (John et al. 2001).

Both cell-free and cell-associated HIV-1 levels have been associated with MTCT (Rousseau et al. 2004). Comparing transmission risk due to cell-free versus cell-associated virus is difficult because these parameters are highly correlated with each other. Recent studies have suggested local mammary HIV-1 replication occurs with evidence that maternal-child cell-cell transfer of HIV-1 is the key route of breast milk MTCT (Van de Perre et al. 2012). This is consistent with the high early postnatal HIV-1 transmission at the time when breast-milk cellularity is highest (Rousseau et al. 2004). However, HAART, which markedly diminishes breast-milk HIV-1 RNA but has minimal effect on cell-associated HIV-1 DNA, significantly decreases MTCT, suggesting that HIV-1 RNA is the more relevant correlate of MTCT (Lehman et al. 2008). It is likely that both cell-free and cell-associated viruses contribute to MTCT.

Host susceptibility: Evidence for the role of host susceptibility includes increased risk among preterm infants who have immature immune development (although reverse causality may explain this association) (John and Kreiss 1996). HIV-1-uninfected, exposed infants have detectable HIV-1-specific immune responses and are born with passively transferred maternal HIV-1

Preventing Mother-to-Child Transmission of HIV-1, Table 1 MTCT cofactors

Mother-transmitter	Interface between mother-infant variations in HIV-1 exposure	Infant-host
Systemic HIV-1 RNA (plasma)	Maternal coinfections (STIs/mastitis) increased HIV-1 replication at surface to which infant is exposed	Prematurity, low birth weight
Genital HIV-1 RNA, DNA	Vaginal delivery, first-born twin	Genetic and innate factors (HLA, SLPI, TLR polymorphisms)
Breast-milk HIV-1 RNA, cell-associated virus	Placental breaches (syphilis, malaria)	Humoral immune responses (HIV-1-specific neutralizing antibody passively transferred)
CD4 count	Breastfeeding	HIV-1-specific cellular immune responses

neutralizing antibodies (Lehman and Farquhar 2007). There is evidence that cellular and humoral HIV-1-specific immune responses contribute some protection from MTCT. Genetic factors may modify MTCT risk. Selected infant and maternal HLA types influence MTCT. Mother-infant pairs who share HLA types are more likely to have MTCT compared to pairs with more HLA discordance, suggesting the importance of diversified immune protection conferred from paternally HLA-derived cellular responses (Mackelprang et al. 2008). Innate factors such as SLPI or defensins in infant saliva may influence MTCT. Some studies have noted an increased susceptibility of female infants to HIV-1 infection.

Interface between mother and infant may influence a likelihood of exposure: The type of encounter between transmitter and host may modify transmission risk by providing more or less exposure to HIV-1 virus. Thus, vaginal delivery has been associated with a higher transmission risk, as has first- vs. second-born twin delivery (John and Kreiss 1996). Both of these findings suggest that passage through the HIV-1-infected birth canal or ascending genital HIV-1 infection following ruptured membranes contributes substantial HIV-1 exposure to infants at delivery, which in turn leads to infection. Alternatively, increased transplacental microtransfusions during labor may explain this association. Genital or breast-milk coinfections (with sexually transmitted infections or mastitis) may increase MTCT by increasing local HIV-1 replication as a result of inflammation, immune activation, and recruitment of HIV-1-infected lymphocytes.

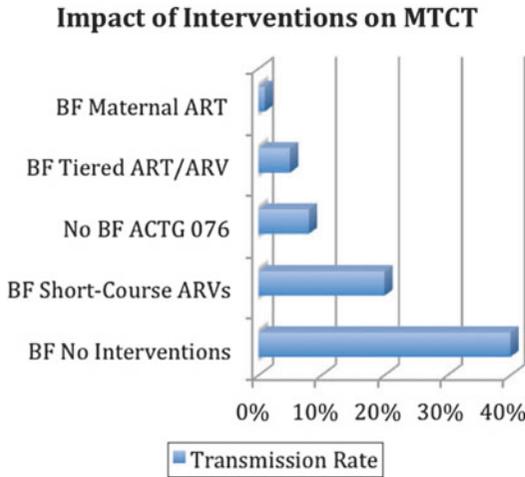
The place at which transmission occurs in the infant is undefined. Speculation from animal models note possibilities including tonsillar or gut infection (Abel et al. 2006). A series of studies noted increased breast-milk HIV-1 transmission when breastfeeding mothers introduced mixed feeding during the first few months postpartum (Young et al. 2011). It was speculated that gut permeability may be compromised by mixed feeding, leading to MTCT; however, there has not been data to support this. A recent study noted increased breast-milk HIV-1 RNA levels following the cessation of breastfeeding or during

mixed feeding when compared to exclusive breastfeeding (Kuhn et al. 2013). This study provides another potential explanation for increased MTCT during mixed feeding - that breast-milk HIV-1 levels increase. Ongoing studies are exploring the impact of exclusive versus mixed breastfeeding or replacement feeding on gut microbiota and immune development, both of which may influence susceptibility to HIV-1.

The breast milk of HIV-1-infected women contains a rich milieu of protective substances, including antimicrobial substances, cytokines, antibodies, and immune cells; breast milk has been noted to contain almost 3,000 substances in proteomic studies (Van de Perre et al. 2012). In murine studies, breast milk protected from HIV-1 infection, illustrating the dual role of breast milk in HIV-1 MTCT in increasing transmission risk because it contains the pathogen but protecting from transmission because of its many immune components (Wahl 2012). Antiretrovirals optimize the benefit/risk ratio of breastfeeding and enable long-term breastfeeding by HIV-1-infected mothers with minimal risk of transmission.

Prevention of MTCT (PMTCT) Research Landmarks and Guidelines

PMTCT has been a dynamic area of progress, following the landmark study ACTG 076, which was the first study in HIV-1 in general to demonstrate that antiretrovirals could decrease transmission (Paintsil and Andiman 2009) (Fig. 2). Infants receiving zidovudine monotherapy in ACTG 076 had a highly significant substantial reduction in MTCT from 24% to 7% (67% reduction), leading to guidelines to provide the zidovudine regimen to HIV-1-infected pregnant women and infants in resource-rich settings. However, the 076 regimen was not implemented in low- and middle-income countries (LMICs) because efficacy during breastfeeding was undefined, intravenous administration posed implementation challenges, and the regimen was cost prohibitive. To address these issues, trials were conducted to evaluate shorter “short-course” antiretroviral regimens without intravenous dosing, in



Preventing Mother-to-Child Transmission of HIV-1, Fig. 2 Mother to Child HIV Transmission Risk with Different PMTCT Regimens. Abbreviations. BF: breastfeeding. Short-course ARV: either short-course zidovudine or nevirapine. Tiered ARV/ART: ART-eligible receive ART, others ARVs also known as Option A

breastfeeding populations. These trials demonstrated that efficacy persisted with “short-course” regimens with some attenuation of efficacy due to regimen differences and further attenuation due to breastfeeding (~50% efficacy without breastfeeding and ~33% efficacy with breastfeeding). Remarkably, the HIVNET 012 trial, in which only a *single* dose of nevirapine (SD NVP) was administered both to mother and infant peripartum, demonstrated 50% efficacy in decreasing MTCT risk over 18 months of breastfeeding. This regimen revolutionized efforts to scale up PMTCT, because of its simplicity and low cost. Over time, combined regimens were used with higher efficacy. A final pivotal insight in PMTCT research came from several clinical trials among breastfeeding infants whose HIV-1-infected mothers did not receive ART in whom infant antiretroviral exposure prophylaxis significantly decreased MTCT (Kumwenda et al. 2008). These were the first studies to demonstrate the proof of concept that exposure prophylaxis could decrease HIV-1 transmission.

A variety of non-ART interventions were examined for effects in preventing MTCT. Elective cesarean section significantly decreased

MTCT versus vaginal delivery in a randomized trial conducted in Europe. Micronutrients, vitamin A, and chlorhexidine washes did not decrease MTCT in RCTs in Africa. In the absence of antiretrovirals, formula feeding was associated with decreased MTCT and HIV-free survival in an LMIC setting. However, cesarean section and replacement feeding became less relevant for PMTCT once antiretroviral approaches were implemented.

Breastfeeding HIV-1 transmission has been a key area of research, because MTCT through this route is substantial (16% among chronically infected and 29% in acutely infected women). Although avoiding breastfeeding spares infants’ exposure to HIV-1-infected breast milk, replacement feeding is risky in LMIC settings, leading to increased mortality and severe morbidity. Addressing breast-milk HIV-1 transmission has been challenging for policy-makers trying to optimize child health outcomes by preventing both HIV-1 and infectious morbidity and mortality. Mixed breastfeeding, replacement feeding, and early cessation of breastfeeding are associated with increased morbidity and mortality. Thus, the finding that maternal ART or infant prophylaxis could make breastfeeding safe has enabled policies to lengthen breastfeeding and avoid pitfalls of no or shortened breastfeeding. Over the past two decades, recommendations by WHO/UNAIDS underwent several changes as research matured – initially women in LMICs were recommended to breastfeed (1992), then to replacement feed if affordable, feasible, acceptable, sustainable, and safe (AFASS), but otherwise to exclusively breastfeed for 6 months then stop (2001), and then to use maternal ART or infant ARV prophylaxis through extended breastfeeding for at least a year (2010). The changes in breastfeeding recommendations have caused confusion for programs, mothers, and health-care providers (Young et al. 2011).

In 2004, funding from the Presidential Emergency Fund for AIDS Relief (PEPFAR) and Global AIDS Program (GAP) resulted in a widely expanded access to combination ART. This enabled women in LMICs who met ART eligibility (initially CD4 <200) to receive ART. Because

Preventing Mother-to-Child Transmission of HIV-1, Table 2 WHO Option A and Option B/B+ (From WHO 2012, April 12 programmatic update)

	Woman receives		Infant receives
	Treatment (for CD4 count ≤ 350 cells/mm ³)	Prophylaxis (for CD4 count > 350 cells/mm ³)	
Option A ^a	Triple ARVs starting as soon as diagnosed, continued for life	Antepartum: AZT starting as early as 14 weeks gestation	Daily NVP from birth through 1 week beyond complete cessation of breastfeeding, or, if not breastfeeding or if mother is on treatment, through age 4–6 weeks
		Intrapartum: at onset of labor, sdNVP, and first dose of AZT/3TC	
		Postpartum: daily AZT/3TC through 7 days postpartum	
Option B ^a	Same initial ARVs for both ^b		Daily NVP or AZT from birth through age 4–6 weeks regardless of infant feeding method
	Triple ARVs starting as soon as diagnosed, continued for life	Triple ARVs starting as early as 14 weeks gestation and continued intrapartum and through childbirth if not breastfeeding or until 1 week after cessation of all breastfeeding	
Option B ⁺	Same for treatment and prophylaxis ^b		Daily NVP or AZT from birth through age 4 to 6 weeks regardless of infant feeding method
	Regardless of CD4 count, triple ARVs starting as soon as diagnosed, ^c continued for life		

Note: “triple ARVs” refers to the use of one of the recommended 3-drug fully suppressive treatment options

^aRecommended in WHO 2010 PMTCT guidelines

^bTrue only for EFV-based first-line ART; NVP-based ART not recommended for prophylaxis (CD4 > 350)

^cFormal recommendations for Option B+ have not been made, but presumably ART would start at diagnosis

MTCT is highest in women with immunosuppression and high viral load, provision of ART to ART-eligible women substantially decreased MTCT. In these “tiered” programs, in which ART-eligible women received ART and ART-ineligible women received zidovudine/nevirapine prophylaxis, MTCT rates decreased to ~5%. Rates below 5% have been noted with either ART to all women or tiered ART/ARV approaches with provision of infant antiretroviral prophylaxis throughout breastfeeding (Jamieson et al. 2012). Remarkably, in a Botswana study of maternal ART administered through pregnancy and 6 months of breastfeeding, overall MTCT was only 1% (Shapiro et al. 2010).

Since 2009, WHO has recommended two options – Option A and Option B/B+ (Table 2). Both provide ART to ART-eligible women; Option A provides combined prophylaxis regimens to ART-ineligible women/infants, while Option B provides ART to all women, even ART-ineligible women. Both Option A and B end after lactation ends, while Option B+

continues with lifelong ART for women. While there has been healthy debate about the relative merits of the two options, there is a momentum to move to Option B/B+ as UNAIDS targets “virtual elimination” of pediatric HIV by 2015. Cost considerations are approximately equivalent; Option B/B+ may contribute decreased sexual transmission to partners, less delay in initiating interventions in pregnancy because there is no need to stage women to determine eligibility, and alignment with treatment program regimens. Recently, the PROMISE study, an RCT comparing Option A to B demonstrated lower MTCT in mother-infant pairs randomized to Option B (presented at CROI 2015).

Mechanisms of PMTCT ART Efficacy

Antiretrovirals result in decreased systemic, genital, and breast-milk HIV-1 RNA levels. In addition, breastfeeding infants ingest maternal antiretrovirals via breast milk, often resulting in

appreciable drug levels. Thus, both transplacental and breast-milk ART provide dual protection through decreased maternal virus and transferred antiretrovirals to the infant which provide prophylaxis. Nevirapine efficacy to decrease transmission resulted from both sustained and decreased maternal HIV-1 and infant prophylaxis – its long half-life resulted in decreased breast-milk viral load for ~2 weeks following a single maternal dose (Chung 2007). In infants whose mothers have not received any antiretrovirals, infant NVP prophylaxis was shown to significantly decrease transmission in randomized trials (Kumwenda et al. 2008). Maternal ART and infant prophylaxis may provide similar protective efficacy for breast-milk HIV-1 transmission among ART-ineligible mothers (Jamieson et al. 2012). Both approaches require adherence and maternal ART needs to have resulted in maternal viral suppression to exert its optimal prevention effect. Thus, for mothers starting ART postpartum, a period of infant prophylaxis for 4–6 weeks is necessary to protect infants until maternal viral suppression occurs.

Nevirapine (NVP) monotherapy, while effective for PMTCT, compromises future ART effectiveness among women. Following nevirapine monotherapy, the majority of women had evidence of NVP resistance mutations. Combining NVP monotherapy with a “tail” of other antiretrovirals decreased the likelihood of resistance and became the recommended approach. Alternatively, the earlier use of other prophylaxis (zidovudine) or ART in Option A or B also decreases the development of NVP resistance.

Implementation of PMTCT

Following the identification of efficacious PMTCT regimens, the broad implementation of PMTCT posed the next challenge. The PMTCT delivery cascade involves diagnosing women with HIV in pregnancy, providing ARV regimens and following women and children to ensure adherence to PMTCT regimens and early infant diagnostic testing. Decentralized point-of-care rapid assays to accelerate the diagnosis of

HIV-1 among pregnant women were critical for programmatic expansion. Similarly, “opt-out” counseling programs with group pretest counseling, which normalized HIV testing only excluding women who asked not to be tested, contributed wider HIV testing coverage. CD4 staging posed a persistent bottleneck, leading to noninitiation or delayed initiation of PMTCT while waiting for CD4 results and prompting enthusiasm for Option B strategies, which did not require CD4 counts. In 2011, Malawi opted for a national program that provided Option B+, with early program data noting ~77% retention at 1 year with increased coverage of HIV-1-infected women compared to the previously used Option A approach (Chimbwandra et al. 2013). In the next decade, many countries are planning to transition to Option B/B+, and WHO/UNAIDS has provided toolkits to facilitate national programs in this transition. While there has been a persistent concern that stigma and partner influence may be barriers to PMTCT implementation, health systems may pose the more critical determinant of uptake. When antenatal clinics reliably offer rapid opt-out HIV testing, maternal HIV test uptake of >90% has been observed.

There is a marked heterogeneity in PMTCT coverage – for example, there is a high coverage in Botswana and South Africa and low coverage in Nigeria and Congo (Fig. 3). Systematic PMTCT evaluation methods enable objective comparisons of PMTCT programmatic performance by measuring infant HIV transmission rates. National surveys of 6-week olds attending routine child health immunization visits (attended by >90% of children) have been designed to incorporate HIV testing; this enables sampling of infants whose mothers attended or did not attend PMTCT, yielding a population infant HIV-1 transmission estimate. Recently, one such country-level PMTCT evaluation in South Africa (which was using Option A at the time) demonstrated low 6-week MTCT rates at a population level of 2.7%, reflecting a high population PMTCT coverage, good adherence to regimens, and effective regimens (Barron et al. 2013). Future evaluation methods will need to assess older infants to



Preventing Mother-to-Child Transmission of HIV-1, Fig. 3 PMTCT Intervention Coverage and Effectiveness in Different Countries. Source: UNAIDS World AIDS Day Report 2012

capture later breast-milk HIV-1 transmissions and estimate HIV-1-free survival.

As PMTCT expands, an increasing number of HIV-exposed uninfected (HEU) infants will emerge. HEU infants have been observed to have increased susceptibility to infections, morbidity, and mortality (Heidari 2011). The reasons for this increased susceptibility are undefined -- HIV-1 exposure has been associated with changes in immune development and vaccine responses. However, HIV-1 exposure often includes ART

exposure, maternal coinfections, and social factors, each of which may influence infant outcomes.

New Directions

HIV-1 MTCT research has been an area of rapid translational impact and has yielded critical insights for HIV-1 prevention in general. The unfortunately high time-limited risk of MTCT in

the absence of interventions enabled relatively small cohorts to quickly identify cofactors for transmission and effective preventive interventions. Thus, MTCT was the first HIV-1 transmission model in which treatment for prevention and exposure prophylaxis for prevention were shown to be effective in clinical trials, foreshadowing trials and results in adult sexual transmission.

Following clinical trials, PMTCT implementation research and advocacy led to programmatic innovations and expansion, which promise to continue to rapidly decrease infant HIV-1 infections worldwide. However, despite the impressive expansion of PMTCT programs, novel strategies for preventing infant HIV-1 are necessary to compensate for residual challenges in coverage, retention, and adherence. Vaccine approaches that combine passive antibody and induction of adaptive responses, analogous to hepatitis B vaccines, remain attractive because of their potential feasibility and scalability. High priorities for future MTCT research include optimization of PMTCT (regimens, outcomes, and programs) and development of infant HIV-1 vaccine strategies.

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Prevention Clinical Trials: Highlights of Evidence and Research

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Introduction

Despite a growing trend in global declines in new HIV infections and AIDS-related deaths since 2005, HIV remains a global health challenge.

The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that 36.7 million people were living with HIV in 2015 and a further 2.1 million people acquired HIV during 2015. About 80% of new HIV infections are sexually acquired and this remains the dominant mode of transmission. About two out of three new HIV infections occur in sub-Saharan Africa and one out of three new infections are in young people aged 15–24 years; notably in young and adolescent women. Reducing sexual transmission of HIV is critical to altering the current epidemic trajectory in many parts of the world but remains an ongoing challenge. Prevention of sexual transmission can be achieved through reduction in the number of discordant sexual acts and/or reduction of the probability of HIV transmission in discordant sexual acts but, in real life, this is a more complex interplay of amongst other issues, lack of knowledge of HIV status, biology, gender-power dynamics, and limited options for women unable to negotiate safer sex practices. A substantial body of literature exists that capture extensive efforts to advance HIV prevention efforts. We focus this chapter only on evidence from randomized controlled trials as highest level evidence to inform programmatic scale-up. Compared to the first 29 years of the HIV epidemic, the last 7 years has witnessed a dramatic transformation regarding HIV prevention, primarily around the prophylactic and systemic use of antiretroviral agents. This chapter reviews the HIV prevention clinical trials that have been conducted up until 2015 for the prevention of sexual transmission of HIV and highlights some of the future directions in HIV prevention science.

Treatment of Sexually Transmitted Infections

There is a bidirectional relationship between HIV and other sexually transmitted infections. HIV transmission and acquisition is enhanced in the presence of other bacterial and viral sexually transmitted infections (STI), particularly ulcerative infections such as syphilis, chancroid, and *Herpes simplex* type-2 virus infection. Genital

Study

HPTN 052 – antiretroviral treatment as prevention (Discordant couples – Africa, Asia, Americas)

IPERGAY – on demand oral Truvada (MSM – France)

PROUD – daily oral Truvada (MSM – United Kingdom)

Partners PrEP – daily oral Truvada (Discordant couples – Kenya, Uganda)

Partners PrEP – daily oral Tenofovir (Discordant couples – Kenya, Uganda)

TDF2 – daily oral Truvada (Heterosexuals men and women- Botswana)

Orange Farm, Rakai, Kisumu – medical male circumcision (Men – South Africa, Uganda, Kenya)

iPrEx – daily oral Truvada (MSM- America's, Thailand, South Africa)

Mwanza trial – sexually transmitted infection treatment (Men and women - Tanzania)

CAPRISA 004 – coital Tenofovir gel (Women – South Africa)

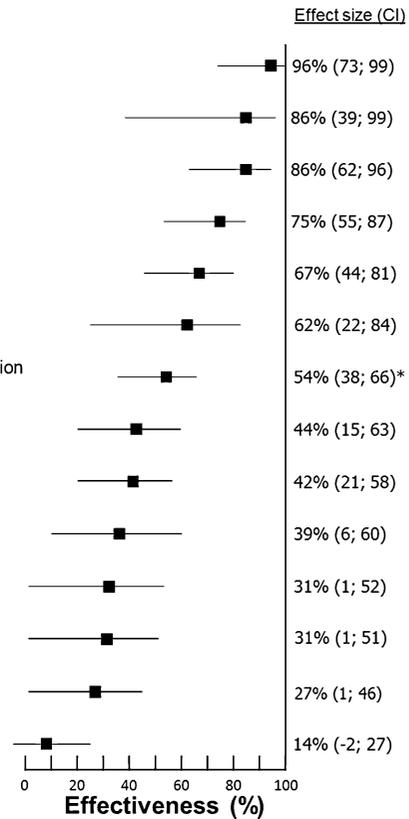
RV144 – HIV vaccine (Men and Women – Thailand)

Ring Study – monthly dapivirine vaginal ring (Women – South Africa, Uganda)

ASPIRE – monthly dapivirine vaginal ring (Women – Malawi, South Africa, Uganda, Zimbabwe)

Project accept – HIV counseling and testing (Men and Women – Africa, Thailand)

*meta analysis of 3 trials



Prevention Clinical Trials: Highlights of Evidence and Research, Fig. 1 Randomized controlled trials demonstrating a reduction in sexual transmission of HIV infection. *Meta-analysis of three studies. Note the Bangkok

tenofovir study among people who inject drugs has not been included as it is not possible to distinguish between HIV transmission that occurs through sex and that which occurs parenterally

ulceration or inflammation caused by STIs increase the infectiousness of HIV positive individuals and the susceptibility of HIV negative individuals.

Nine clinical trials have assessed the impact of STI treatment on the incidence of HIV infection, with some variation in approach, but, to date, only one trial has demonstrated a significant impact on preventing HIV infection (Ng et al. 2011). The Mwanza community randomized controlled trial, conducted in rural Tanzania among 12,537 individuals, showed that improved STI case management at primary health care level reduced incident HIV infections by 42% (Grosskurth et al. 1995) (Fig. 1). This was the first randomized trial to demonstrate an impact of a preventive intervention on HIV acquisition in the general population.

Although none of the eight subsequent STI treatment trials have replicated this finding, the treatment of STIs remains an important public health priority and is included in HIV prevention efforts programmatically as STIs are a major contributor to the global burden of disease, causing substantial illness, severe medical complications, and infertility.

Medical Male Circumcision

Since the 1980s, over 30 observational studies have suggested a protective effect of male circumcision on HIV acquisition in heterosexual men. These studies have been confounded by a number of factors including religion, behavior, and timing

and age of circumcision. In 2002, three independent randomized control trials were initiated in sub-Saharan Africa. Uncircumcised, HIV uninfected males from the general population in South Africa (N = 3,274), Uganda (N = 4,996), and Kenya (N = 2,784) participated in these trials. All three trials demonstrated a consistent HIV prevention benefit of 50–60% of forceps guided medical male circumcision in reducing female-to-male transmission of HIV (Auvert et al. 2005; Bailey et al. 2007; Gray et al. 2007) (Fig. 1).

Although male circumcision has limited immediate benefit to women, it could potentially impact on HIV incidence rates in women in the long-term through diminished exposure to HIV because of lower HIV levels among circumcised men. Evidence for the impact of circumcision on the epidemic trajectory at community level is starting to accumulate. Scale-up of voluntary medical male circumcision (VMMC) from 12% in 2007 to 53% in 2010 in one South African community has led to a 19% reduction in HIV prevalence and up to 61% reduction in incident HIV infection (Auvert et al. 2013).

Notable progress is being made in scaling up voluntary medical male circumcision (VMMC) programs as an HIV prevention strategy in 14 priority countries with HIV burden and low male circumcision rates. Approximately 11.6 million adult African men were circumcised for HIV prevention by the end of 2015, but more needs to be done to realize its public health benefit at a country level. Targeting of VMMC to young men, demand creation for, and uptake of VMMC by early adopters are some of the innovative approaches being utilized in sub-Saharan Africa to increase uptake and thereby impact HIV infection rates in these settings. The recent introduction of nonsurgical technologies such as the ShangRing and PrePex devices may facilitate task-shifting of circumcisions from doctors to mid-level health professionals, including nurses. These innovations and strategies are helping countries accelerate access to VMMC services. Data on the preventive benefits of VMMC in MSM populations are conflicting, but a Chinese observational study suggests that a clinical trial is needed (Qian et al. 2016).

Antiretroviral Treatment as Prevention (TasP)

Remarkable progress has been made in scaling-up antiretroviral therapy (ART) for the treatment of AIDS, with an estimated 18.2 million people receiving ART by June 2016. In addition to averting an estimated 7.6 million deaths globally since 1995, ART has the added benefit of preventing new HIV infections through the suppression of viral replication in the infected individual. Seminal papers published by Quinn et al. (2000) and Fideli et al. (2001) highlighted the positive correlation of plasma viral load and the risk of female and female-to-male transmission of HIV. A systematic review and meta-analysis of studies of HIV transmission among heterosexual couples, where the HIV-positive partner had been on ART for at least 6 months, estimates the transmission rate to be <13/100,000 unprotected sex acts. Observational data from the PARTNERS study, which included both heterosexual and men who have sex with men (MSM) couples, show zero-linked transmission events in discordant couples when the HIV-positive partner has a viral load <200 copies/mL.

The HPTN 052 randomized controlled trial among 1,763 HIV-discordant couples from nine countries, comparing early therapy (immediate ART initiation) to delayed therapy (ART initiation after a decline in the CD4 count or the onset of AIDS-related symptoms), showed that early initiation of ART in HIV-positive patients with CD4 counts between 350 and 550 cells/mm³ reduced HIV transmission by 96% (95% CI: 73–99.5) (Cohen et al. 2011) (Fig. 1). The reduction in HIV transmission based on this strategy is durable and ongoing follow-up for 5 years of this cohort revealed a 93% (95% CI, 78–99.9) lower risk of linked partner infection than was delayed ART (Cohen et al. 2016), thus providing compelling evidence of the prevention benefits of antiretroviral treatment also referred to as Treatment as Prevention (TasP).

Although there was some preliminary evidence from rural KwaZulu-Natal showing that a modest 30–40% ARV treatment coverage and initiation at CD4 counts <200 copies/mL,

resulted in a 34% reduction in new HIV infections, a large cluster-randomized trial assessing the universal test and treat (UTT) approach was unable to demonstrate a population level impact. The ANRS 12249 TasP trial, which was implemented in 22 rural communities in KwaZulu-Natal, South Africa, showed that although there was a high acceptance of home-based HIV testing and high levels of viral suppression among individuals on ART, overall linkage to care was suboptimal and there was no overall reduction in HIV incidence (Iwuji et al. 2016). Further, although countries like Botswana have reached the UNAIDS HIV treatment targets of 90-90-90, they continue to experience high community HIV incidence rates (Abdool Karim 2016). These results highlight the need for a more nuanced understanding of transmission dynamics, treatment coverage rates needed and timing thereof for epidemic control. Importantly, it is unlikely that TasP on its own is likely to achieve epidemic control but will need to be combined with a local understanding of the epidemic, an understanding of what the drivers of transmission are and targeting an appropriate combination of interventions most suited for the evolving epidemic at a local level.

Oral Pre-exposure Prophylaxis (PrEP)

The use of antiretroviral (ARV) agents as pre-exposure prophylaxis (PrEP) to reduce HIV transmission from infected mothers to their unborn infants has made a substantial impact on reducing HIV infection in infants. It was not until the early 2000s that this approach started to be explored to prevent sexual transmission of HIV.

Since 2010, the evidence for the protective benefits of PrEP has been consistently growing (Fig. 1). Results from seven randomized trials assessing prevention benefits of prophylactic use of oral tenofovir-based ARV agents provide compelling evidence that ARVs can prevent sexual transmission of HIV in men who have sex with men and heterosexual men and women in daily and intermittent dosing strategies ranging from 44% to 86%. Daily oral PrEP has also been

shown in one study to be effective in a population of people who inject drugs (Choopanya et al. 2013).

The first oral PrEP study to demonstrate an impact on HIV prevention was the iPREX trial, which showed that the daily oral tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) combination (Truvada[®]) reduced HIV incidence by 44% (95% CI: 15–63) among 2,499 men or transgender women who have sex with men (Grant et al. 2010). Evidence for the effectiveness of daily oral PrEP in heterosexual men and women comes from results of the PartnersPrEP trial (Baeten et al. 2012) and the Botswana TDF2 trial (Thigpen et al. 2012). The PartnersPrEP trial, which included 4,758 HIV discordant couples from Kenya and Uganda, showed that daily oral TDF and TDF-FTC reduced HIV incidence by 67% (95% CI: 44–81) and 75% (95% CI: 55–87) respectively, while the Botswana TDF2 trial, conducted among 1,200 heterosexual men and women from the general population, found that daily oral TDF-FTC reduced HIV incidence by 62% (95% CI: 22–83) (Fig. 1).

Two additional trials – FEMPrEP and the VOICE – conducted exclusively among women were not able to demonstrate a protective effect against HIV due to suboptimal adherence (Marrazzo et al. 2015; Van Damme et al. 2012). The HIV incidence in the FEM-PrEP study, which was conducted in 1,951 women aged 18–35 years from South Africa, Kenya, and Tanzania, was 4.7 per 100 person-years in the TDF-FTC group and 5.0 per 100 person years in the placebo group. Drug level testing revealed that <40% of women had evidence of recent pill taking. The Vaginal and Oral Interventions to Control the Epidemic (VOICE) trial assessed daily oral TDF, daily oral TDF-FTC, or daily 1% tenofovir vaginal gel (discussed below in the microbicide section) as PrEP against HIV infection in 5,029 women at 15 trial sites in South Africa, Uganda, and Zimbabwe. The effectiveness in the VOICE trial was –49.0% with TDF and –4.4% with TDF-FTC. Tenofovir drug levels were only detectable in 30% and 29% of available plasma samples from participants randomly assigned to receive TDF and TDF-FTC, respectively.

Given the anti-HIV specificity of PrEP, adherence is essential for its success. There is a strong correlation between high rates of adherence and effectiveness. Given that adherence can be challenging, even in a clinical trial setting, high levels of adherence may be difficult to achieve in “real world” settings where PrEP may be implemented in underdeveloped public health care facilities without adequate attention to adherence support. Not only will poor adherence contribute to sub-optimal protection, but it may also impact on the emergence of drug resistant strains of HIV akin to experience with tuberculosis.

The IPERGAY trial assessed an “on demand” PrEP dosing strategy in a cohort of HIV uninfected MSM wherein participants took two pills 2–24 h before each act of sexual intercourse, another pill 24 h after sex, and a fourth pill 48 h after sex. An 86% reduction in the incidence of HIV with on demand PrEP (95%CI: 39.4–98.5%, $P = 0.002$) (Molina et al. 2015) was achieved compared to placebo. These results have provided the first evidence that intermittent oral PrEP is highly effective in preventing HIV infection in men who have sex with men.

One of the concerns about widespread scale up of PrEP is the potential for risk compensation (also referred to as behavioral disinhibition), where PrEP users may reduce their use of higher efficacy HIV prevention strategies (like condoms) or may engage in riskier sexual practices. Risk compensation is a potential concern when implementing any new suboptimal HIV prevention strategy and is not specific to PrEP. However, risk compensation could potentially undermine and even reverse the beneficial effects of PrEP, as shown by mathematical models on HIV epidemics in Botswana, Kenya, and southern India (Vissers et al. 2008). The Pragmatic Open-Label Randomized Trial of Pre-exposure Prophylaxis (PROUD) study in MSM utilizing 13 sexual health clinics in England showed that there was no evidence of increased risky sexual behavior among MSM taking daily oral PrEP and led to an 86% reduction in HIV incidence (Fig. 1).

This series of scientific breakthroughs in HIV prevention, combined with the approval in 2012 of the first antiretroviral drug (TDF-FTC) as PrEP

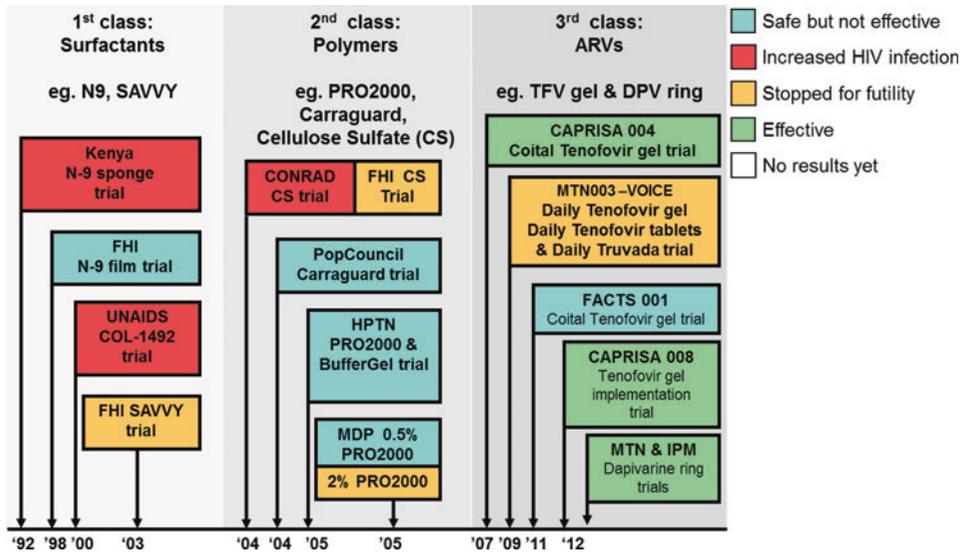
for reducing the risk of sexually acquired HIV infection by the U.S. Food and Drug Administration, has made the use of ARV drugs, as part of a comprehensive HIV prevention package, a reality, and has created newfound hope that the epidemic can be controlled, particularly among MSM. The use of PrEP in women appears to be a bit more complex and needs to be urgently unravelled.

Microbicides Including for the Prevention of HIV

Microbicides are topical agents applied in the genital tract to prevent HIV infection and were initially focused on vaginal products for HIV prevention in women in recognition of the urgent and growing need for women initiated technologies given this gap in current safer sex approaches that include abstinence, behavior change, use of male and female condoms, and counselling and testing which are all dependent on male partner initiation and or cooperation. Since then microbicide development has expanded to include topical agents that could be used rectally by men and women. Over the past 25 years of microbicide development, 15 advanced clinical trials of 8 candidate products (some tested as multiple doses and formulations) have been completed. To date only the CAPRISA 004 tenofovir gel trial and the two dapivirine ring trials have demonstrated a significant reduction in HIV acquisition in women (Fig. 2).

Early microbicide development focused on surfactants, e.g., nonoxynol-9 (Advantage 24; Columbia Research Laboratories, Rockville Center, NY) and SAVVY[®] (C31G, Cellegy Pharmaceuticals Inc., Huntingdon Valley, PA, USA), which act by disrupting cell membranes. Several formulations of nonoxynol-9 were evaluated but none were shown to provide protection against HIV and the phase III trial showed that the gel formulation of nonoxynol-9 may have increased the risk of HIV infection among women who used the product most frequently.

The next generation of microbicide candidates evaluated was vaginal defense enhancers, e.g. BufferGel, and attachment and fusion



Prevention Clinical Trials: Highlights of Evidence and Research, Fig. 2 Randomized controlled trials assessing microbicide candidates

inhibitors, e.g. Carraguard, PRO2000, and Cellulose Sulphate. Although the HPTN 035 trial, which assessed 0.5% PRO2000 compared with placebo, showed a signal for protection, the much larger MDP 301 trial, also testing PRO2000, showed no protective effect against HIV. Trials of Cellulose Sulphate were stopped in 2007 due to safety concerns.

The current category of microbicides that are being assessed in clinical trials includes antiretroviral agents that inhibit specific steps in the HIV life cycle.

Tenofovir gel was the first antiretroviral drug that was shown to significantly reduce the risk of both HIV and HSV-2 acquisition in women. In 2010, the CAPRISA 004 tenofovir gel trial showed that HIV acquisition was reduced by 39% and HSV-2 by 51% when tenofovir gel was used before and after sex (Abdool Karim et al. 2010). Despite these very encouraging results, even with high levels of adherence, tenofovir gel was shown to provide a moderate 54% protection and in women with detectable drug levels of >1,000 ng/ml tenofovir gel's effectiveness reached 74% (S. S. Abdool Karim et al. 2011). Two other trials – the VOICE and FACTS 001 trials – which also assessed tenofovir gel, showed no protective effect

against HIV. The VOICE trial, which evaluated a daily dosing regimen, showed that the effectiveness of tenofovir gel was 14.7% (95% CI: -21;40) (Marrazzo et al. 2015). The lack of protection observed in this study was primarily due to the low levels of adherence; estimated, based on detectable drug levels, to be 25% (Marrazzo et al. 2015). The Follow-on African Consortium for Tenofovir Studies (FACTS) 001 trial (CONRAD 2011), which included 2,059 South African women and assessed the same dosing strategy as the CAPRISA 004 trial, showed no overall HIV prevention benefit of the gel. However, a cohort analysis of 214 participants in the TFV-treated group showed a 52% reduction in HIV infection when tenofovir was detected in genital fluid.

Research on tenofovir gel is continuing. Tenofovir gel is currently being studied by the Microbicide Trials Network as a rectal application in MSM in several countries, including South Africa. CAPRISA is in the process of completing the CAPRISA 008 trial, which assesses gel adherence in women receiving tenofovir gel in conjunction with family planning. This real world implementation study may shed additional light on adherence when using a product that users know works. Further, a vaginal ring with both

tenofovir and a contraceptive is being developed with the aim of improving tenofovir adherence in women who are also strongly motivated to prevent pregnancy.

Focus in the microbicide field has also shifted toward developing products that are less dependent on user compliance and products that can meet multiple sexual reproductive health needs, for example STI and pregnancy prevention. Data from two long-acting antiretroviral intravaginal ring trials, which release drugs slowly over a 28 day period, were released in 2016 and showed that the dapivirine vaginal ring reduced HIV incidence overall by 27% (95% CI: 1–46%) in the Microbicide Trials Network-funded ASPIRE trial and by 31% (95% CI: 1–51%) in the International Partnership for Microbicides Ring trial (Baeten et al. 2016; Nel et al. 2016) (Fig. 2). However, the results show that protection differed by age; with no protection observed in young women below the age of 21, possibly due to lower rates of adherence in this age group (Baeten et al. 2016). More research is needed to fully understand and overcome the remaining challenges for sustained adherence in this high-risk population. An open label post-trial access study is ongoing and will hopefully contribute to our understanding of the contextual factors that impact on adherence in young women.

Long-Acting Biologicals

There are two categories of promising long-acting products for HIV prevention in early stages of clinical development. The first category includes prophylactic use of ARV based injectable agents and the second category is the use of broadly neutralizing antibodies as immunotherapy.

Long-acting antiretroviral injectable agents such as rilpivirine (TMC278) and cabotegravir (GSK1265744), which can be administered every 2–3 months, are in early stages of clinical testing as potential microbicide / PrEP agents and offer the advantage of being less user dependent.

The potential of immunotherapy using broadly neutralising antibodies is discussed in the “[Vaccines](#)” section below.

Vaccines

There continues to be a compelling need for a safe and efficacious vaccine to prevent HIV infection. Developing such a candidate, however, has proved challenging. Initial HIV vaccine efforts were focused on generating neutralizing antibodies using recombinant monomeric envelope gp120 (AIDSVAX) as immunogen. This vaccine did not induce neutralizing antibodies and the phase III trials failed to show protection against HIV acquisition. Vaccines that focused on eliciting cellular immune responses, e.g., the adenovirus vector-based T cell vaccine, also failed to show a protective effect and may have increased risk of HIV infection in some individuals. In 2009, results from the RV144 trial in Thailand, which showed a protective effect of 31.2% (95% CI, 1.1 to 52.1; $P = 0.04$) has provided renewed hope that an HIV vaccine is possible (Rerks-Ngarm et al. 2009).

Since the RV144 trial, important progress is being made in the vaccine field. Specifically, new sites for neutralization, including a site at the gp120–gp41 interface, and isolation of even more potent neutralizing monoclonal antibodies to the V2 apex and the high mannose patch on gp120 have been identified. These neutralization sites will be important targets for a HIV vaccine (Corey et al. 2015). Preclinical studies have also shown that broadly neutralizing antibodies, while effective in preventing a SHIV challenge in monkeys, may also have therapeutic benefits.

Progress is also being made in moving vaccine candidates into early clinical testing. The most advanced candidate includes the mosaic Ad26 vectors that are likely to be used in conjunction with a trimeric envelope protein or mosaic MVA boost, as well as clade C ALVAC and NYVAC vectors that will be used in conjunction with an adjuvanted bivalent clade C gp120 protein immunogen. Clinical trials of an adeno-associated virus expressing a broadly neutralizing antibody directed at the V2 apex and passive transfer studies of potent neutralizing monoclonal antibodies have also been initiated. A recombinant CMV-HIV vector is also in development, which has shown over 50% efficacy in preclinical studies.

Structural and Behavioral Interventions

Behavior Change Intervention

A number of studies have assessed the impact of behavioral change interventions to prevent HIV. A systematic review in 2010 of 11 behavior change studies, assessing eight unique interventions, showed that only one study resulted in a reduction of HIV incidence. This study was conducted among 541 female sex workers in India and included group educational and motivational sessions over 6 months, and was shown to reduce HIV incidence after 1 year of follow up (IRR = 0.33, 95% CI: 0.15–0.72) (Bhave et al. 1995). More recently, the SHARE (the **S**afe **H**omes and **R**espect for **E**veryone) trial, a community-level mobilisation intervention in Uganda to change attitudes, social norms, and behaviors related to intimate partner violence (IPV) was shown to be associated with a 33% reduction in HIV incidence (aIRR 0.67, 95% CI: 0.46–0.97, $p = 0.036$) (Wagman et al. 2015).

Cash Incentives for HIV Prevention

A novel strategy being investigated is the use of cash incentives to reduce HIV risk. A systematic review of cash transfer studies in 2012 identified 16 studies, 10 of which have been completed. Most of the studies have been conducted in adolescents and most report reductions in risky sexual behavior such as delaying sexual debut, staying in school, and using condom. One cluster trial in Malawi of cash incentives for school attendance showed that weighted prevalence of HIV at 18 months was lower in the intervention communities (Baird et al. 2009) but none of the studies have demonstrated a reduction in HIV incidence. Data from two randomised trials assessing the impact of cash incentives on HIV incidence have recently become available. However, neither of the trials were able to demonstrate an impact of cash transfers on HIV incidence. The HPTN 068 Swa Koteka study ($n = 2,448$), which provided a cash incentives for school attendance, found no difference in HIV acquisition between the young women who received the cash transfer and those that did not (Pettifor et al. 2012). The CAPRISA 007 cluster randomised controlled

trial, which was undertaken in 3,217 consenting male ($n = 1,517$) and female ($n = 1,700$) students in grades 9/10 in 14 schools in rural KwaZulu-Natal, showed that conditional cash incentives for meeting any combination of four conditionalities (annual HIV testing, school performance, participation in a HIV prevention programme and participation in a community project) was associated with a 30% reduction in herpes simplex virus type 2 (HSV-2) incidence but did not have sufficient statistical power to demonstrate an impact on HIV incidence (Abdool Karim et al. 2015). The implementation of cash incentive programmes for HIV prevention in young women would need to be evaluated in the specific context and would most likely need to be complemented by other effective HIV prevention strategies. Results from the ongoing Iringa combination prevention community cluster randomized trial (Pettifor et al. 2012) in Tanzania among 1,800 15–24 year old girls, which is assessing the impact of unconditional cash transfer for parent/guardian and child on HIV incidence, is eagerly awaited.

HIV Counselling and Testing

Knowledge of HIV status is a vital gateway to treatment and prevention services but remains a challenge. HIV counselling and testing has been shown in a large multicenter study ($n = 4,293$), conducted in Kenya and Tanzania, to reduce risky sexual behaviors (The VCT efficacy study group 2000). Men and women randomized to receive HCT had significantly lower rates of unprotected intercourse with their primary partners than those receiving only health information (The VCT efficacy study group, 2000). A large cluster-randomized controlled trial, Project Accept, conducted in 34 communities in four sites in Africa and 14 communities in Thailand, showed modest reductions in HIV incidence combined with increases in HIV testing. HIV incidence in the intervention group was 1.52% compared to 1.81% in the control group, with an estimated reduction in HIV incidence of 13.9% (relative risk [RR] 0.86, 95% CI: 0.73–1.02; $p = 0.082$). HIV incidence was significantly reduced in women older than 24 years (RR = 0.70, 0.54–0.90; $p = 0.0085$), but not in other age or

sex subgroups. Community-based voluntary counselling and testing increased testing rates by 25% overall (12–39; $p = 0.0003$), by 45% (25–69; $p < 0.0001$) in men and 15% (3–28; $p = 0.013$) in women (Coates et al. 2014).

Conclusion

Over the past 20 years, over 40 randomized controlled trials to prevent HIV infection have been undertaken but, to date, only 16 have demonstrated significant reductions in HIV. The proof of concept evidence is only the first step. Programmatic scale-up of what works is key to translate science to action and public benefit. Medical male circumcision is already being implemented and scaled up as a prevention option for men in high HIV burden countries. The use of oral antiretroviral drugs, as treatment or as PrEP, has created newfound optimism in HIV prevention. ARVs increase options for HIV prevention, especially for specific high-risk populations such as MSM and HIV serodiscordant couples. There are no magic bullets but these scientific advances in combination could advance efforts to achieve epidemic control. The quest for women initiated HIV prevention technologies remain urgent and long-active products that are less dependent on user compliance are promising. The development of a safe and effective HIV vaccine will substantially advance the progress toward eliminating HIV infection.

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Prevention Counseling and Other Strategies in the HIV Care Setting

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Definitions

Prevention counseling is a form of psychological, educational, and practical support intended to prevent a particular issue, event, or condition. This type of counseling incorporates education and psychotherapy and can be provided for an individual, pairs, or a group. This type of counseling may focus on primary or secondary prevention and may be conducted by psychologists, psychiatrists, mental health counselors, nurses, physicians, social workers, prevention case managers, and other professionals. Prevention counseling is an important component of HIV counseling (CDC 2010), which also includes the provision of information.

HIV prevention counseling is “an interactive process of assessing risk, recognizing specific behaviors that increase risk of acquiring or transmitting HIV, and developing a plan to take specific steps to reduce risks” (CDC 2006). HIV prevention counseling focuses on counseling

strategies that either support and encourage (1) people living with HIV/AIDS (PLWHA) to make decisions and engage in behaviors that reduce their risk of transmitting HIV to others (secondary prevention) or (2) people who are at risk for HIV to make decisions and engage in behaviors that reduce their risk of contracting HIV (primary prevention). It may also be focused on tertiary HIV prevention which promotes health and prevention of HIV disease progression. Overall, HIV prevention counseling relies on interventions aimed at changing behaviors, including counseling and testing in various settings and formats (Mizwa and Gwebu-Storer 2010).

Primary HIV Prevention Counseling

The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) support prevention counseling across all settings for persons at high risk for HIV. CDC recommends that patients receive information about HIV testing, infection, and the meaning of test results – separate from or in conjunction with counseling (Mizwa and Gwebu-Storer 2010).

HIV Prevention Counseling Regarding HIV Testing

Information regarding the HIV test, estimated time to receive results, and the associated benefits and consequences can be provided in a face-to-face meeting with a counselor, in a brochure, pamphlet, or video (CDC 2006), or via telephone. Regardless of the type of HIV test, clients should be given information about the routes of HIV transmission, strategies to prevent transmission, the lay meaning of test results, and need for confirmatory testing if the screening test is reactive and where to obtain additional information and services, including treatment.

Fundamentals of HIV Prevention Counseling

HIV prevention counseling fundamentals include performing a personalized risk assessment, acknowledging and encouraging ongoing prevention efforts, clarifying myths and misconceptions about HIV risk and transmission, and maintaining a focus on risk reduction (CDC 2006). It is crucial

to tailor counseling strategies to the needs and situation of the client and to help each client establish realistic and achievable behavior change goals and a strategic plan that includes concrete strategies to reduce HIV risk (CDC 2006). Also critical to prevention counseling is the need to address and reduce the internalization of stigma by providing education about HIV in a nonjudgmental manner and by treating PLWHA with respect and compassion.

Disclosure

Disclosure refers to the revelation of information to another person or party. For PLWHA, disclosure of HIV status is often a stressful topic as they consider when and to whom to disclose. In some cases, disclosure is inherent to the nature of a client–professional relationship. In other cases, automatic disclosure may occur due to state reporting laws that mandate notification of positive sexually transmitted infections (STIs), including HIV, to local state health departments. Similarly, in many states, people who test positive for STIs or HIV are legally responsible for notifying their sexual partner(s) of their positive status and the partner’s potential exposure. PLWHA also face the choice of whether or not to disclose their HIV status to relatives, friends, or other members of their social networks. They may therefore need counseling to help them make these decisions, including understanding state regulations on and other potential consequences of HIV disclosure. Counseling about HIV disclosure can also highlight its role in promoting secondary prevention as it emphasizes the responsibility of an HIV-positive person to inform sexual partners of his/her status and create the opportunity for engagement in risk reduction behaviors.

Secondary and Tertiary HIV Prevention Counseling (Prevention for Positives)

CDC recommends that HIV care clinics incorporate prevention into clinical practice. Prevention counseling in the HIV care setting occurs across the continuum of HIV care, beginning at the point of entry and continuing throughout care with a focus on preventive maintenance.

Entry into HIV Care: Early HIV Prevention Counseling

Prevention counseling for newly diagnosed patients should commence immediately following diagnosis. Patients should be screened for transmission behaviors initially and at subsequent visits, in a straightforward, nonjudgmental manner. Counseling at the early stages of diagnosis and care may include assessment of transmission risk factors, provision of education about transmission, determination of coping resources, counseling regarding HIV disclosure, and adherence to antiretroviral treatment as a strategy to reduce transmission. In the early stages of HIV care, counseling is best offered through a supportive, collaborative environment that helps patients come to terms with their diagnosis while emphasizing the importance of safe behaviors to protect their health and the health of their partners.

Preventive Maintenance and AIDS Prevention Counseling

As PLWHA live longer, fuller lives due to advances in technology and medication, consideration should be paid to health maintenance across the life span. Preventive maintenance refers to the ongoing strategies utilized to maintain health among PLWHA and prevent progression from HIV to AIDS. Cognitive-behavioral interventions (CBIs) are a useful strategy, as they have been found to be effective in improving mental health and to relate to immune functioning and CD4 counts (Crepaz et al. 2008). CBI helps to buffer the impact of stress and depressive symptoms on immune function and can slow HIV disease progression. By focusing on the interaction between thoughts, feelings, and behaviors, CBI teaches participants stress management and coping skills that may be utilized in many aspects of life.

Similarly, AIDS prevention counseling focuses on the importance of adherence to treatment to maintain or enhance immune function and prevent the development of resistant strains of HIV, as well as on behavioral modification and risk reduction. PLWHA are counseled about symptoms that are suggestive of an immunocompromised status and warrant a visit to a healthcare provider.

PLWHA are also counseled on the importance of maintaining regular healthcare visits to assess their health and risk status and to monitor HIV viral load levels, CD4 cell counts, and other laboratory markers – regardless of how well they might feel. Depending on each PLWHA's CD4 cell count level, he/she will be counseled on the importance of beginning and maintaining AIDS prophylaxis treatments. PLWHA with a CD4 cell count less than 200 cells/ μ L will be prescribed prophylaxis for *Pneumocystis jiroveci* pneumonia (known as "PJP"), and those with CD4 cell counts less than 50–100 cells/ μ L will be prescribed prophylaxis for *Mycobacterium avium* complex (MAC) and toxoplasmosis. PLWHA are also counseled regarding exposures they should avoid if they do become immunocompromised. Along with counseling, many HIV care professionals regard HIV treatment as a critical form of prevention.

HIV Prevention and Counseling Strategies and Methods

A number of HIV prevention and counseling strategies are available, including treatment as prevention (TasP), preexposure prophylaxis (PrEP), postexposure prophylaxis (PEP), behavioral interventions, prevention case management (PCM), peer counseling, motivational interviewing, group counseling, and couples/partner counseling.

Treatment as Prevention (TasP)

Treatment as prevention (TasP) operates under the assumption that transmissibility of the HIV virus is reduced when there is less viremia in the body. Effective treatment with antiretroviral therapy (ART) results in lower concentrations of the virus in seminal and vaginal fluids and is considered to be an avenue for averting sexual HIV transmission. Evidence for the potential utility of TasP was first demonstrated through prevention of vertical transmission of HIV from pregnant mothers to their fetuses/neonates; treatment with ART during pregnancy and 6 weeks after birth reduces vertical transmission by up to 66%. Further evidence for the effectiveness of TasP was seen in observational studies of serodiscordant

heterosexual couples, in which one partner is HIV-positive and the other partner is HIV-negative, and indicated that treatment with combination ART (3 or more antiretrovirals in combination) could limit transmission of the virus to HIV-negative partners as much as 80–98%. Population-level studies outside the USA also indicate combination ART may lower community HIV viral load. The most compelling evidence to date for the potential of TasP was provided by the HIV Prevention Trials Network (HPTN) 052 clinical trial. HPTN 052 found that giving combination ART immediately to an HIV-positive partner resulted in a 96% reduction in phylogenetically linked transmissions compared to the standard of care group. HPTN 052 was conducted with heterosexual partners, and given that the transmissibility for penile–vaginal sex is lower than that of penile–rectal sex, these results, although promising, are not generalizable to epidemics driven by penile–rectal sex. In order to capitalize on findings from HPTN 052, attention must be given to HIV testing and linkage and engagement in care over the disease course. Nearly 80% of persons in HIV care are estimated to have suppressed HIV viral load; however, 25% of HIV-positive persons are not in HIV care. Efforts by HIV care settings to identify, link, and retain HIV-positive individuals in clinical care are essential to TasP (Forsyth and Valdiserri 2012).

Preexposure Prophylaxis (PrEP)

PrEP refers to an HIV prevention method in which HIV-negative persons take daily medication to reduce their risk of contracting HIV. This method of prevention is used among persons who are considered high risk and has been found to be effective among populations of men who have sex with men (MSM) and heterosexual men and women. In July 2012, the US Food and Drug Administration (FDA) approved the HIV antiretroviral medication tenofovir for use as PrEP. Tenofovir belongs to the antiretroviral class of nucleotide analogue reverse transcriptase inhibitors (NRTIs), which block reverse transcriptase (an enzyme crucial to HIV viral production).

Postexposure Prophylaxis (Emphasis on Healthcare Workers)

Postexposure prophylaxis (PEP) refers to treatment started immediately after exposure to a pathogen in order to prevent infection and disease progression. In the case of HIV infection, PEP is a course of antiretroviral drugs taken after possible or suspected exposure to HIV. Exposures can be occupational or behavioral, but PEP is most often provided to healthcare workers who are occupationally exposed. PEP should be administered as soon as possible and is most effective within 72 h of exposure. Generally, a person on a PEP regimen will take 2–3 types of antiretroviral drugs for a 28-day period (www.AIDS.gov).

Behavioral Interventions

Strategies to change the knowledge, attitudes, behaviors, or practices of individuals in order to limit health risks are known as behavioral interventions. Changes in behavior can be enabled through environmental approaches in clinical settings, messages from healthcare providers, or through referral to other groups providing prevention services. Environmental approaches include flyers with prevention risk and transmission information and posters containing prevention messages displayed in waiting or exam rooms. Straightforward, brief prevention messages delivered by providers can be of benefit to all HIV-positive patients, particularly since many lack specific knowledge on risks and methods for preventing transmission. These messages may include abstinence from sex and injection drug use and safe sexual practices such as consistent condom use. Prevention messages may be tailored to meet the needs of patients with specific identified risk behaviors or positive STD tests. Reinforcement of prevention messages may need to be subsequently carried out more intensely or for a longer duration by nurses, social workers, or health educators. Underlying issues that may impede successful implementation of safer behaviors are often outside of the expertise and resources of HIV clinicians. In these situations, behavioral interventions involve referral for services such as intensive HIV prevention interventions, medical services for family planning,

contraceptive counseling, substance abuse treatment, mental health services, and social services. Referrals for patients' self-identified priorities are more likely to be successful.

HIV prevention interventions that have been found to significantly reduce unprotected sex, especially among PLWHA, include those that (1) are guided by behavioral theory; (2) have more than 2/3 of sessions focused on HIV transmission behaviors; (3) provide skills-building; (4) include intensive session delivery (>10 sessions, >20 h); (5) address issues related to coping with HIV status, adherence, and risk behaviors; and are delivered (6) in one-on-one session(s), (7) by healthcare providers or professional counselors, or (8) in HIV care setting or setting where PLWHA receive services or (9) for greater than 3-month duration (Crepaz et al. 2006; Lyles et al. 2007; Temoshok and Wald 2008). Also important is the integration of HIV prevention intervention and counseling services into multidisciplinary services, including social services (Crepaz et al. 2006; Lyles et al. 2007; Temoshok and Wald 2008; Fisher and Smith 2009).

Prevention Case Management

HIV prevention case management (PCM) approaches combine aspects of traditional case management with HIV prevention counseling strategies. PCM has been described by CDC and others as an intensive, client-focused, multi-session intervention designed to increase clients' personal risk management abilities. PCM promotes behavioral changes that reduce the chance of HIV transmission. Case management settings offer direct opportunities for professional staff to use PCM techniques while simultaneously assisting clients with housing, counseling, and other material needs. Generally, HIV case managers are professionally trained social workers or counselors who have received additional training or have expertise in HIV prevention. Although few studies have assessed the impact of prevention case management approaches, some have demonstrated efficacy in reducing risk transmission behaviors including unprotected vaginal intercourse, insertive anal intercourse, and needle sharing among HIV-positive persons and their

negative or serostatus unknown partners (Gasiorowicz et al. 2005). Further, PCM approaches are well received among specific subgroups of PLWHA. HIV-positive MSM who participated in an intervention assessing the feasibility of a PCM program indicated that they experienced a comfortable environment and a nonjudgmental stance from counselors and appreciated the resources their case managers were able to provide.

Peer Counselors/Positive Care

Peer counselors are often semiprofessional or trained laypersons who share one or more characteristics with the individual or group intended to receive the intervention. Peer-delivered counseling or interventions are intended to create an opportunity for the client(s) to connect to both the content being delivered and the shared experience(s) of the peer counselor. An HIV-positive peer sometimes delivers secondary HIV prevention interventions to PLWHA. Similarly, HIV prevention interventions designed for high-risk populations such as MSM may also be delivered by HIV-positive MSM. Overall, positive peer counseling is well received by PLWHA (Driskell et al. 2010; Safren et al. 2011) and has been shown to reduce HIV transmission-associated risk behavior (Safren et al. 2011; Simoni et al. 2011).

Motivational Interviewing

Motivational interviewing (MI) is a person-centered counseling method used to address ambivalence about change and to elicit and strengthen a person's own motivation for and commitment to change (Miller and Rollnick 2010). The MI approach emphasizes three key elements including (1) a collaborative partnership between counselor and client, (2) exploration of the client's thoughts about changing his behavior, and (3) recognition and support of clients' autonomy as a change agent. The major principles that guide MI include expressing empathy (acknowledging the challenge of change), supporting self-efficacy (focusing on previous successes and highlighting the client's innate abilities and strengths), "rolling with resistance" (avoiding struggling/arguing with a client if he

presents resistance to change), and developing discrepancy (enabling clients to recognize the mismatch between values/goals and current behavior). Counselors employ several core behaviors during motivational interviewing in order to facilitate the process: primarily the use of open-ended questions, affirmations of clients' strengths, reflective listening, and summaries of all or parts of the counseling session. MI has been successfully used to address behavior change regarding a variety of topics (substance use, sexual risk reduction, medication adherence), in varied settings (inpatient care, outpatient clinics care centers), and with a number of different populations (children, adults). Among PLWHA, use of MI has been shown to reduce sexual risk behavior and also substance use, but to a lesser extent (Naar-King et al. 2012).

Group Counseling

Group counseling is delivered by a trained facilitator or counselor in a group format often with participants with similar issues or characteristics, such as MSM or HIV-positive women. As a method of HIV prevention counseling, it is typically conducted with small groups of 10–12 and relies on the sharing and exchange of experiences among participants.

Couples/Partner Counseling

Couples/partner counseling is conducted with couples or sexual or needle-sharing partners. This type of counseling has the benefit of simultaneously counseling the complete sexual relationship or needle-sharing dyad. Counselors are able to identify attitudes and behaviors of each partner that might increase the risk of both. They can also emphasize the importance of risk reduction behaviors and help couples to learn skills or engage in activities such as role-playing to facilitate safe behavior negotiation. HIV testing is another important component in couples HIV prevention counseling. Partners who test positive may need counseling to support their response to an HIV-positive status and/or ongoing support to address denial, blame, or anger (Curran et al. 2012). If both partners are HIV-positive, it is important for counselors to educate the couple

about their ability to transmit other STIs or different strains of the virus to each other. Couples counseling emphasizes the importance of each individual knowing the HIV viral load and CD4 cell count of their partner in order to better understand their risk for HIV disease progression.

Prevention Counseling Special Considerations

Serodiscordant Couples

Behavior change strategies available to serodiscordant couples include discussions regarding reduction of outside partners, abstinence, and correct and consistent condom use (Curran et al. 2012). Counselors emphasize the importance of HIV medication adherence and viral suppression to reduce the risk of HIV transmission to the HIV-negative partner. The option of PrEP, including the associated benefits and risks, for the HIV-negative partner may also be discussed.

Family Planning/Pregnant Women

Cases of perinatally acquired HIV have been declining for a number of years, largely due to increases in routine HIV testing of women during pregnancy, availability of antiretroviral therapy for maternal treatment and perinatal prophylaxis, and delivery options that reduce chances of transmission. Accordingly, the rate of perinatal transmission of HIV has dramatically diminished to less than 2% in the USA and Europe (Birkhead et al. 2010; Panel on ARV 2012). Perinatal HIV transmission reduction strategies operate at a system-wide level and include increasing availability of early HIV testing, increasing availability of rapid HIV testing during labor and delivery, and advocating for clinical standard of care changes that incorporate HIV testing into prenatal care (Fowler et al. 2010).

In the HIV care setting, both men and women living with HIV should be provided with family planning education and counseling, including information about their contraceptive choices, options to reduce the HIV-related risks associated with conception (particularly for serodiscordant couples), and the prevalence of perinatal,

antepartum, and maternal–infant HIV transmission. For PLWHA who express an interest in conceiving a child, healthcare providers can discuss options based on existing family planning standards for the planning phase, pregnancy, and during/after delivery. In the planning phase, family planning counseling for PLWHA and their partner may include an emphasis on medication adherence for the seropositive partner and the use of PrEP for seronegative partners in order to reduce HIV viral load and the risk of HIV transmission during unprotected intercourse. For seroconcordant HIV-positive partners, medication adherence counseling emphasizes HIV viral suppression and prevention of HIV-resistant strains. Counseling should include a discussion of the benefits of ARV drugs for pregnant women and the risks of adverse events to the woman and infant. Providers may also discuss options such as artificial insemination, in vitro fertilization (IVF), or surrogate mothers. Above all, a pregnant woman's informed decision on prevention of mother-to-child transmission of HIV should be respected.

Adolescents/Young Adults

Adolescents and young adults (AYA) face unique developmental challenges that lend themselves to a variety of prevention counseling approaches. Many comprehensive sexuality education programs including information around STI/HIV prevention have been implemented in a variety of settings. Generally, approaches taken with youth fall into one of several categories: curriculum-based programs, STI/HIV education programs focusing on parents and families, video- or computer-based interventions, clinic-based reproductive health programs, school-based health centers, or community-wide STI/HIV or pregnancy prevention programs. Across intervention types, curriculum-based education programs appear to show the greatest strength of evidence for positive effects on sexual behavior. A comprehensive review of 48 STD/HIV education programs found that nearly half of interventions reviewed showed efficacy in delaying initiation of sex, reducing number of sexual partners, and increasing condom use; 29% reduced frequency of sex;

and 69% impacted one or more sexual risk behaviors. Educational programs impact behavior through affecting psychosocial mediators such as HIV/STD knowledge, perceived risk of HIV/STDs, values and attitudes toward sexual health, and self-efficacy to avoid risky sexual behaviors and engage in safer ones (Kirby et al. 2009)

Older Adults (50+)

Although most HIV infections occur among the young, nearly one-fourth of all HIV/AIDS cases in the USA are among persons over the age of 50. Stigma associated with ageism leads to less discussion and knowledge about HIV risks among older adults. Compared to their younger counterparts, older adults living with HIV have a more severe HIV disease course and shorter survival rates, poorer health indicators at diagnosis, shorter AIDS-free intervals, a higher number of opportunistic infections, and earlier development of tumors and lesions. Risk in this group may be increased due to stigma around sexual behavior, an already compromised immune system, or age-related health problems (Linsk 2000). HIV rates among older women have surpassed that of men, and due to lack of fear of pregnancy, older women may not demand that partners use a condom (Maes and Louis 2003). Symptoms of HIV/AIDS may be misdiagnosed as signs of aging or attributed to another disease or condition. Moreover, older adults are less likely to have received prevention messages from healthcare providers and are less likely to be screened for HIV and receive early intervention. Since older adults are often assumed not to be sexually active and not to be at risk for HIV, routine testing for HIV is relatively uncommon. This results in delayed diagnosis and treatment at an early stage when HIV can be most readily treated and decrease risk of transmission to others. It is important to remember that recommendations for HIV prevention among older adults are the same as those for younger individuals.

Mental Illness/Substance Use

Mental illness and substance use concerns are important factors to consider when implementing

HIV prevention strategies with PLWHA. Specific mental health concerns may include stress relating to PLWHA's HIV diagnosis or physical health, relationships with partners and family, and stigma associated with being HIV-positive. Consequently, the prevalence of mood and anxiety disorders is elevated among populations of HIV-positive persons and should be addressed within the context of HIV prevention activities. Studies have shown a negative association between depressive symptoms and medication adherence, and within some subgroups, depressive symptoms also predict risky sexual behavior. Combined mental health and HIV prevention programs, particularly those utilizing cognitive behavioral techniques, have been demonstrated to have some success in reducing both sexual risk taking and in improving medication adherence.

Similarly, HIV prevention strategies for drug users should take into account the related social and psychological factors that influence the behaviors of PLWHA. Poverty, policies criminalizing drug use, and access to drug treatment are some of the factors contributing to the spread of HIV among drug users. Drug users, particularly of injection drugs, are at increased risk of HIV transmission as a result of sharing practices and the associated increase in risky sexual behaviors common among persons under the influence of alcohol or drugs. Behavioral interventions aimed at HIV prevention with both seronegative and seropositive drug users include drug abuse treatment programs, harm reduction approaches, and community outreach programs.

Prisoners/Criminal Justice System

HIV prevalence is over 5 times greater in the incarcerated population than in the general population. Although diagnosis and treatment of HIV in correctional institutions pose unique challenges, incarcerated individuals are likely to benefit significantly from such efforts. Since non-Whites are overrepresented in the prison population, HIV prevention efforts among the incarcerated could also impact HIV/AIDS disparities. Most prisons do not

test for HIV routinely, despite CDC's recommendation for routine opt-out HIV testing in correctional settings. Far more prisons use a risk-based HIV testing approach that is highly underutilized due to stigma and lack of confidentiality. In the few settings that have implemented opt-out testing, large proportions of inmates were tested, many of whom had never been tested previously. Rapid testing, which offers the benefit of results in about 20 min, assists with providing test results in prisons with high rates of turnover.

Benefits of HIV treatment in the general population have been replicated in correctional setting with suppression of HIV viral loads prior to release. However, provision of care is expensive, and adherence to medications, particularly in institutions requiring inmates to retrieve their medications in "pill lines" that seriously undermine confidentiality, remains a barrier to effect treatment. Linkage to care post-release is difficult and often undersupplied.

Reentry into the community is a time of high stress often leading to risky behavior. Successful case management approaches have focused on HIV risk reduction, substance use counseling, and linkage services as primary prevention post-release. Couples-based prevention efforts may be an additional avenue for prevention with incarcerated individuals and their partners (Beckwith et al. 2010).

Conclusion

Prevention counseling in the HIV care setting may be offered across the spectrum and continuum of HIV care – from HIV testing and primary HIV prevention to entry into HIV care and treatment and secondary and tertiary HIV prevention. There are various HIV prevention counseling strategies, methods, foci, and formats, which can be delivered in a variety of HIV care settings and have been shown to be effective with several at-risk or vulnerable populations. Overall, HIV prevention counseling is used to promote HIV risk reduction and to promote HIV medication adherence and health outcomes.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Behavioral Aspects of HIV Treatment as Prevention](#)
- ▶ [Clinical Ethics in HIV/AIDS Prevention, Care, and Research](#)
- ▶ [HIV Counseling and Testing, Prevention of HIV](#)
- ▶ [HIV Testing and Counseling](#)
- ▶ [Prevention for People Living with HIV](#)

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Prevention for People Living with HIV

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Definition

Prevention for people living with HIV integrates prevention, treatment, medical care, and support to improve and maintain physical, mental, sexual, and reproductive health as well as the well-being of people with HIV; to prolong survival; and to reduce the risk of transmitting HIV and acquiring other sexually transmitted infections (STIs).

History

In the early 1980s, behavioral risk-reduction methods that decreased the risk of HIV exposure during sexual contact, drug use, and pregnancy were the main strategies for reducing HIV transmission and acquisition of new infection. Throughout the first 15 years of the HIV epidemic, prevention efforts primarily focused on changing risk behaviors of persons who were at heightened risk for HIV infection. During this time, there was less emphasis on prevention for persons with HIV as no effective treatment was available. Additionally, there was concern that focusing on transmission prevention for persons with HIV would further stigmatize a population that was already facing discrimination and life-threatening illness.

The availability of highly active antiretroviral therapy (HAART) in the mid-1990s led to a new era of HIV prevention. Many HIV-positive persons had successfully lowered their viral load and achieved much-improved survival through strict adherence to their HIV regimens. Consequently, there was a dramatic decline in the incidence of AIDS and AIDS-related deaths in places where HAART was widely available. HIV was no longer considered a fatal disease but instead thought of as a treatable chronic infectious disease. The use of antiretroviral therapy (ART) to prevent HIV transmission from pregnant women to their newborns was also a major breakthrough and resulted in steady declines in the number of perinatally infected infants in many countries.

Treatment advancement also fostered a growing effort in the 2000s and early 2010 to identify people with HIV earlier; link them into HIV care; provide treatment, care, and support to improve the health of persons with HIV; and prevent further transmission of HIV. In 2001, the US Centers for Disease Control and Prevention (CDC) introduced the Serostatus Approach to Fighting the HIV Epidemic (SAFE) program, an effort to scale up HIV testing and identify HIV-positive persons who do not know their status (Janssen et al. 2001). The CDC’s “Advancing HIV Prevention” (AHP) effort in 2003 further addressed other health-care needs of person with HIV to include STI screening, substance abuse treatment, and prevention of opportunistic infections with a clear goal to reduce the number of new HIV infections by changing the behavior of persons with HIV (CDC 2003). CDC, Health Resources and Services Administration, National Institutes of Health, and HIV Medicine Association of the Infectious Diseases Society of America published guidelines for clinical providers to incorporate HIV prevention into the medical care of persons living with HIV (CDC et al. 2003). In the same year, the US President’s Emergency Plan for AIDS Relief (PEPFAR) was announced (U.S. State Department 2014) to fund prevention programs to expand access to ART within resource-limited countries with high HIV/AIDS

prevalence rates. In 2004, the Global HIV Prevention Working Group published *HIV Prevention in the Era of Expanded Treatment Access* (Global HIV Prevention Working Group 2004). This publication called for increased attention to prevention for persons with HIV as one key part of a broader HIV prevention agenda with expansion of treatment access. It also recommended to provide greater support to networks of persons with HIV and stressed the critical importance of overcoming stigma. All these earlier efforts were, however, criticized by their definitions of “prevention” – which focused almost exclusively on the prevention of transmitting HIV to other persons. The recent guidelines by the World Health Organization (World Health Organization 2008) were more centered on empowering persons with HIV by providing them with needed prevention, treatment, care, and support to stay healthy, avoid acquiring new sexually transmitted infections, delay HIV disease progression, and avoid transmitting HIV to others. The US National HIV/AIDS Strategy (NHAS) released in 2010 has emphasized increasing access to care and improving health outcomes of persons with HIV as one of the main goals, in addition to the goals of reducing HIV incidence and HIV-related health disparities (Office of National AIDS Policy 2010). Both WHO guideline and NHAS also emphasize the use of evidence-based strategies in delivering the comprehensive prevention for persons with HIV.

A Comprehensive Approach to Prevention with People Living with HIV

To date, many behavioral and biomedical interventions are shown to improve the health of persons with HIV and, at the same time, reduce HIV transmission to others. The most effective way of having the highest impact on the individual’s and community’s health is a comprehensive approach that combines prevention, treatment, care, and support together by tailoring to the needs of persons with HIV. This approach involves not only persons with HIV and HIV medical care providers but also community-based HIV service providers,

health departments, private and public sector entities, and the general public. A comprehensive approach includes efforts to enable individuals with HIV to become aware of their serostatus through HIV testing, be linked to and retained in HIV care, initiate and adhere to HIV treatment, receive risk screening and risk-reduction interventions, and be offered partner services, STD services, reproductive health care and perinatal transmission prevention, and other medical and social services that influence HIV transmission (such as substance abuse treatment and mental health services). Elements of a comprehensive prevention approach are outlined below.

Addressing Contextual Factors and Social Vulnerabilities

Factors such as poverty, homelessness, violence and abuse, and discrimination as a result of one’s age, gender, race, ethnicity, socioeconomic status, location of residence, literacy level, and sexual orientation shape the lives and health of persons with HIV and their access to prevention, treatment, care, and support. These factors often make people vulnerable to HIV in the first place and continue to impede their ability to adopt strategies to protect their health and partner’s health. HIV infection may also worsen some of these factors, making persons with HIV even more vulnerable to disparities. Understanding how these important contextual issues influence the use of prevention services and potential HIV transmission risk behavior of people living with HIV is critical. Providers and organizations working in clinical and nonclinical settings, policy makers, and affected communities need to be aware of these contextual issues and work together with persons with HIV to address them.

Knowing One’s Seropositive Status

Many HIV-positive persons substantially reduce risk behaviors after learning that they are HIV-positive (Marks et al. 2005), as many believe that they have a responsibility to protect their sex partners from HIV. However, 18% of the people with HIV in the United States are not aware of

their seropositive status (Hall et al. 2013). As a result, they miss important opportunities to initiate HIV treatment before the virus does significant damage to the immune system and potentially unknowingly pass the HIV virus to others. Several strategies are recommended to improve early detection of HIV infection and increase the proportion of tested persons who learn their test results, including reducing stigma about HIV testing, detecting HIV early on with tests for acute infection, making HIV testing more available and accessible (e.g., mobile testing, home testing), implementing HIV testing as part of routine health care, using more rapid testing technologies, and offering HIV testing and services to partners of those who are tested HIV seropositive (World Health Organization 2008).

Improving Early Entry to HIV Care and Retention in Care

After HIV diagnosis, timely entry into HIV medical care and retention in care are essential to the provision of effective ART (Thompson et al. 2012). Earlier entry into care and better retention in care have been shown to reduce the risk of developing HIV opportunistic infections, increase survival rates, improve access to psychosocial and preventive services which promotes continuity of medical care, and improve overall quality of life. However, of persons with HIV living in the United States, only 66% were linked to HIV care and only 37% were retained in care (Hall et al. 2013). Those figures are comparable with global data (Thompson et al. 2012). Several strategies may improve early entry into care and retention in care, including using HIV testing and surveillance data to identify persons who have never initiated care; offering services that promote linkage to and retention in care through collaborations among HIV testing providers, community-based HIV prevention providers, HIV care providers, case managers, and health departments; providing a brief, strengths-based case management that encourages persons with HIV to recognize and use their own abilities and strengths to access resources and solve problems; delivering intensive outreach for individuals not engaged in

medical care within 6 months; using peer or paraprofessional patient navigators; offering ancillary services (e.g., transportation, child care, mental health and substance abuse treatment) to address barriers to entry to care and retention in HIV care; and having monitoring system in place to track HIV care visits and sending reminders for any missed appointments (Higa et al. 2012; Thompson et al. 2012).

Increasing HIV Treatment Initiation and Adherence

HIV treatment guidelines recommend ART for all persons with HIV. Receiving ART earlier in the course of the disease and maintaining long-term adherence to ART provide added benefit to the individual's health and reduce the risk of transmitting HIV to partners. Inconsistent adherence may lead to treatment failure, compromise immune function, or result in mortality. Suboptimal adherence can also promote viral resistance that limits future treatment options for the patient and newly infected partners. Several effective strategies are recommended to improve HIV medication adherence, including involving patient in decisions about treatment regimens; creating a multidisciplinary team to support long-term adherence (e.g., nurse, case manager, social worker, pharmacist, counselor); offering ART regimens that are highly effective but reduce pill burden, dosing frequency, and dietary restrictions as much as possible; providing education and reminder tools to support good adherence; and providing referrals for services that address factors that may impede adherence, such as lack of health insurance or other resources to cover ART costs, drug and alcohol use, and mental illness (Thompson et al. 2012; CDC 2014).

Implementing Risk Screening and Interventions to Reduce Risk Behavior

Adopting and maintaining safer behaviors over a lifetime can be challenging for persons with HIV, especially when they feel healthy. Approximately 30–55% among various subgroups of HIV-positive persons (e.g., heterosexual men, women, men who have sex with men) reported unprotected sex after

their HIV diagnoses that put them at risk for potential STIs or put their partners at risk for HIV (Blair et al. 2011). Several behavioral risk-reduction interventions aim to reduce risk behaviors of persons with HIV by promoting behaviors such as condom use, not sharing drug injection equipment, and avoiding unprotected sexual practices. Some intervention characteristics are strongly associated with reducing the frequency of unprotected sex, including the use of motivational enhancement; providing skills building (e.g., correct use of male and female condom, role play for negotiating condom use); delivering to individuals by health-care providers or professional counselors in settings where persons with HIV receive medical care or prevention, or social services; and specifically addressing multiple facets of coping with being infected, including mental health, medication adherence, and care issues (Crepaz et al. 2006). Additionally, brief individually tailored prevention messages derived from risk screening during routine HIV care visits are also effective in reducing risk behaviors among persons with HIV. Sexually active persons with HIV should be offered regular STI screening and promptly treated if diagnosed with STI (CDC 2014). Offering HIV-positive persons of reproductive age with services for family planning and pregnancy care can improve the reproductive health of persons with HIV and also reduce potential sexual transmission and perinatal transmission (CDC 2014).

Providing Other Medical and Social Services to Enhance the Well-Being of Persons with HIV

As treatment has evolved to transform HIV into a more manageable chronic infectious disease, an increasing number of persons with HIV face emotional and physical challenges as they cope with managing HIV/AIDS over their lifetime. One of the foremost concerns for persons with HIV is telling other people about one's seropositive status. Some people may respond to the serostatus disclosure with love and support while others may not be as accepting. In most cases, sharing one's HIV status is a personal choice. However, in the case of sexual relationships, it can be a legal requirement in some states or countries. It is

important to offer support and information to persons with HIV to promote successful serostatus disclosure to sex partners to minimize risk of discrimination, prosecution, and other negative consequences. Other issues affecting the well-being of persons with HIV include relationship dynamics, sexuality, reproductive health, pregnancy prevention, mental health, employment, and basic needs (e.g., housing, food). Providing needed health and social services to address these issues increases the capacity of HIV-positive persons to take care of themselves and improve the overall quality of life.

Resources of Evidence-Based Prevention Strategies

Several guidelines emphasize the use of evidence-based prevention strategies and provide information on the strategies to support status awareness, early entry into care, retention in care, treatment initiation, long-term adherence to ART, safe behavior, and good quality of life among people living with HIV (World Health Organization 2008; Office of National AIDS Policy 2010; CDC 2014). In addition to these guidelines, the CDC's Prevention Research Synthesis Project conducts ongoing systematic reviews to identify evidence-based interventions and best practices, including those for persons with HIV (CDC and Prevention Research Synthesis (PRS) Project 2014). The CDC's dissemination effort of High-Impact Prevention Interventions and Strategies (formerly known as Diffusion of Effective Behavioral Interventions) provides a list of evidence-based interventions and public health strategies that have intervention packages and training available and also technical support to agencies that implement evidence-based interventions.

Conclusion

As the number of persons who are living with HIV grows, it is essential that HIV prevention, treatment, care, and services also expand to meet their needs. HIV medical care and prevention activities with people living with HIV play a critical role in HIV prevention. Providing good medical care to

HIV-positive persons and helping them to retain in routine HIV care and adhere to ART medication, keep viral load suppressed, and reduce risky behaviors can impact not only individual's health outcomes but also public health. Persons with HIV share some common experience, but they are not a homogeneous population. Various contextual factors affect subgroups of persons with HIV in different ways. Special consideration for the individual needs should be taken into account when providing prevention, treatment, care, and support. Maximizing the connections between prevention, treatment, care, and support will enhance the health and quality of life of persons with HIV. Everyone has a role to play and the responsibility is shared for making treatment and prevention available, reducing stigma and discrimination, and reducing HIV transmission.

Disclaimer The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the US Centers for Disease Control and Prevention.

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Prevention of Alcohol-Related HIV Risk Behavior

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Definition

HIV continues to be a major global public health concern. In order to stem the epidemic, prevention efforts must increase; programs targeting high-risk populations and behaviors are essential. Alcohol use is practiced and accepted worldwide. Alcohol is known to reduce inhibition and has been associated with increased risk-taking behaviors. Identifying strategies to prevent alcohol-related HIV risk-taking behaviors is one way to potentially decrease the spread of HIV.

Alcohol consumption is a common practice among all races, ethnicities, and cultures worldwide. Alcohol is responsible for approximately 4% of all deaths and 5% of the global burden of disease; low-income countries and poor populations are disproportionately affected (Beaglehole and Bonita 2010). Drinking patterns vary and include daily use, heavy drinking (more than 1–2 drinks per day), and binge drinking (more than 4–5 drinks on one occasion) (CDC 2014). Excessive drinking encompasses heavy and/or binge drinking and is associated with short- and long-term health risks (CDC 2014). Fifty-one percent of US adults aged 18 and over describe themselves as “regular drinkers” (Schiller et al. 2012). There is a growing population of underage drinkers in the USA, in both urban and rural settings (Yan et al. 2007).

In virtually all settings, alcohol use has been associated with an increase in risk-taking behaviors. Globally, alcohol use has been linked to concentrated HIV epidemics and to overall morbidity and mortality associated with HIV (Braithwaite et al. 2014; Fritz et al. 2010). Alcohol

is known to impair judgment and reduce inhibition, increasing the potential for other risk-taking behaviors, such as unprotected sex or injection drug use (Rehm et al. 2011; Bryant et al. 2010; Cook and Clark 2005). Under the influence of alcohol, persons often have a reduction in risk perception, making them more vulnerable to violence, coercion, and sexual risk. Additionally, the ability to negotiate safe-sex practices (condom use) is often impeded when under the influence of alcohol (CDC 2014). Changes in libido and sexual functioning associated with alcohol use may lead to a greater likelihood of receptive anal intercourse or condom failure (Bryant et al. 2010; Meade and Weiss 2007).

The exact nature of the relationship between alcohol consumption, risk-taking behavior, and specifically HIV risk is not well defined or understood. The research has not reached consensus on the causality of the interactions between the different variables contributing to individual risk taking (Rehm et al. 2011; Woolf and Maisto 2009; Cook and Clark 2005). Regardless, alcohol consumption has been associated with increased risk-taking behaviors, particularly increased sexual risk behaviors.

Populations at Risk

Individual personality traits and behavioral factors, such as risk taking and sensation seeking, influence a person’s use of and responses to alcohol (Bryant et al. 2010; Meade and Weiss 2007). Specific populations are known to have an increased likelihood of alcohol misuse and other risk-taking behaviors. Societal norms, cultural influences, establishments, and other contextual factors may impact the consumption of alcohol and subsequent associated behaviors (Li et al. 2010).

Severe Mental Illness

Persons suffering from severe mental illness (SMI), including depression, are particularly vulnerable to alcohol use and abuse. The effects of

alcohol may exacerbate mental illness; others may seek alcohol to mitigate symptoms. Persons with SMI may engage in more risky activities, including unprotected intercourse, multiple partners, trading sex for money or goods, and, more infrequently, injection drug use (Meade and Weiss 2007).

Adolescents and Young Adults

Adolescents and young adults represent a significant at-risk population for alcohol use/abuse and sexual risk taking. This age group also represents the greatest percentage of sexually transmitted infections, a potential contributor to increased HIV transmission (Wen et al. 2012). Adolescents and young adults are known to have multiple sexual partnerships (current and lifetime) as well as inconsistent and infrequent condom use. Risky encounters are more often reported while under the influence of alcohol, particularly binge or heavy drinking (Wen et al. 2012).

Men Who Have Sex with Men

Across the globe, MSM report high levels of current and episodic heavy drinking (Finlayson et al. 2011). As many as 85% of uninfected US MSM in a large, longitudinal study reported current drinking; of those 59% reported binge drinking. Heavier, episodic drinking in uninfected MSM was associated with socioeconomic status, a history of substance abuse, depression, and/or childhood sexual abuse. Men who have sex with men (MSM) represent a significant percentage of both new and current HIV cases in the USA (CDC 2012; Woolf and Maisto 2009). Heavy alcohol consumption has been identified as a significant risk factor for risk-taking behaviors, specifically unprotected receptive anal intercourse, sex with casual partners, multiple partners, and exchange of sex for money or goods (Woolf and Maisto 2009). How alcohol consumption contributes to risk taking and HIV acquisition in this population warrants further exploration.

Female Sex Workers

Given the stressors of sex work, female sex workers (FSW) may use alcohol as a means to self-medicate, increasing the likelihood of risky sexual transactions. Both parties (worker and client) may use alcohol to increase confidence and decrease inhibitions associated with transactional sex (Li et al. 2010). FSW may seek clients in alcohol-serving venues and may be expected to consume alcohol as part of the transaction. FSW are also at risk for coercion and violence and may have difficulty with condom negotiation; these risks are increased under the influence of alcohol (Li et al. 2010).

People Who Inject Drugs (PWID)

People who inject drugs (PWID) are at higher risk for HIV than the rest of the general, or even at-risk, population. Progress has been made at reducing the rate of transmission, primarily through needle-exchange programs. Alcohol has not been adequately addressed as a contributing factor to increased risk behavior in this group (Arasteh et al. 2008). PWID often report multiple sexual partners and unprotected and transactional sex. The increased risks associated with alcohol use among PWID have been documented globally (Arasteh and Des Jarlais 2010). Non-injecting partners of PWID are also at substantial, yet often unknown, risk for HIV.

Venues

While alcohol is a legal substance served in socially and culturally acceptable settings, alcohol-serving establishments may contribute to the HIV epidemic in a variety of ways. Venues may serve as “sexual networking sites” in both formal and informal ways; drinking establishments are a source of recreation and social networking among friends, and venues may provide opportunities to meet potential new sex partners and/or participate in commercial sex (Bryant

et al. 2010; Kalichman 2010; Woolf and Maisto 2009).

Interventions

Despite the association between alcohol consumption, increased risk taking, and a potential for increasing the spread of HIV, interventions specifically addressing alcohol use and its relationship to HIV acquisition are lacking (Fritz 2009; Fritz et al. 2010). The majority of interventions aimed at reducing HIV acquisition are designed to address specific, individual-level, HIV risk behaviors (e.g., sexual risk); alcohol use is not identified as a distinct variable that has been shown to significantly increase the risk of new infection. The combined impact of personal, behavioral factors, the environment, and societal and policy norms are not well integrated into the current intervention approaches and literature. The majority of interventions targeting alcohol-related HIV risk reduction fall into three categories: (1) youth-focused curriculum-based approaches, (2) individual counseling, and (3) venue-based interventions (Fritz 2009). The most successful HIV risk-reduction interventions, not specific to alcohol use, are those that have expanded beyond the individual level and incorporate social and structural factors underlying HIV risk (Finlayson et al. 2011; Harrison et al. 2010; Noar 2008). Few interventions target alcohol use specifically in PWID or persons with SMI; persons with substance use dependence or mental health issues may require concurrent substance abuse treatment in order to effectively mitigate HIV risk.

Adolescents and Young Adults

School-based curricular programs addressing HIV prevention, targeting adolescents and young adults, have been implemented in South Africa, modeled after US programs, with varying levels of success (Harrison et al. 2010; Fritz 2009). Incorporating the “causal pathways” of HIV risk,

such as alcohol use, sexual coercion or violence, and cultural or population norms and views, has had the greatest impact on success of programs, particularly in South Africa. The most successful interventions in South Africa, modeled after successful US programs, have incorporated the following key components: adopting structural approaches to change the context of individual risk (e.g., life-skills training) and changing social norms through group interventions and collaboration (Harrison et al. 2010; Noar 2008; Kalichman et al. 2007). Implementation in the school setting is also more effective when outside, trained personnel are utilized to deliver the interventions; older mentors were shown to be more effective than same-age peer mentors (Harrison et al. 2010).

This combined curricular-behavioral approach was not effective with US college students in reducing the frequency of unprotected sex or number of partners (Dermen and Thomas 2011). This population is known to engage in high-risk behavior including excess alcohol consumption; efforts to understand and address this issue in the USA are worthwhile.

Men Who Have Sex with Men

In the 1990s, an approach using “popular opinion leaders” was used to change behaviors in high-risk groups, to reduce the risk of HIV transmission. Specifically, trusted, well-liked individuals within a social network were recruited and trained to deliver a targeted intervention to reduce HIV risk; for example, safe-sex behaviors (condom use) was identified and promoted as the “new norm” within a group (Fritz 2009; Kelly et al. 1991). This approach was initially used with men frequenting gay bars; however, it has been adapted and implemented with other populations (Fritz 2009; Kalichman 2010). Characteristics of interventions resulting in behavioral change (safer sex practices, reduced alcohol intake) include a theory-based approach, interpersonal and life-skills training, support of key community members, multiple intervention sessions

using more than one method (e.g., counseling and group discussion), and consistent follow-up. This combination approach reduced the incidence of unprotected anal intercourse and number of sexual partners and increased condom use in US MSM (Herbst et al. 2005; Johnson et al. 2008). Alcohol consumption, as an independent or compounding variable, was not a specifically targeted variable in these interventions. Recent research further supports an integrated approach, incorporating HIV prevention content with behavioral risks and risk-reduction strategies in order to effect behavioral change and risk reduction (Finlayson et al. 2011; Johnson 2008).

STI Clinic Populations

Patients receiving care in US STI clinics have higher rates of alcohol misuse and are at higher risk for HIV (Carey et al. 2010). A combined brief and intensive behavioral intervention showed sustained success measured by lower STI rates, increased condom use, fewer episodes of unprotected sex, and improved knowledge and attitudes at 12 months. This two-step approach incorporated individualized counseling and risk-reduction strategies using appropriate screening tools (AUDIT), motivational interviewing, and a skills component. Personal triggers and strategies to minimize sexual risk behaviors were incorporated; alcohol use was not included as a specific variable in this study (Carey et al. 2010).

Female Sex Workers

The literature is mixed on the effectiveness of behavioral interventions to reduce HIV risk among female sex workers, particularly in Southeast Asia. The focus of behavioral interventions is on promoting condom use and increasing testing efforts, with the goal of reducing the spread of HIV; however, with the exception of the 100% Condom Use Project, implemented in Asian countries beginning in the 1990s to promote condom use with sex workers, these programs have shown little change in condom use or STI/HIV

testing (Ota et al. 2011; Tan et al. 2011). Successful programs addressed individual life skills, peer-to-peer partnerships, and those targeting sex-work establishments (Tan et al. 2011). Alcohol use or reduction efforts were not specifically addressed in any of the intervention studies with sex workers.

People Who Inject Drugs

There are limited studies addressing interventions to reduce alcohol-related risk among injection drug users. An intervention using motivational interviewing was particularly effective with PWID who participated in a needle-exchange program (Stein et al. 2002). The goals of motivational interviewing were to identify hazardous drinking behaviors, identify the relationships between alcohol and increased risk taking, and set goals for behavior change. Follow-up sessions focused on goal attainment and overcoming barriers to success.

Venue and Policy-Based Interventions

The legality of alcohol and the socially acceptable nature of alcohol consumption likely inhibit the development and implementation of effective interventions to reduce use. Policy measures have been proposed in the USA, such as increasing the tax on alcoholic beverages (Shuper et al. 2010), banning alcohol from college campuses (Cook and Clark 2005), or changing the minimum drinking age in the USA. However, these measures have not received significant support or been evaluated for their effectiveness in HIV prevention.

Given the overlap between alcohol use and risky sexual behavior, engaging alcohol-serving venues in HIV risk-reduction intervention efforts is appropriate (Kalichman 2010). Strategies can be employed in venues that will not have a direct negative impact on alcohol sales, such as making condoms available in restrooms and displaying positive messages regarding safe-sex practices. Training employees on alcohol-related HIV risk and engaging respected members of the

community, who frequent specific venues, to act as mentors for patrons to change the social norms, by promoting risk-reduction behaviors, such as safe-sex practices and reduced alcohol consumption, may also positively influence sex risk behaviors (Kalichman 2010). Venues can also increase awareness and demonstrate support by hosting events related to alcohol and HIV risk and prevention strategies. Addressing alcohol use in alcohol-serving venues, as a specific risk factor for HIV transmission and acquisition, is warranted.

Conclusions

The relationship between alcohol use and increased sexual risk behavior is well documented; the clinical significance between alcohol and HIV risk cannot be underestimated. Despite this association, interventions to reduce HIV risk do not target alcohol use/abuse; it remains a secondary, or hidden, factor.

Alcohol use/abuse is a modifiable risk factor for the acquisition and progression of HIV. Reducing problem drinking in all populations is necessary to combat the global HIV epidemic. Screening for alcohol misuse, using approved tools (e.g., alcohol use disorders identification test (AUDIT) or screening, brief intervention, referral for treatment (SBIRT)), should be incorporated into primary care visits or other healthcare settings (e.g., community programs); prompt identification of persons at risk can lead to recommendation for participation in risk-reduction programs. Integration and collaboration between disciplines as well as policymakers is an important aspect of intervention development and implementation. Alcohol, as a significant factor in the global HIV epidemic, must be more effectively addressed.

Cross-References

- ▶ Behavioral Aspects of HIV Treatment as Prevention
- ▶ Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk

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Prevention of Mother-to-Child Transmission of AIDS

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Definition

The role of antiretroviral treatment (ART) in pregnant and breastfeeding women for prevention of mother-to-child transmission (pMTCT) of HIV has become simple: control of maternal HIV viremia with highly active combination ART effectively blocks MTCT. Details, however, are complex. Lifetime optimal health for mother and child must be balanced with potential toxicities associated with such treatment. The pharmacokinetics of ART in pregnancy and transfer of ART through the placenta and in breast milk are also important considerations in optimizing the health of mother and infant. Management of opportunistic infections in the setting of pregnancy, and limitations in access to, and lack of safety data for newer combination therapies also impact the complexity of managing HIV in pregnancy. ART as preexposure prophylaxis (PreP) for serodiscordant couples planning pregnancies and the use of PreP in serodiscordant couples during pregnancy and breastfeeding have emerged as strategies in which HIV treatment is the primary prevention.

Introduction

The very first clinical trial studying the use of antiretroviral agents for prevention of HIV transmission occurred in the context of the prevention of mother-to-child transmission (pMTCT) in the

National Institutes of Health (NIH) Pediatric AIDS Clinical Trials Group protocol 076. It was a resounding success despite the limited armamentarium of azidothymidine (AZT) alone, decreasing transmission by 67% using a three-part dosing schedule beginning in the third trimester, continuing intrapartum, and incorporating treatment in the delivered infant (Connor et al. 1994). By the late 1990s, investigators knew that vertical transmission was strongly linked with maternal viral load at the end of pregnancy. Subsequent modifications of the 076 approach to using antiretroviral therapy (ART) during pregnancy for pMTCT have included everything from single-dose treatment at labor (with attendant dangers of selection for ART resistance in women and infected babies) to the advent of currently recommended highly active combination ART for all women with HIV. Almost every clinical trial of pMTCT has raised significant questions about how to simultaneously optimize maternal health and survival, prevent vertical transmission, and establish the maternal and fetal safety of new classes of drugs used during pregnancy. Ultimately, scientists, caregivers, and women living with HIV have come to realize that controlling maternal HIV viral load is the key to transmission prevention.

HIV transmission from mother to child can occur during pregnancy, around the time of delivery, and during breastfeeding. Population estimates of mother-to-child HIV transmission rates in the absence of ART are 25–30% during pregnancy and delivery and an additional 14–15% during breastfeeding. HIV exposure is thought to occur around the time of birth, with rupture of membranes; immune activation associated with delivery, especially when complicated by chorioamnionitis; and increased fetal exposure to maternal blood, both via the placenta and during passage through the birth canal. Optimized maternal ART provides effective preexposure prophylaxis by minimizing fetal exposure to maternal virus and by blocking infection of HIV-naïve fetal cells. Recent evidence from resource-limited regions suggests that continuing effective maternal ART also reduces breast milk transmission. Postexposure prophylaxis for infants requires

that ART reaches fetal and infant tissues at sufficient levels to prevent viral replication in the fetus. For the fetus, this requires transplacental passage of drugs (McCormack and Best 2014); for infants, ART can be administered directly.

Antiretroviral Therapy: What to Start and When to Start

The approach to any HIV treatment has generally been modeled to answer two questions: when to start and what to start? For women around the world, the diagnosis of HIV infection often occurs in the antenatal clinic. Depending on the gestational age at which maternal HIV is discovered, there may be some urgency to the question of when to start. The current approach of “test and treat” may be nowhere more urgent than in the pregnant woman newly diagnosed with HIV infection. The time on therapy required to achieve complete virologic suppression for effective prevention of transmission depends on maternal stage of disease, maternal viral load at presentation, an individual’s capacity to rapidly adjust to living with HIV, adherence, pill burden, and other maternal factors as well as on the choice of regimen. For women already on effective therapy at the time of conception, maintaining virologic suppression for maternal health and to prevent transmission is essential. When nausea and vomiting of early pregnancy interfere with ART adherence, anti-nausea medication prior to ART dosing is appropriate. For women starting or restarting therapy, postponing initiation of ART until later in the second or early third trimester both increases the likelihood of a successful start and decreases the chance of selecting for ART resistance to missed or vomited doses.

Treatment guidelines for pMTCT have evolved along with HIV treatment on the basis of many large-scale strategy studies. In 2013, the World Health Organization (WHO 2013) issued updated international guidelines for pMTCT which were consolidated into a single document of treatment and prevention recommendations, driven by CD4, disease stage, disease complications, pregnancy, and breastfeeding, with the goal

of harmonizing provision of ART for both treatment and prevention regionally. The options outlined in these guidelines are still being used in most countries with access to ART (WHO 2013). Very recently (September 30, 2015), the WHO again issued updated guidelines based partly on trial data indicating that initiating treatment while CD4 counts are still high reduces death, AIDS, and severity of non-AIDS disease. The new WHO guidelines call for immediate universal treatment for all people with HIV regardless of CD4 or disease stage and preexposure prophylaxis (PreP) for those at substantial risk. This would increase treatment goals to include all 37 million people currently living with HIV worldwide and harmonize with the current test-and-treat approach to HIV treatment in high-income (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015) and some middle-income countries.

The answer to the question of what ART to start is constantly evolving as new drugs and combination therapies become available. The primary goal of ART in women with HIV infection, as in anyone with HIV infection, is constant and complete HIV suppression, which optimizes both maternal health and pMTCT. In pregnant women and women of childbearing potential, fetal safety also influences the choices of what to start, as discussed below. The choice of ART depends on regimen availability and supply chain requirements to maintain availability throughout the period of pregnancy and breastfeeding. In addition, women may have different levels of tolerance than men for components of any recommended regimen, and there are known genetic polymorphisms in different racial groups that may influence tolerance. Availability of alternatives and adherence support and assurance of compatibility with other required medications (i.e., antituberculous therapies, opportunistic infection prophylaxis, and, for the nonpregnant woman, hormonal contraceptives) are also considerations in regimen choice. Unfortunately, experience over many years has shown us that despite high adherence to HIV treatment throughout pregnancy, many women are at risk for inadequate treatment adherence after delivery when

newborn care and ART prophylaxis often take precedence in family life. There are substantial data indicating that despite successful pMTCT with treatment during pregnancy, if maternal health fails after delivery, even uninfected infants and children are at increased risk of morbidity, mortality, and, if they survive, growing up as orphans.

Both the WHO and the United States Public Health Service (Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission 2015) specify that treatment for pregnant women and women of childbearing potential is combination antiretroviral therapy, typically with two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI). WHO guidelines further specify that the protease inhibitor combination of ritonavir-boosted lopinavir is an alternate regimen to the currently recommended first-line regimen of fixed-dose efavirenz (EFV) + tenofovir (TDF) + lamivudine (3TC) or emtricitabine (FTC). The prevalence of transmitted drug resistance varies widely around the world, and so it is recommended to test for HIV drug resistance prior to initiating therapy whenever possible. However, in women diagnosed late in pregnancy, the timing of ART initiation may not allow for such results to be returned, so the choice of agents may depend on some assessment of local resistance transmission patterns if that information is available. The risks of selection for drug resistance mutations using single-dose nevirapine for pMTCT have been well documented with a median overall maternal risk of approximately 38% (Arrivé et al. 2007) and 75% for infants infected despite prophylaxis. Using sensitive assay techniques to detect lower-frequency populations of resistant virus, these numbers are even higher. Consequently, the strategy of treatment only at onset of labor is no longer endorsed. Unfortunately many women in the developing world received some variation of partial treatment regimens in the context of pMTCT, placing them at risk for selection of drug-resistant virus. The assessment of archived resistance mutations in subpopulations of virus is not widely available at present.

Avoiding selection of drug resistance by optimizing continuity of complete therapeutic regimens for pMTCT preserves future maternal treatment options, including pMTCT for subsequent pregnancies.

Pharmacokinetics of Antiretroviral Therapy in Pregnancy

Part of preserving future treatment options for mothers includes optimizing pharmacokinetics during pregnancy so that drug levels do not drop sufficiently to allow loss of virologic control and/or emergence of resistance on treatment. Physiologic alterations in pregnancy affect absorption, distribution, metabolism, and elimination of many drugs, including ART (Buckoreellal et al. 2012; Gilbert et al. 2015), and changes occur even from first to third trimester. Pregnancy slows gastric emptying and intestinal motility, resulting in nausea, vomiting, and slower absorption. Changes in gastric pH may affect some drugs such as atazanavir, which requires acid for adequate absorption. Intravascular volume increases by 50%, total body water by 8 L, and total body fat by 4 kg, all affecting the apparent volume of distribution for hydrophilic and lipophilic drugs, respectively. Hepatic and placental metabolic pathways are influenced by both progesterone and estrogen in pregnancy including the cytochrome P450 isozymes and the UGT1A1/3 (uridine-5'-diphospho-glucuronosyl) transferases, both of which are important pathways in ART metabolism. Similarly, renal clearance is increased in pregnancy, which may lead to lower concentrations of renally excreted drugs or active metabolites.

Studies of ART pharmacokinetics in pregnancy have accelerated with the participation of two large study groups: the International Maternal Pediatric Adolescent AIDS Clinical Trial Network (IMPAACT) and Pharmacokinetics of Newly Developed Antiretroviral Agents in HIV-Infected Pregnant Women (PANNA). In the NRTI class overall, pharmacokinetic studies demonstrate adequate serum levels at standard dosing. Although most of these drugs are active

intracellularly, there is little data in pregnant women on intracellular drug levels. Tenofovir disoproxil fumarate, a nucleotide, is one NRTI which has demonstrated significantly increased clearance and therefore a fall in serum levels during pregnancy, though, in most patients studied, it exceeds the therapeutic target concentration in serum at standard doses throughout pregnancy. It also acts primarily intracellularly. A modified tenofovir molecule, tenofovir alafenamide, which reaches high intracellular concentrations with lower serum concentrations, is soon to be released. All of the drugs in this class show adequate placental transfer to the fetus. In the NNRTI class, which includes nevirapine, efavirenz, rilpivirine, and etravirine, most data indicate that though there is an increased clearance most likely associated with hepatic enzyme changes, trough concentrations remain above the therapeutic target and no dosage adjustments have been recommended to date. Placental transfer is comparable to NRTIs. Protease inhibitors, including the DHHS-preferred atazanavir and darunavir and the alternative, lopinavir, have been studied in pregnancy and most often show reduced plasma exposures, especially in the third trimester of pregnancy, likely due to a combination of enhanced hepatic metabolism and increases in the volume of distribution. Therefore, a boosting agent such as ritonavir is recommended with the use of any protease inhibitor in pregnancy. (Cobicistat has not been studied to date in pregnancy and is therefore currently not recommended; however, some practitioners may consider its use in fixed-dose combination pills to lower pill burden.) Protease inhibitors, including ritonavir, are subject to many drug interactions. An example of this is the interaction of tenofovir and atazanavir in which atazanavir levels are substantially decreased in the third trimester, even with ritonavir boosting, leading some experts to recommend dose increases in the second and third trimester, with similar recommendations for lopinavir/ritonavir dose increases in second and third trimester for treatment-experienced patients (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015). As a class, protease inhibitors exhibit low placental transfer. Entry inhibitors

such as enfuvirtide and maraviroc have not been extensively studied in pregnancy, and they cross the placental barrier poorly, if at all, but may have utility in women with preexisting drug resistance or intolerance to other agents. Integrase strand transfer inhibitors (INSTIs) are the most recently developed category of ART, and raltegravir has recently been added to the list of preferred agents for initial therapy in HIV-infected pregnant women following sufficient evaluation of pharmacokinetics, safety, and efficacy (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015). The rapid fall in HIV viral load seen with INSTI-based combination therapy has also led to its use in women diagnosed with HIV late in pregnancy. Importantly, caregivers and women should recognize that despite reassuring FDA pregnancy category ratings, limited prospective birth outcome data, especially birth defect data, on INSTI-based regimens are currently available, as detailed below.

Teratogenicity and Antiretroviral Agents

Risk of birth defects among infants exposed to ART during pregnancy has been an ongoing concern since AZT first became available. The Antiretroviral Pregnancy Registry (APR 2015) is a voluntary, prospective drug registry in which health-care providers register pregnant women taking ART before the outcome of the pregnancy is known. With almost 20,000 women enrolled and outcomes of 17,332 live births reported, it is the largest registry in history. No apparent increase in birth defects has been found, and an independent advisory committee summarizes, "APR finds no apparent increases in frequency of specific defects with first trimester (or other) exposures and no pattern to suggest a common cause; however, potential limitations of registries should be recognized" (Advisory Committee Consensus 2015). The French Perinatal Cohort study of 13,124 exposed children has also been reassuring despite their finding of a small increase in heart defects associated with AZT (no longer a component of WHO first-line therapy) and a

questionable association of early efavirenz exposure with a small increase in neurologic defects (Sibiude et al. 2014).

Efavirenz has been the subject of intense scrutiny ever since an unpublished, preclinical cynomolgus monkey study of 20 pregnancies showed a high prevalence of central nervous system and clefting abnormalities. Six retrospective case reports of central nervous system birth defects in humans have appeared. Such retrospective birth defect data must be interpreted with caution as they are subject to overwhelming selection bias. Neither APR nor a large meta-analysis of prospectively collected data in large populations of women and their infants has revealed any evidence of an increased risk of birth defects following first trimester exposures (Ford et al. 2014). Nonetheless, pending additional data, the USPHS continues to recommend avoiding initiation of EFV-based regimens in the first 8 weeks of pregnancy or in women who are contemplating pregnancy. Within the APR, a small but statistically significant increase was seen in overall birth defects for didanosine and nelfinavir, both rarely used currently in pregnancy, though there is no pattern to the defects observed, so the clinical significance is limited. Longer term follow-up of ART-exposed infants continues, and to date, no pattern of late-onset complications has been seen, though such studies are limited.

Despite such reassuring findings overall, birth defect and other pregnancy outcome data for ART that have entered the market since 2006 are very limited. In the APR January 2015 interim report, outcomes following first trimester exposure to raltegravir (180), rilpivirine (110), elvitegravir (18), cobicistat (18), and dolutegravir (3) are insufficient to detect even a potential twofold increase in all birth defects. Newer drugs and fixed-dose combinations offer low pill burden, low toxicity, enhanced tolerability, effectiveness against resistant virus, and markedly superior adherence compared to older ART which dominate the published pregnancy literature. Newer drugs and combinations are widely used outside of pregnancy and today are probably included in a

majority of pregnancy treatment plans. The need for prospective enrollment of pregnant women in clinical trials and/or APR is urgent: retrospective reports can create false alarms and dangerous treatment interruptions, while lack of prospective data could result in missing an early teratogenic signal, should any occur. Guidelines urge providers to submit cases to the APR (APRegistry.com).

Birth outcomes including low birth weight and preterm delivery in the context of maternal HIV infection and treatment are an area of controversy. Both are associated with increased morbidity and mortality particularly in the developing world. After a number of conflicting reports on the association of protease inhibitors with preterm births, more recent studies have raised additional concerns about any combination ART and preterm delivery, especially when ART was initiated pre-conception (Mofenson 2015); however, most studies are biased by enrollment after delivery and comparison of treated and untreated women in the very recent era of limiting HIV treatment to individuals with advanced disease. Careful monitoring of birth outcomes in light of newer guidelines calling for universal treatment is urgently needed and will be critical to the clarification of the risks as well as benefits associated with pregnancy in a test-and-treat environment.

Comorbidities and HIV Treatment in Pregnancy

Comorbid conditions are common in the setting of HIV infection, and strategies for pMCTC in women with concomitant tuberculosis, hepatitis B and/or C infection, malaria, or opportunistic infections present challenges in sequencing initiation of therapy to avoid immune reconstitution inflammatory syndrome, though this has been studied very little in pregnancy. The choice of NRTI therapy in pregnancy may also depend on coinfection with HBV, and avoidance of HBV flares if NRTIs are subsequently changed in pregnancy. Drug-drug interactions of antituberculous drugs with ART may be unpredictable especially in the

setting of pregnancy-induced changes in metabolism. Newer drugs are emerging, particularly in treatment of tuberculosis, and the study of these agents in women of reproductive age living with HIV, especially in pregnancy, and birth outcomes of ART-exposed infants and children will be essential to preserving maternal-child health and the effectiveness of pMTCT (McIlleron et al. 2015).

Treatment During Delivery, the Neonatal Period, and Breastfeeding

The virologic goal of maternal treatment is complete and durable virologic suppression; however, this may not be achievable in an individual pregnancy. At the onset of labor, the antepartum ART regimen is continued intrapartum for most women, and in settings where it is available and safe, intrapartum IV AZT, as well as elective cesarean delivery, before the onset of labor may be recommended to augment pMTCT if viral load at delivery is likely to be >1000 copies/ml. At the same time, data are clear that even with viral load above 1000, women taking combination ART are unlikely to transmit (Katz et al. 2010).

Regardless of the type of maternal ART, the third component of pMTCT includes 6 weeks of prophylaxis for HIV-exposed infants, although recently experts in the USA and Europe have shortened the duration of infant AZT prophylaxis to 4 weeks if the mother is fully treated at the time of delivery. In cases of less than optimal maternal virologic suppression or known or suspected ART resistance, the addition of a short course of nevirapine to AZT prophylaxis has been shown to be safe.

Breastfeeding presents another opportunity to use treatment to prevent mother-to-child transmission of HIV infection. Current recommendations in the developed world are for complete avoidance of breastfeeding (Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission 2015), while in the developing world, in the absence of safe, reliable alternatives to breastfeeding, extensive data supports exclusive breastfeeding for 6–12 months with

simultaneous maternal ART for the entire duration of breastfeeding, though again, with newer recommendations for universal treatment, lifelong ART would include treatment for the duration of breastfeeding. Infant prophylaxis with AZT and up to 6 months of NVP has also been studied to prevent breast milk transmission in the setting of maternal treatment interruption postpartum. Use of breast milk substitutes in resource-limited regions, as well as early weaning and mixed formula, and breastfeeding have all been linked with increased morbidity and mortality among both uninfected and infected newborns and are not recommended outside of industrialized countries (Jamieson et al. 2012).

Preexposure Prophylaxis for Seronegative Women

Acute HIV infection occurring during pregnancy or breastfeeding is associated with a very high risk of mother-to-child transmission, presumably secondary to the extremely high levels of maternal viremia. Although treatment can be initiated rapidly in this setting, prevention is always better. Preexposure prophylaxis (PreP) with tenofovir or a fixed-dose combination of tenofovir and emtricitabine in HIV-seronegative women at risk, in addition to counseling and support for safer sex practices and effective treatment of HIV-infected sexual partners, could avoid acute infection. Pregnancy and breastfeeding are not themselves contraindications to initiation or continuation of PreP. Indeed, heterosexual transmission risk, male to female and female to male, is significantly increased during pregnancy. Monitoring for risk, continuing to offer retesting, and PreP during pregnancy for women at risk after an initial seronegative result in early pregnancy are strategies that may be used for pMTCT in uninfected women.

Achieving Pregnancy

Discordant couples desiring children face many challenges. First, partners should each know

their HIV status. Assisted reproductive technologies for prevention of transmission such as in vitro fertilization, so-called sperm washing, and artificial insemination are available to some discordant couples in resource-rich regions but not to most. Low-technology interventions to minimize sexual transmission during conception exposures include optimized control of the infected partners HIV disease with ART, semen analysis to detect azospermia and subclinical epididymitis, limiting unprotected intercourse to conception attempts only, and at-home artificial insemination for infected women with uninfected partners. Regardless of whether conception was achieved in a high- or low-technology setting, strict safer sex practices during pregnancy are universally indicated, and ongoing pre- and postexposure prophylaxis, especially for uninfected pregnant women, should be discussed and offered.

Summary and Conclusions

Prevention of mother-to-child transmission is possible in virtually 100% of women with the present regimen of antiretroviral therapies, obstetrical practices, and support and correlates with suppression of maternal HIV. Understanding the pharmacokinetics of antiretrovirals and drug-drug interactions during pregnancy and breastfeeding in both mother and infant is vital for effective pMTCT, which includes postpartum health of both mother and child. As new drugs emerge in the treatment of HIV, evaluation of efficacy and risks in the setting of pMTCT is also vital both to offer new and potentially better therapies to all persons with HIV but also to keep treatment options open for women with resistant virus. Finally, for seronegative women at risk during pregnancy and breastfeeding, the use of preexposure prophylaxis throughout may be the most effective way to pMTCT of HIV infection. Continuing to study the use of antiretrovirals in women of childbearing age will increase the ability to use these drugs effectively and safely for the health of women and children.

Cross-References

- ▶ Behavioral Aspects of HIV Mother-to-Child Transmission
- ▶ MTCT HIV-1 Transmission Update: Transmission Routes and Mechanisms
- ▶ Pregnant Women: Care and Treatment
- ▶ Preventing Mother-to-Child Transmission (PMTCT): Prevention of HIV
- ▶ Preventing Mother-to-Child Transmission of HIV-1

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Primary Effusion Lymphoma

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Definition

Primary effusion lymphoma (PEL) is a rare non-Hodgkin's lymphoma that characteristically presents as a malignant effusion in the body cavities

of patients with AIDS and occasionally in other patients with an underlying immunodeficiency. The malignant PEL cells are infected with Kaposi's sarcoma-associated herpesvirus (KSHV, also known as human herpesvirus-8, HHV8) and are often coinfecting with Epstein-Barr virus (EBV). PEL cells display morphologic features of large-cell lymphomas and are of B-cell lineage. The diagnosis of PEL is characteristically made in AIDS patients presenting with a malignant effusion in the pleural, pericardial, or peritoneal cavity characterized by the presence of KSHV-infected cells with characteristic morphology and phenotype. The clinical course of PEL is generally aggressive, with a mean survival of less than 1 year, despite chemotherapy.

Introduction

PEL was recognized as a distinct clinical entity when it was discovered that certain non-Hodgkin's lymphomas arising in the pleural, pericardial, or peritoneal cavity of HIV-infected patients were infected with KSHV, often in conjunction with the related herpes virus Epstein Barr Virus (EBV) (Cesarman et al. 1995). By definition, PEL cells are infected with KSHV. In 70–90% of cases, PEL cells are coinfecting with EBV. KSHV viral sequences were first identified in Kaposi's sarcoma-associated Herpesvirus (KSHV) or Human Herpesvirus 8 (HHV-8) tissues and were subsequently detected in a proportion of lymphoid tissues affected by multicentric Castleman's disease (MCD) and in PEL cells (Chang et al. 1994). KS, MCD, and PEL are the three principal malignancies caused by KSHV that arise in AIDS patients. Clinical descriptions of PEL in patients with AIDS preceded by about 5 years the discovery of KSHV, and since then it was suspected that these lymphomas might constitute a distinct entity because of their unusual presentation in body cavities, histology, and phenotypic characteristics. PEL may give rise to solid tumor masses inside the body cavities by adhesion to the mesothelial layer and may infiltrate surrounding tissues; these forms are referred to as "solid PEL" (Menon et al. 2012). More

infrequently, KSHV-infected large-cell lymphomas resembling PEL have been observed in HIV-infected individuals without malignant effusions. Because these KSHV-infected lymphomas resemble PEL in morphology, have phenotypic and genetic characteristics, and are often coinfecting with EBV, the 2008 World Health Organization classification described them under the category of PEL as they may represent part of a spectrum of PEL types (Menon et al. 2012).

Epidemiology and Pathogenesis

PEL comprises only about 4% of all HIV-associated non-Hodgkin lymphomas (AIDS-related Primary Central Nervous System Lymphoma; and Burkitt-like Lymphoma; Diffuse Large B-Cell Lymphoma; Plasmablastic Lymphoma), which are estimated to develop in 5–20% of HIV-positive patients, with a relative risk 60–200-fold higher than that of the general population (Yarchoan et al. 2005). The development of high-grade B-cell non-Hodgkin's lymphoma in an HIV-positive individual is considered an AIDS-defining illness; this occurs in 3–4% of HIV-infected individuals. The introduction of highly active antiretroviral therapy (HAART) (Antiretroviral Medications, Adult Care and Treatment) has decreased the incidence of HIV-associated non-Hodgkin's lymphomas, particularly the incidence of primary central nervous system lymphomas (AIDS-related Primary Central Nervous System Lymphoma) (Yarchoan et al. 2005).

Studies of PEL pathogenesis have focused on KSHV, since infection with this herpesvirus defines the illness, but the mechanisms by which KSHV promotes tumorigenesis are currently incompletely understood and are likely complex. KSHV is a gammaherpesvirus, which is endemic in sub-Saharan Africa (where the seroprevalence ranges between 50% and 70% of the population) and in the Mediterranean basin (where the seroprevalence ranges between 20% and 30% of the population); in North America KSHV infection among blood donors is believed to range between 1% and 3% but is higher in certain risk groups

(e.g., men who have sex with men). Some aspects of the natural history of KSHV are incompletely understood, including how the virus is transmitted to humans, in which cells it replicates, where it establishes latency, and how the immune system controls primary and latent infection. The strong association of KSHV malignancies with HIV infection has prompted investigation into the role of HIV infection. HIV does not infect PEL cells, but T-cell immunodeficiency associated with AIDS is believed to contribute to KSHV tumorigenesis by reducing immunity to the virus and to virus-infected cells, and by promoting expression of growth-promoting cytokines and growth factors. In addition, one of the HIV-1 proteins, the TAT protein (Tat Expression and Function), may facilitate KSHV infection of target cells (Yarchoan et al. 2005). The observation that the majority of PEL are coinfecting with EBV has suggested a contribution by EBV, a herpesvirus that infects most adult individuals worldwide. Unlike KSHV, which has not been shown to immortalize any cell type in culture, EBV is a potent B-cell-immortalizing herpesvirus. However, EBV is not always present in PEL cells, suggesting that it is not necessary. Nonetheless, EBV may contribute to PEL tumorigenesis. Comparative biochemical profiling of PEL cells infected with KSHV alone or coinfecting with KSHV and EBV has failed to identify major differences, except for increased activation of the MAPK (mitogen-activated protein kinase) pathway in cells infected with KSHV alone. In spite of its inability to immortalize normal cells of B-cell lineage in culture, KSHV is required for the survival and growth in culture of established PEL cell lines (Guasparri et al. 2004).

KSHV exists in PEL cells as an oligoclonal or monoclonal episome and in PEL usually does not undergo complete lytic replication resulting in the production of infectious viral particles. Rather, PEL cells are generally latently infected with KSHV, expressing only a few viral gene products. Thus, attention has focused on these KSHV latency genes (Speck and Ganem 2010). LANA (latency-associated nuclear antigen-1) is one such gene product, which tethers the viral DNA to the host-cell DNA, and this ensures viral DNA

persistence during PEL cell replication. Viral cyclin (vCYC), which is closely related to cellular cyclin D2, can inhibit the cell cycle inhibitor p27 KIP1 promoting cell cycle progression. Viral FLICE inhibitory protein (vFLIP) is required for the continuous growth and survival of PEL cells in vitro; vFLIP activates the NF κ B pathway, thereby promoting the expression of many cellular genes that can contribute to PEL survival and growth (Speck and Ganem 2010). Viral IL-6 (vIL-6), which shares similarity with cellular IL-6, is an autocrine growth factor for PEL cells in culture (Jones et al. 1999). The viral G-protein-coupled receptor (vGPCR) stimulates cell proliferation and promotes the expression of Vascular Endothelial Growth Factor (VEGF), the pro-angiogenic and vascular permeability factor VEGF (Speck and Ganem 2010). The viral Bcl2 homologue, vBcl-2, blocks cell apoptosis (Speck and Ganem 2010). All these KSHV latency genes have the potential to contribute to PEL malignant phenotype as they promote cell progression through the cell cycle resulting in uncontrolled cell growth and prevent cell death. In spite of this pattern of viral gene expression and gene function, and in contrast to their sustained and progressive growth in the body cavities, PEL is not easily propagated in culture once removed from the patients' body cavities, whether or not PEL cells are infected with KSHV alone or are coinfecting with EBV. This situation differs drastically from the ease with which B cells naturally infected with EBV can be propagated in vitro as immortalized cell lines, suggesting an important contribution of the tumor microenvironment in supporting PEL cell growth. As a consequence, only few PEL cell lines are currently available, which provide the only cells naturally infected with KSHV that can be propagated in vitro.

PEL cells resemble post-germinal center B cells because they display immunoglobulin gene rearrangement and somatic hypermutation; gene expression profile analysis suggests that PEL cells are plasmablastic (Menon et al. 2012). Consistent with this, PEL cells express the plasma cell-associated CD138/syndecan-1 marker and CD45 but lack expression of common B-cell-associated antigens (including surface

immunoglobulin, CD19, and CD20). They also generally lack the T-cell markers CD4 and CD8, although they may express cytoplasmic CD3. A number of activation markers, including CD30, CD38, CD71 and HLA-DR, are usually detected in PEL. Cytogenetic analysis has revealed no common chromosomal aberration in PEL, but Myc is often amplified (Bubman et al. 2007). Morphologically, PEL cells appear plasmablastic, immunoblastic, or anaplastic lymphoid cells.

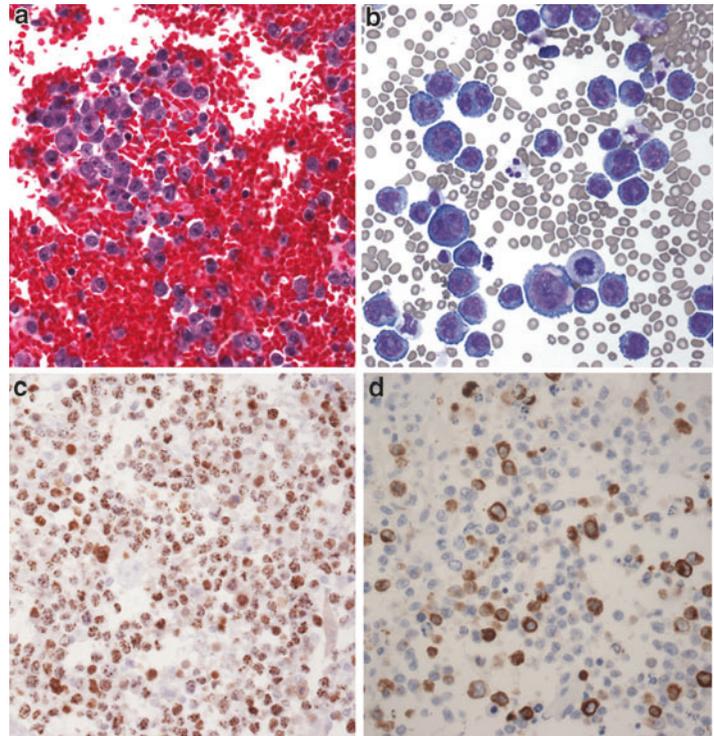
Clinical Presentation, Diagnosis, and Treatment

Patients with PEL are usually HIV-positive men, often with AIDS, presenting with symptoms resulting from accumulation of the malignant effusion in the pleural, pericardial, or peritoneal cavity, including dyspnea (pleural and pericardial PEL) and abdominal distension (peritoneal PEL). In cases of extracavitary PEL localized to the

gastrointestinal tract, soft tissue, or other extranodal sites, patients may present with symptoms reflecting PEL tissue location. A proportion of patients with PEL have preexisting or concomitant KS and/or MCD, the principal other malignancies associated with KSHV infection. Occasionally, PEL arises in pharmacologically immunosuppressed HIV-negative patients who have received a solid organ transplant. The diagnosis of PEL is based on histological, virological, immunophenotypic and molecular characteristics of the cells or tissue. Typically, PEL cells are derived from the effusions as cell suspensions and are processed as cytospin preparations or embedded as a cell block. In cases of solid PEL, the diagnostic sample is a tissue fragment. Histologically, PEL cells are large, resembling plasmablastic (eccentric nuclei and abundant cytoplasm), immunoblastic (round central nuclei with prominent nucleoli), or anaplastic (very large cells with pleomorphic nuclei) lymphoid cells (Fig. 1). All these features can be simultaneously present, resulting in PEL having pleomorphic

Primary Effusion Lymphoma,

Fig. 1 Morphology, KSHV infection, and vIL-6 expression in PEL cells. Characteristic pleomorphic morphology of PEL cells from two AIDS-associated pleural effusions (**a**, **b**). KSHV-LANA (**c**) and vIL-6 (**d**) detection in PEL cells from a case of AIDS-associated extracavitary PEL (The images from the files of the laboratory of Pathology, CCR, NCI are a gift of Dr. S. Pittaluga)



morphology. Demonstration of KSHV infection is required for diagnosis of PEL, which is usually accomplished by immunohistochemical detection of KSHV-LANA. Evidence of EBV infection is usually obtained by *in situ* hybridization of EBV-EBER (EBV-encoded RNAs). The EBV latent membrane protein-1 (LMP1) is usually not detected in PEL. Immunophenotyping reveals the expression of CD138, CD45, and cytoplasmic CD3, in the absence of mature B-cell markers. The differential diagnosis in cases of PEL includes other types of non-Hodgkin's lymphomas associated with lymphomatous effusions; many histologic subtypes of non-Hodgkin's lymphomas can present as a neoplastic effusion. Pyothorax-associated diffuse large-cell B-cell lymphoma, which develops in the pleural cavity in the setting of pyothorax in elderly HIV-negative patients, is EBV positive but KSHV negative. The correct diagnosis of PEL requires integration of clinical presentation and correct interpretation of histological, virological and immunophenotypic characteristics of the tumor cells. The initial patient evaluation includes a complete blood count and comprehensive chemistry panel together with appropriate staging to estimate disease burden and provide a baseline to assess response to treatment.

First-line therapy of AIDS-associated PEL often consists of combination chemotherapy with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone)-like regimens, generally added to HAART antiviral treatment (Antiretroviral Medications, Adult Care and Treatment) (Yarchoan et al. 2005). Complete remissions have been observed in a proportion of patients (43–57% remissions). However, the 1-year overall survival rate is reported at 39.3% and the mean survival of patients with PEL is less than 1 year, despite treatment. There is evidence that the anti-CD20 monoclonal antibody, rituximab, can have activity in PEL, even though PEL cells generally express no or very low levels of CD20. The most frequent causes of death are PEL progression as a consequence of drug resistance, and complications related to HIV infection, including opportunistic infections.

Treatment of PEL arising after solid organ transplantation presents unique challenges: T-cell immunosuppression is required to prevent graft rejection but immunosuppression can be harmful to control KSHV infection. Thus far, 12 cases of posttransplant PEL have been reported: 7 post-kidney, 3 post-heart, and 2 post-liver transplantations (Riva et al. 2012). It is important to note that at least in some of these cases, the transplant recipients likely became infected with KSHV after transplantation, having acquired the virus from the KSHV-infected cell graft. In these cases, primary KSHV infection in severely immunosuppressed individuals may have played a critical role in PEL development. Treatment recommendations for posttransplant PEL include a combination of reduced immunosuppression, antiviral therapy and CHOP chemotherapy. The mTOR inhibitor rapamycin, which is often used as immunosuppressant after transplantation, has shown some efficacy in the treatment of experimental PEL, probably attributable to inhibition of VEGF translation. However, two cases of post-transplant PEL have been reported in patients on rapamycin raising concerns.

Chemotherapy at either standard or high dose has rarely cured PEL and has generally not provided long-term survival. Thus, much effort is being placed on novel approaches based on improved molecular understanding of PEL. One of the difficulties lies in the ability to recruit sufficient numbers of PEL patients for enrollment into clinical trials, which are often necessary to appreciate therapeutic advances. However, novel approaches to PEL treatment have been tested in some patients, others have been suggested from preclinical studies, and some are currently being tested or are planned. Antiviral therapy with intracavitary cidofovir was reported to prolong survival in 4 PEL patients (Luppi et al. 2005), suggesting that KSHV replication may contribute to PEL disease progression. Whether the drug targets the limited or periodic KSHV replication occurring in PEL cells or other KSHV-infected host cells is currently not clear. In part based on this experience with cidofovir, attempts have been made to purposely promote viral replication in

PEL cells and combine this approach with anti-viral agents: the antiseizure medication valproate, which promotes KSHV replication, was combined with cidofovir or foscarnet, resulting in increased PEL cell death *in vitro*. However, induction of viral replication in PEL cells by use of phorbol esters compromised the PEL proapoptotic effects of the chemotherapeutic agents doxorubicin and etoposide (Sarek et al. 2012). A different approach was based on the observation that the KSHV latency gene vFLIP, a potent activator of the NF κ B pathway, is necessary for PEL survival and growth *in vitro*. The proteasomal inhibitor bortezomib, which inhibits NF κ B signaling, promoted apoptosis of PEL cells *in vitro* (An et al. 2004). In addition, Nutlin-3, an activator of p53, induced massive cell death in PEL cells injected subcutaneously in mice (Sarek et al. 2012).

Another approach has been directed at modifying the PEL tumor microenvironment by targeting VEGF, which promotes vascular permeability and contributes to the accumulation of body cavity effusions that accompany a number of malignancies. PEL cells express VEGF at high levels, and VEGF is present at high concentrations in PEL effusions. In a preclinical model of PEL growing in the mouse peritoneal cavity, antibody neutralization of VEGF delayed PEL progression. In addition, the mTOR inhibitor rapamycin, which inhibits VEGF translation, was effective at reducing PEL growth in this preclinical mouse model. However, drug resistance developed rapidly, in part due to accelerated PEL secretion of the autocrine growth factor IL-10 (Gasperini and Tosato 2009). The combination of VEGF and IL-10 neutralization has been proposed but has yet to be tested. Besides IL-10, vIL-6 is an autocrine growth factor for PEL and an inducer of VEGF production, which is detected at high concentrations in PEL effusions (Sakakibara and Tosato 2011). Unlike human IL-6, which activates the common signal transducer gp130 only after associating with the non-signaling IL-6 receptor chain, vIL-6 binds directly gp130 and activates its phosphorylation

and downstream signaling. Since gp130 is expressed in virtually all cells, whereas the IL-6 receptor has a more limited cell distribution, vIL-6 has the potential to activate all cells in the body, thus potentially contributing directly or indirectly to PEL cell growth. Neutralization of vIL-6 by either monoclonal antibodies or soluble gp130 has been proposed to reduce PEL progression, particularly in situations in which PEL is associated with KSHV-associated MCD or related MCD-like syndrome, where vIL-6 is believed to play an important pathogenetic role. Another experimental treatment approach to PEL has been based on the observation of the importance of the STAT3 signaling pathway, which is active in PEL cells at least in part due to PEL activation by the autocrine growth factors IL-10 and vIL-6 (Aoki et al. 2003). Expression of a dominant-negative form of STAT3, which serves as a competitive inhibitor of STAT3 binding to target DNA sequences, not only prevented STAT3 activity in PEL but also induced prominent apoptosis *in vitro*. Consistent with a role of active STAT3 in PEL survival, the JAK2 synthetic inhibitor tyrphostin AG490, which inhibits STAT3 phosphorylation, promoted PEL cell death *in vitro*.

Conclusion

In summary, PEL is a rare malignancy usually occurring in patients with AIDS characterized by KSHV-infected large-cell lymphoma cells. Despite chemotherapy, the prognosis is poor. It is hoped that an improved understanding of the role of KSHV in PEL pathogenesis and of the biochemical pathways sustaining PEL progression will identify new targets for treatment.

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Progressive Multifocal Leukoencephalopathy and HIV

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Definition

Progressive multifocal leukoencephalopathy (PML) is an opportunistic infection of the central nervous system (CNS), caused by the reactivation of the polyomavirus JC (JCV) and the infection of brain cells, and characterized by progressive focal demyelination and necrosis (Hirsch et al. 2013). Despite diagnosis can be achieved promptly, there is no specific therapy for PML, and, before the introduction of combination antiretroviral therapy (cART), the disease was almost invariably fatal within a few months. Nowadays, cART-induced immune reconstitution is able to halt disease progression in more than half of the cases, although PML survivors will most often retain disabling neurological deficits (Cinque et al. 2009).

Epidemiological Aspects

Before the advent of cART, PML developed in 3–7% of patients with AIDS, and it was the cause of up to 18% of fatal CNS diseases (Cinque et al. 2009). In the cART era, the incidence of PML has decreased of about tenfold, from 0.7 to 0.07 per 100 person year of follow-up in the period 1994–2002, representing, however, the lowest decrease of incidence among all opportunistic infections of the CNS (D'Arminio Monforte et al. 2004). While median CD4 count at diagnosis

of PML was around 50 cells per μL in the pre-cART era, it is around 100 cells per μL in recent years (Casado et al. 2014), and PML can also develop in patients with CD4 counts greater than 200 cells per μL , in the setting of initiating cART and immune reconstitution and, although more rarely, in long-term-treated patients with full viral suppression.

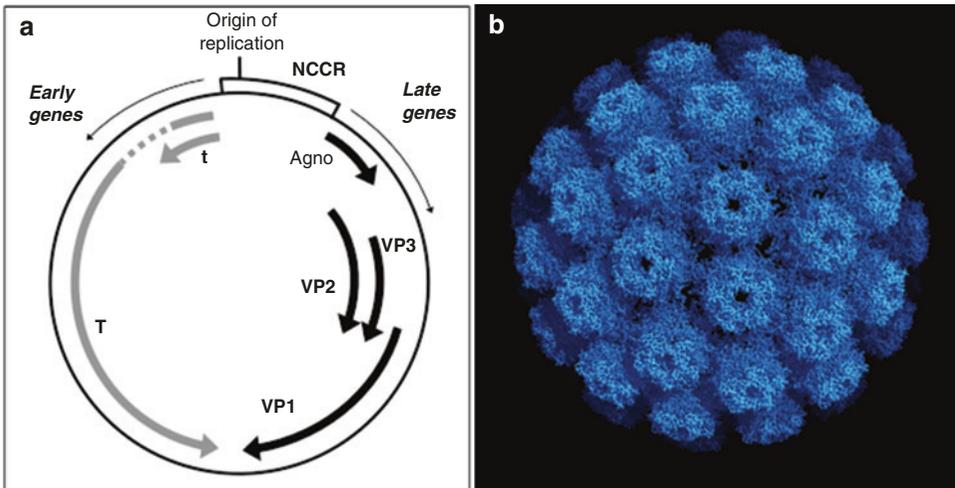
Etiology and Pathogenesis

JCV is a small DNA virus belonging to the family of human polyomaviruses (Fig. 1). JCV infection is ubiquitous, with a seroprevalence among healthy people of 39–69%, depending on the sensitivity of serologic assays. Primary infection occurs without identified symptoms, and ingestion of contaminated water or food has been suggested as major mode of transmission. Following primary infection, JCV may establish a chronic asymptomatic carrier state, with

persistence in the urinary tract and excretion of viral DNA in urine of 20–30% of healthy adults. Because JCV has been found in the bone marrow and CNS of subjects without PML, these tissues have also been suggested as possible sites of JCV persistence (Hirsch et al. 2013).

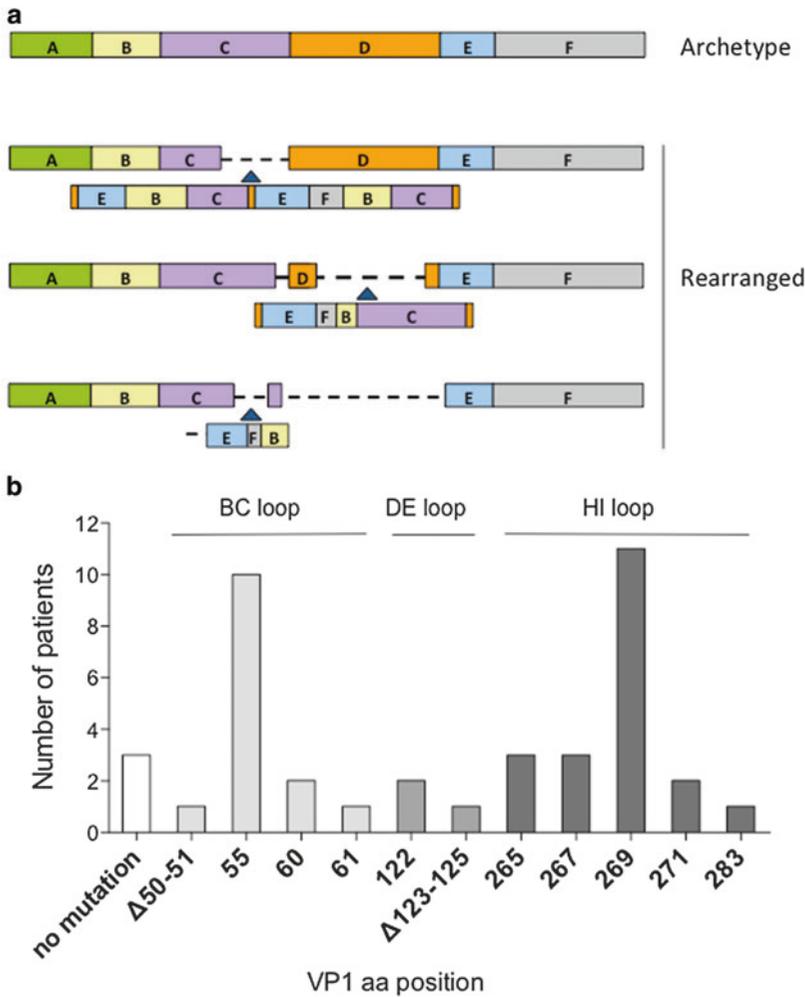
The loss of the immune control against JCV is essential in favoring JCV reactivation, either in the periphery or in the CNS, and allowing viral propagation in the CNS with consequent brain disease. The importance of immune defenses is clear from both the context of PML in patients with immune defects and its remission after reversion of the immune defect with cART. Indeed, remission of PML after cART is often associated with increased JCV-specific T-cell and B-cell responses in blood and cerebrospinal fluid (CSF) (Hirsch et al. 2013; Gheuens et al. 2011).

Beyond the immune defect, virus factors are also involved in the development of PML. These involve rearrangements of the JCV noncoding control region (NCCR) and mutations in the



Progressive Multifocal Leukoencephalopathy and HIV, Fig. 1 The polyomavirus JC (JCV) (a) Schematic representation of JCV genome. The noncoding control region (NCCR) contains the origin of replication, viral promoter-enhancing sequences, and binding sites for cell transcription factors. The early genes encode the large T antigen (T-Ag), which initiates viral DNA replication and regulates early and late gene transcription, and the small T antigen (T-Ag). The late genes code for the major

viral capsid protein-1 (VP-1), which mediates cell entry and is likely the main target of both humoral and cellular immune responses, the minor capsid proteins VP-2 and VP-3, and the agnoprotein (agno). (b) Three-dimensional atomic-level structure of the major capsid protein VP-1 of simian virus 40 (SV40), closely related to JCV and fully resembling JCV structure at picture resolution (Courtesy of Thilo Stehle, University of Tübingen, Tübingen, Germany)



Progressive Multifocal Leukoencephalopathy and HIV, Fig. 2 Genomic organization of the JC virus noncoding control region (NCCR) and frequency of viral protein-1 (VP-1) mutations in clinical specimens. (a) Schematic representation of JC virus NCCR. In “archetype” NCCR (found in urine of all healthy subjects and PML patients), there is an ordered sequence of six genomic fragments, designated with the alphabet letters from A to F (total length 267 bp). In “rearranged” NCCRs (found in brain tissues, cerebrospinal fluid

(CSF), and plasma of PML patients), the archetype sequence is completely reorganized with deletions, insertions, duplications, and mutations, and each PML case will present a unique pattern of rearrangement (figure shows three examples). (b) Frequency of VP-1 mutations in CSF samples of 40 PML patients (Adapted from Gorelik et al., Ref. 5). 37/40 PML cases presented one of these mutations. The numbers on the x-axis denote the amino acid substitution position in VP-1; BC, DE, and HI loops denote the outer VP-1 loops

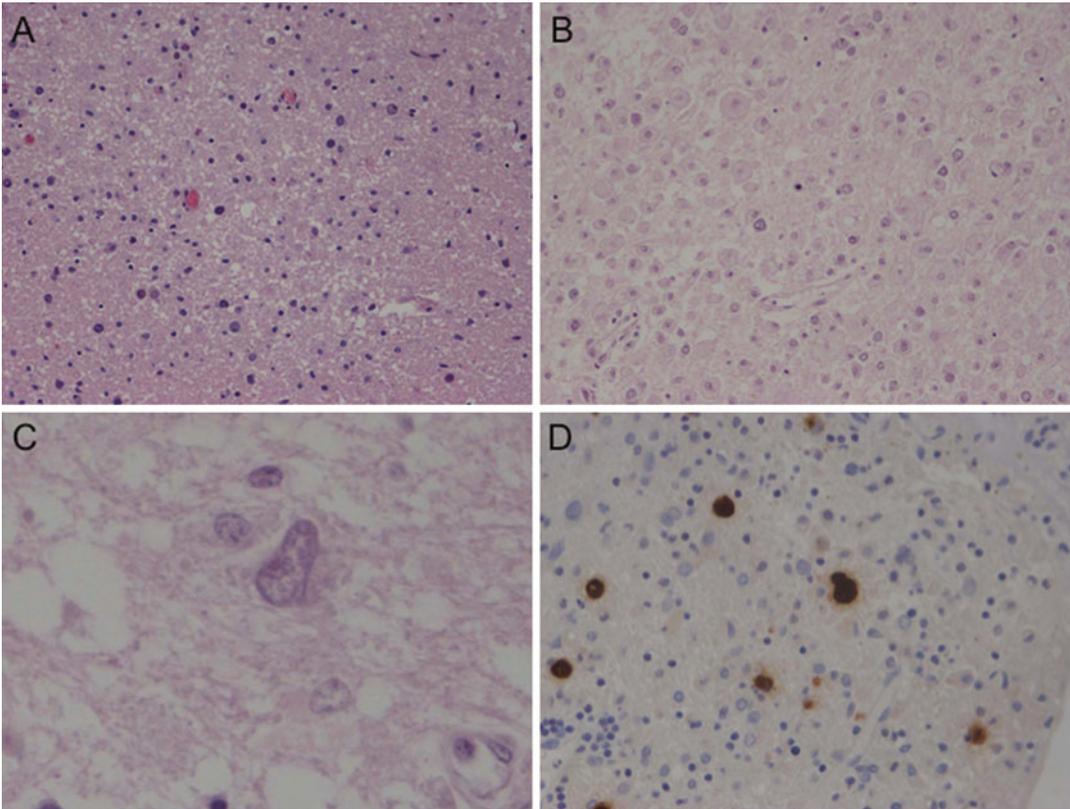
region coding for the major surface protein VP-1 (viral protein-1) (Fig. 2). Whereas JC virus NCCR sequences are conserved (“archetype”) in wild-type JC virus, i.e., the virus excreted in urine from both healthy subjects and PML patients, sequences are completely subverted (“rearranged type”) in the virus found in brain tissue and CSF

of patients with PML. Because NCCR contains sequences that regulate virus transcription as well as binding sites for cell proteins, it has been suggested that DNA rearrangements in this region may affect both virus replication and its tropism for host cells (Hirsch et al. 2013). In addition to NCCR rearrangements, the virus found in the brain

tissue and CSF of patients with PML also contains one of several amino acid mutations of the external capsid protein VP-1. These “PML-specific” mutations are located in the external VP-1 loops, which are both part of the JCV binding pocket for the sialic acid cell receptors, and involved in immune recognition. Therefore it is likely that VP-1 mutations affect cell binding and might also be involved in mechanisms of viral immune escape. Of note, these mutations are not present in wild-type viruses, including the virus excreted in urine from PML patients, indicating that these are selected within the patient upon JCV reactivation at the time of PML (Gorelik et al. 2011).

Neuropathology

PML is characterized by multiple areas of demyelination, varying in size and stage of development. Initial foci of demyelination expand and might coalesce into larger areas that can evolve into cavitory necrosis. All CNS regions can be involved, although spinal cord lesions are rare. Classically, oligodendrocyte nuclei are enlarged and densely basophilic, filled with eosinophilic inclusions, and stained by immunocytochemistry and in situ hybridization for JCV proteins and nucleic acid, indicating productive lytic infection (Fig. 3). Astrocytes also appear enlarged,



Progressive Multifocal Leukoencephalopathy and HIV, Fig. 3 Histopathological findings in PML. (a) Early progressive multifocal leukoencephalopathy (PML) lesion: white matter vacuolization, enlarged oligodendrocytes with viral nuclear inclusions, and no inflammatory infiltrate (hematoxylin–eosin, $\times 100$). (b, c) Late PML lesion: diffuse demyelinating lesion with abundant lipid-laden macrophages and few infected

oligodendrocytes (b, hematoxylin–eosin, $\times 100$) and a bizarre astrocyte with atypical multilobated, or “pseudoneoplastic,” nucleus (c, hematoxylin–eosin, $\times 40$). (d) In situ hybridization with JCV-specific probe: nuclear staining of infected oligodendrocytes (JC Virus BioProbe, hematoxylin counterstaining, $\times 100$) (Courtesy of Manuela Nebuloni, University of Milan, Italy)

sometimes with multiple or multilobate hyperchromatic nuclei, resembling neoplastic cells, the so-called “bizarre” astrocytes, and also harbor JCV gene products. Foamy macrophages, though not specific to PML, are present in many cases as a response to myelin breakdown. Very recently, the use of a novel animal model of PML, i.e., a chimeric mouse engrafted with human glial progenitor cells and infected intracerebrally with JCV, has shown that astrocytes and glial progenitor cells were the principal CNS targets for infection, with demyelination occurring only secondarily, due to JCV T antigen-triggered apoptosis of oligodendrocytes (Kondo et al. 2014).

One of the classic pathological features of PML is the presence of little or no inflammation. However, “inflammatory” forms can be observed. These usually occur at onset of PML in patients with relatively high CD4 cell numbers or, more typically, develop after starting cART, in the context of immune reconstitution. The inflammatory forms are characterized by either diffuse or focal perivascular mononuclear infiltrates. These consist mostly of CD8+ T lymphocytes and monocytes or macrophages; B lymphocytes, plasma cells, and CD4 T cells are also present, but usually in smaller numbers (Martin-Blondel et al. 2013).

JCV has also been identified in cerebellar granule cells, either together with other PML lesions or as unique neuropathology in the context of cerebellar atrophy. This picture characterizes the so-called cerebellar granulopathy, which is associated with a progressive cerebellar degeneration. Recently, an encephalitic form with JCV infection of cortical neurons and a meningitis, with JCV infiltration of subarachnoidal space, have also been described in HIV-negative patients (Miskin and Koralnik 2015).

Clinical Manifestations

The classical presentation of PML is with focal neurological deficits, usually with insidious onset and steady progression. Deficits vary depending on the location of the lesion, including visual field loss and hemianopsia (due to involvement of occipital lobes or optic radiations), hemisensory

defects or hemiparesis (frontal and parietal lobes), aphasia (language-dominant hemisphere), dysmetria and ataxia (cerebellar hemispheres and peduncles), or as a more diffuse encephalopathy, with cognitive deficits or behavioral alterations. Focal symptoms often begin as partial deficits that worsen with time (e.g., arm weakness evolving to hemiparesis), which reflect the spread of individual lesions concentrically or along white matter tracts. Seizures develop in nearly 20% of PML patients, usually in association with lesions adjacent to the cortex, while headache and fever are usually absent.

The inflammatory forms described above, if sufficiently severe, can be accompanied by additional symptoms and signs, which characterize the so-called immune reconstitution inflammatory syndrome (PML-IRIS). In general, IRIS is defined by the presence of immune reconstitution, tissue inflammation, and new onset or worsening of clinical disease that would not be expected from the natural course of the disease itself. The immune reconstitution develops in the context of a decrease of plasma HIV-1 RNA, most often with an increase of CD4+ T cells, following initiation of cART. As with other cART-associated IRIS events, PML-IRIS is also observed either as “paradoxical” or “unmasking.” In paradoxical PML-IRIS, inflammation and clinical worsening develop in relation to existing lesions a few weeks to months after cART initiation. In this setting, it is important to distinguish the clinical worsening associated with IRIS from classical progressive PML without inflammation, since the former may be ameliorated by treatment with corticosteroids. In the unmasking form, PML emerges in previously asymptomatic patients after the start of cART with an inflammatory picture on MRI. Unmasking PML-IRIS is presumed to represent the effects of a restored immune response to subclinical JCV brain infection, with resultant local inflammatory responses and clinical symptoms.

Before the introduction of cART, PML was almost invariably fatal. Median survival from diagnosis was of a few months, although continuous progression over a year or more was observed occasionally in patients with high CD4+ T-cell counts. In the cART era, the survival of PML has

increased substantially, with 38–62% survival rates at 1 year (Cinque et al. 2009). However, mortality remains ten times higher than the combined mortality of all AIDS-related diseases making PML the second most lethal AIDS defining opportunistic disease, after non-Hodgkin lymphoma (Antiretroviral Therapy Cohort Collaboration ART-CC et al. 2009).

Diagnosis and Monitoring

The diagnosis of PML is based on three subsequent steps: recognition of symptoms, radiological identification of the lesions, and confirmation of JCV etiology by CSF analysis or, more rarely, brain biopsy (Table 1).

The clinical recognition involves the identification of the above-described focal or, more rarely, diffuse, neurological deficits with steady progression. The time course of the disease, over several weeks, may provide a diagnostic clue because the other major opportunistic focal brain disorders (cerebral toxoplasmosis and primary CNS lymphoma primarily) usually progress within hours to days.

Neuroimaging

Magnetic resonance imaging (MRI) enables the detection and characterization of distinct white matter lesions in CNS areas corresponding to the clinical deficits. CT scan is less sensitive in detecting PML and less useful in delineating its characteristic anatomic pathology. PML lesions are most common in the subcortical white matter, the white matter of the cerebellar peduncles or hemispheres, and in the brain stem. However, they can involve any part of the central nervous system, including the deep gray matter and very rarely the spinal cord. Because the lesions involve demyelination, these are typically hyperintense (white) on T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences, but hypointense (dark) on T1-weighted sequences, which indicate tissue destruction (Fig. 4). At onset of PML, there is classically either no or only minimum contrast enhancement, and, unlike other major CNS-OIs, primarily toxoplasmosis and

Progressive Multifocal Leukoencephalopathy and HIV, Table 1 Diagnostic criteria and definitions in PML

Criteria for PML diagnosis

a. Confirmed:

Clinical picture consistent with PML

MRI findings consistent with PML

JCV-DNA detected in CSF or the presence of specific pathology at brain biopsy or postmortem examination (with identification of JCV proteins by immunohistochemistry, of JCV or cross-reacting polyomavirus DNA by in situ nucleic acid hybridization, or of JC virions by electron microscopy)

b. Presumptive:

Clinical picture consistent with PML

MRI findings consistent with PML

JCV-DNA not detected in CSF (either nondetectable or lumbar puncture not performed) and brain pathology not available

PML outcome

a. PML progression:

Worsening of clinical picture

Enlargement of old MRI lesions and appearance of new lesions, with persistent activity (hyperintense signal in T2 and FLAIR sequences and high-signal intensity rim at lesion periphery on DWI sequences)

b. PML remission:

Improvement or stabilization of clinical picture

Loss of activity of MRI lesions (disappearance of the hyperintense signal in T2 and FLAIR sequences and of the high-signal intensity rim at lesion periphery on DWI sequences)

JCV-DNA not detected in CSF

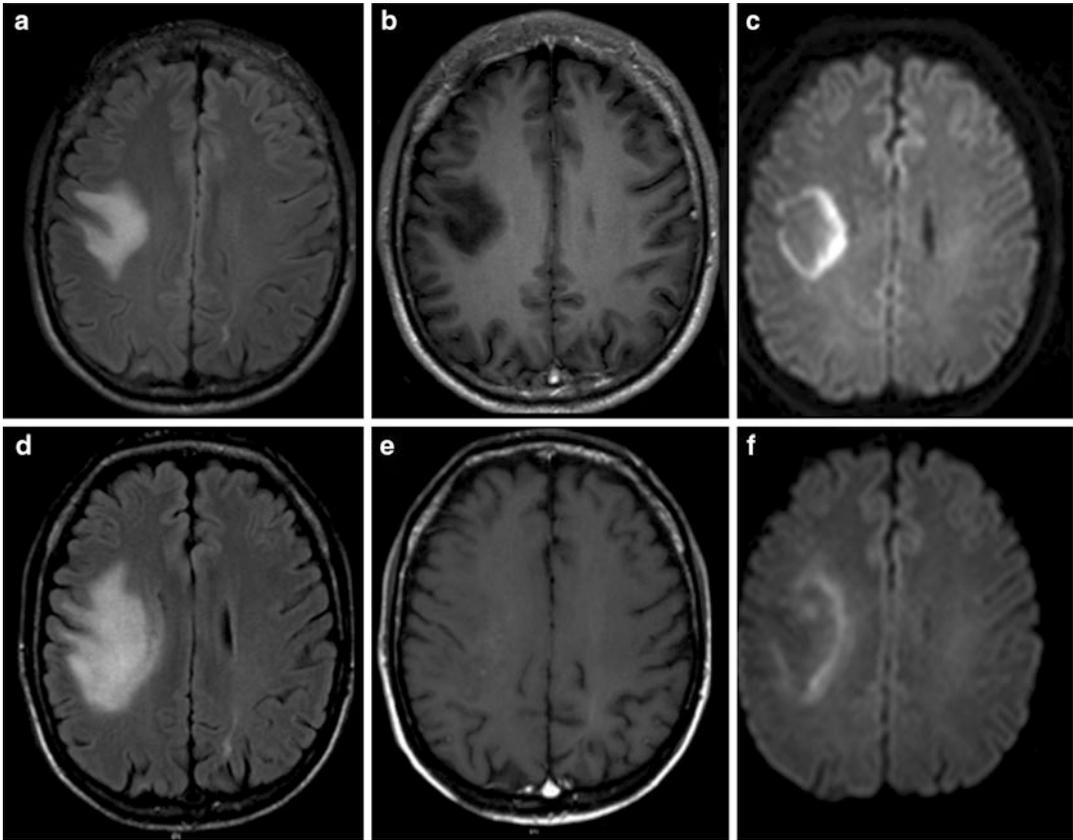
PML-immune reconstitution inflammatory syndrome (IRIS)

Immune reconstitution following cART initiation, with a decrease of plasma HIV-1 RNA, and usually also an increase of CD4+ T cells

Signs of brain inflammation by MRI or at brain biopsy

New onset (unmasking IRIS) or worsening (paradoxical IRIS) of clinical disease that would not be expected from the natural course of PML

primary CNS lymphoma, there is no mass effect or displacement of brain structures. Lesion appearance at MRI may also help distinguish PML from other white matter diseases, in particular HIV encephalitis, which is characterized by more diffuse and symmetrical central white matter changes that are either not detected or resulting only in a subtle signal alteration on T1-weighted sequences.



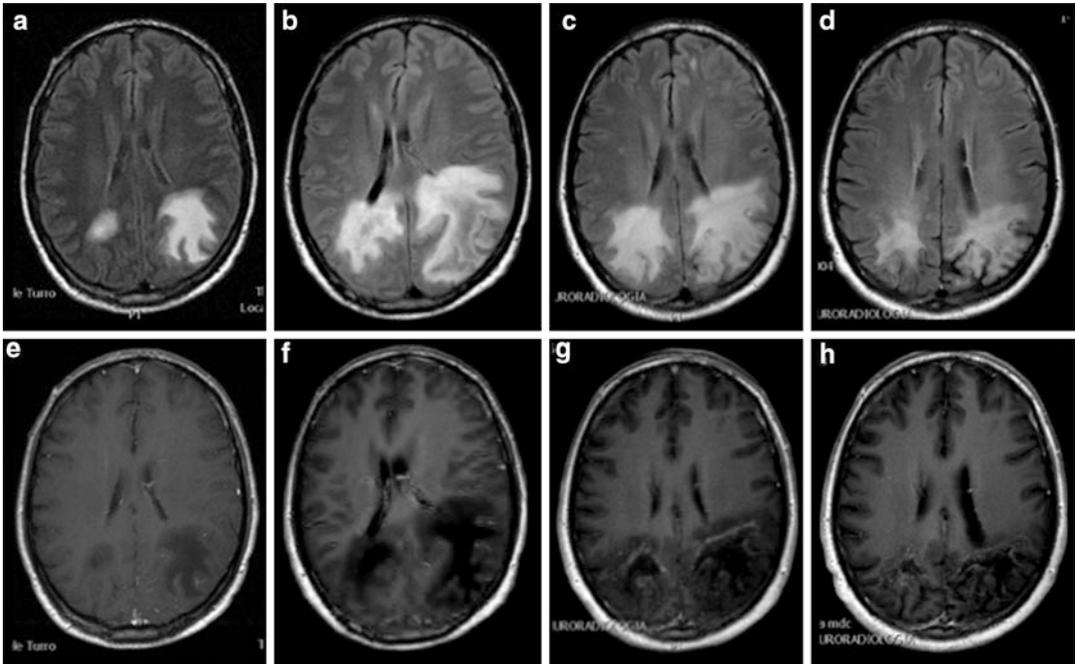
Progressive Multifocal Leukoencephalopathy and HIV, Fig. 4 Progression of disease by magnetic resonance imaging (MRI) in PML. Axial brain images at the time of PML diagnosis (a–c) and showing lesion evolution after 4 weeks (d–f). (a, d) Fluid-attenuated inversion recovery (FLAIR) sequences, showing focal hyperintense signal alteration in the subcortical white matter of

the right frontal region; (b, e) T1-weighted sequences showing hypointense signal alteration (*black*), with no enhancement after gadolinium administration; (c, f) diffusion-weighted images (DWI), showing high-signal intensity at the advancing edge of the active lesion and low-signal intensity in the center of the lesion

P

Contrast enhancement of PML lesions becomes more frequent after cART initiation with immune reconstitution and disease remission. This type of contrast enhancement is frequently described as “dot-like” perivenular, with a thin or reticulated appearance adjacent to the edge of the lesions. However, contrast enhancement may also present with a thick peripheral component, which is associated with the inflammatory forms (PML-IRIS), and can be accompanied by edema, mass effect, with or without displacement of brain structures (Post et al. 2013; Fig. 5). In these cases the distinction from the other CNS-OIs may be more difficult.

Advanced neuroimaging techniques, such as diffusion-weighted imaging (DWI), diffusion tensor imaging (DTI), and proton MR spectroscopy (MRS), may provide additional diagnostic information (Shah et al. 2010). DWI shows the degree of diffusion of water molecules in brain structures; thus more recent lesions and the advancing edges of established lesions show high-signal intensity (low water diffusion), reflecting active infection and cell swelling. By contrast, older lesions and the centers of larger lesions show low-signal intensity (high water diffusion), reflecting areas of necrosis and/or reparative gliosis. Diffusion tensor imaging (DTI) also shows the degree of



Progressive Multifocal Leukoencephalopathy and HIV, Fig. 5 Inflammatory PML by magnetic resonance imaging (MRI). Three different aspects of inflammatory PML observed at different time points in a patient with a simultaneous diagnosis of HIV infection and PML. (A–D), Axial fluid-attenuated inversion recovery (FLAIR); E–H, axial T1-weighted sequences after gadolinium administration. (a, e) At PML diagnosis, cART-naïve (CD4 cell count, 323/ μ L; HIV-RNA 5810 c/mL) left parieto-occipital and right parietal lesions with surrounding edema determining mass effect; there is no contrast enhancement of the lesions. (b, f) After 18 days of cART (clinical worsening): increase of lesion size, with edema and mass effect; intravenous dexamethasone is started at 8 mg tid for 10 days followed by oral taper,

followed by reduction of the perilesional edema. (c, g) After 12 weeks of cART (clinical worsening following previous improvement; CD4 cell count, 402/ μ L; HIV-RNA <1 c/mL): gadolinium T1-weighted sequences show the presence of thick contrast enhancement on the periphery of the lesions; intravenous methylprednisolone 1 g/day for 5 days is started, followed by clinical improvement and reduction of the enhancement. (d, h) After 18 weeks of cART (clinical stabilization; CD4 cell count, 344/ μ L; HIV-RNA <1 c/mL): gadolinium T1-weighted sequences show the presence of dot-like contrast enhancement within and in the periphery of the lesions; FLAIR images show reduction of lesion size and signal intensity and atrophy

white matter fractional anisotropy (FA), which reflects the tendency of water molecules to move preferentially in one direction of the space, in an asymmetric fashion. This is a sensitive tool for the assessment of myelination and white matter integrity with the potential to detect white matter injury before conventional MR imaging and DWI. By MRS, PML lesions typically show decreased N-acetylaspartate (NAA), or NAA to creatine ratio, reflecting neuronal and axonal damage, and increased choline, or choline to creatine ratio, related to the myelin breakdown, with the greatest changes at the center of lesions. The NAA

to creatine ratio is usually lower in PML than in other white matter lesions, and it might help in the differential diagnosis with HIV-1 encephalitis. Lactate and lipid signals, related to necrosis, may also be high in PML lesions.

Laboratory Assessment

The definite etiological diagnosis of PML is obtained, in the appropriate clinical and radiological context, through polymerase chain reaction (PCR) analysis of CSF for the presence of JCV-DNA or, more rarely, by brain biopsy (Table 1). Among cART-untreated patients with

neurological disease, the diagnostic sensitivity of JCV-DNA PCR in CSF is of 72–92% and specificity of 92–100%. Because the ability to detect JCV-DNA increases with PML progression, lumbar puncture is usually repeated if the first PCR analysis is negative, but diagnostic suspicion remains high. However, the likelihood of detecting JCV in CSF is reduced in patients on cART. The JCV-DNA load in CSF varies greatly, between the lower limit of detection (usually at around 50–100 copies/mL) to over 10 million copies/mL. This likely reflects the degree of JCV replication in the brain and in cART-untreated patients that may provide information on the patient's prognosis, with higher copy numbers predicting shorter survival. Measuring JCV-DNA copy number in the CSF can also be useful for monitoring disease progression during cART, with JCV-DNA clearance being associated with PML remission (Cinque et al. 2009).

At brain biopsy, PML is identified by the characteristic tissue cytopathology, including oligodendrocytes with intranuclear inclusions, bizarre astrocytes, and lipid-laden macrophages, with identification of JCV proteins by immunohistochemistry, of JCV or cross-reacting polyomavirus DNA by *in situ* nucleic acid hybridization, or of JC virion electron microscopy. In IRIS-PML histopathology typically demonstrates perivascular mononuclear inflammatory infiltration, with rare JCV-positive cells.

Other Laboratory Assessments

By the use of PCR, JCV-DNA can also be detected in blood, including peripheral blood mononuclear cells (PBMCs) and plasma. In PBMCs, JCV-DNA can be found in both PML patients (in 10–60% of cases) and in subjects without the disease (in up to 20% of cases); therefore this test is unlikely to provide a sensitive and specific diagnostic method. In plasma, on the contrary, JCV is detected in about one half of HIV-positive PML patients, with highly variable levels, but only occasionally and at low copy numbers in controls without PML, thus providing, in case of a positive result, a useful tool for disease monitoring. In urine, JCV-DNA is detected in about 20–30% of both healthy and

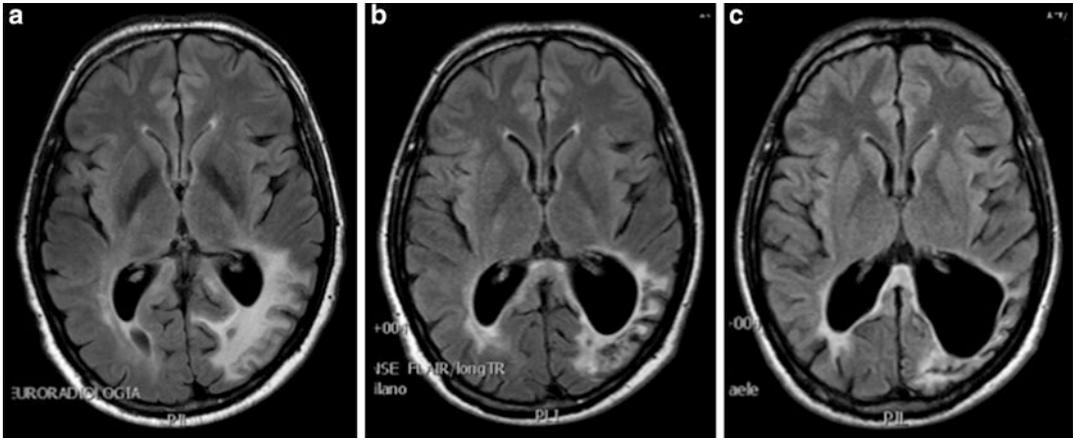
immunocompromised individuals without PML, at a wide range of copy numbers and with no differences between patient status, and therefore it is not useful in diagnosis (Hirsch et al. 2013).

Serologic testing is not diagnostically useful because of high anti-JCV IgG prevalence in the general population. Finding a JCV-specific intrathecal antibody, synthesis might provide additional diagnostic support, especially in patients with undetectable JCV-DNA in CSF; however this approach has not yet entered the clinical practice.

JCV-specific CD4+ and CD8+ T-cell immune responses have largely been assessed in PML, mainly in the context of research studies. Laboratory approaches have involved *ex vivo* stimulation of patient's blood cells with JCV or virus peptides, either individually or as peptide pools, more often covering the external capsidic, highly immunogenic VP1 region. A number of methodologies have been used including among the others, the functional cytokine secretion and ELISPOT assays. At the time of diagnosis, most of the patients with HIV-1-related PML show low or undetectable responses, which increase in frequency and magnitude during cART immune reconstitution. The diagnostic sensitivity and specificity of these assays has not been formally assessed, but the large variability of responses suggests that these assessments are unlikely to become diagnostically useful. Nevertheless, they may prove valuable for monitoring immune reconstitution following cART initiation.

Treatment

There is no specific therapy for JCV infection or PML, and, at present, cART remains the only proven effective approach to treatment of HIV-1-related PML (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2013). By reversion of the immunosuppression, cART stops progression of PML in about half of patients. Unfortunately, neurological deficits frequently persist following PML remission, because of irreparable loss of brain tissue, with approximately half of PML survivors retaining moderate to severe disability. Although several factors may



Progressive Multifocal Leukoencephalopathy and HIV, Fig. 6 Lesion evolution following PML remission. Axial fluid-attenuated inversion recovery (FLAIR) images following PML remission show progressive atrophy of the brain areas with PML lesions and

secondary, ex vacuo, enlargement of lateral ventricles in the case illustrated in Fig. 5: after 7 months (a), 13 months (b), and 36 months (c) from the diagnosis of PML

be associated with worse prognosis, including low CD4+ T cells, high plasma HIV-RNA levels, high CSF JCV-DNA level, and the presence of lesions in the brain stem, the prognosis is usually unpredictable at disease onset. In clinical practice, cART should be started immediately in untreated patients with PML and be optimized for virologic suppression in patients with PML who have received ART but remain HIV viremic. There is no evidence supporting cART intensification for PML or the use of antiretroviral drugs that better penetrate the CNS. On the other hand, it is reasonable to use drug combinations that achieve the fastest and most effective control of HIV-1 replication and immune reconstitution (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2013).

Untreated PML or progressive disease despite cART is characterized by continuous neurological worsening usually within weeks to months, in parallel with enlargement of initial lesions and onset of new ones. JCV-DNA levels in CSF may either increase or remain stable. On the contrary, disease remission is achieved with stabilization or improvement of clinical symptoms and clearance of JCV-DNA from the CSF, likely reflecting suppression of viral replication in brain lesions. The dynamics of JCV-DNA decay in CSF varies

largely among patients, likely depending on the amount of JCV-DNA at diagnosis and the degree of immunological restoration. MRI improvement usually follows clinical and virological changes, showing loss of activity of old lesions, with a decrease of the hyperintense signal in T2 and FLAIR sequences and disappearance of the high-signal intensity rim at the lesion periphery on DWI sequences, and frequent appearance of contrast enhancement. The formation of scar tissue that follows disease remission is associated with progressive atrophy of the brain structures (Fig. 6). By MRS, disease stabilization is associated with decreasing choline to creatine, increasing NAA to creatine, and increasing myoinositol to creatine ratios, the latter signaling glial activation and resolution of the lipid and lactate signals.

Corticosteroids are used in PML-IRIS to reduce local inflammatory reactions, and it is justified in patients with both progressing clinical deficits and signs of inflammatory disease. PML patients who initiate cART with evidence of immune reconstitution at MRI, such as contrast enhancement, might not need steroid treatment if they are clinically stable or improving. The dosage and duration of corticosteroid treatment for PML-IRIS are not established. One common approach, based on treatment of multiple sclerosis flares, is to begin

Progressive Multifocal Leukoencephalopathy and HIV, Table 2 Drugs used for treatment of PML

Drug name	Rationale for use	Highest clinical evidence of efficacy/nonefficacy	Current recommendations or status of development
Cytarabine	Anti-JCV in vitro activity	No survival benefit of IV or intrathecal administration in a controlled clinical trial. Significant toxicity	Not recommended
Cidofovir	Anti-JCV in vitro activity	No survival benefit from a meta-analysis of five published studies. Significant toxicity	Not recommended
Brincidofovir/ CMX001 (hexadecyloxypropyl- cidofovir)	Anti-JCV in vitro activity	Disease remission in one patient with ICL with subsequent administration of IL-7	Requires additional investigation
Mefloquine	Anti-JCV in vitro activity	No virological or clinical benefit in a phase I/II clinical trial	Not recommended
Mirtazapine, risperidone (5HT2a receptor inhibitors)	Serotonergic 5HT2a receptor can serve as cellular receptor for JCV in glial cell culture	No survival benefit from retrospective analyses and large clinical experience	Not recommended
Topotecan (Topoisomerase inhibitor)	Anti-JCV in vitro activity	No survival benefit from evaluation of individual case reports. Significant toxicity	Not recommended
Interferon-alpha	Immunomodulatory effect	No survival benefit from large retrospective analysis	Not recommended
Interleukin-7 (IL-7)	Immunomodulatory effect (thymic development and post-thymic survival, proliferation and maturation of T cells)	Disease remission in individual PML patients with ICL or receiving immunosuppressive drugs	Clinical trial planned in lymphocytopenia-related PML
Adoptive cell transfer	Improves JCV-specific T-cell immunity	Disease remission in a single patient with PML receiving immunosuppressive drugs	Requires additional investigation
Viral protein-1 (VP-1) as therapeutical vaccine	Improves JCV-specific B- and T-cell immunity	Disease remission in three PML patients (two ICL, one receiving immunosuppressive drugs) with simultaneous administration of IL-7	Requires additional investigation

Notes

ICL idiopathic CD4+ lymphocytopenia

See references Shah et al. 2010; Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2013; Alstadhaug et al. 2014

with a 3–5-day course of intravenous (IV) methylprednisolone at 1 g per day, followed by an oral prednisone taper (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2013). The possible development of systemic and immune effects should be monitored carefully during steroid therapy. Contrast-enhanced MRI may be helpful in documenting resolution of

inflammation and edema and control for IRIS recrudescence. Importantly, cART should be continued during this period.

The history of specifically targeted treatments for PML is disappointing. Indeed, developing a treatment for PML is challenging, because of the lack of availability, in the past, of an animal model for the disease and of the difficulties to design and

carry out a clinical trial – due the low incidence of PML, its rapid progression, and its potential response to immune reconstitution interventions. A number of known drugs are effective in inhibiting JCV replication in cell culture systems, and several of these have also been administered to patients with PML, either in the context of structured clinical studies or of uncontrolled clinical practice. However, there is no drug that can influence substantially the natural history of PML (Table 2). Recently, more promising approaches have been suggested with the aim to restore either general or JCV-specific host immunity. These have included human recombinant interleukin-7 (IL-7), a cytokine that induces thymic T-cell development and post-thymic proliferation and maturation of both naive and memory T cells (Alstadhaug et al. 2014; Sospedra et al. 2014), adoptive infusion of JCV-specific cytotoxic T cells (Mani et al. 2014), and a JCV-VP1-based “vaccine,” which may be able to stimulate both T- and B-cell-specific responses (Sospedra et al. 2014).

Conclusion

PML remains a relevant opportunistic disease in persons with HIV infection. The only effective way to prevent the disease is to prevent progression of HIV-related immunosuppression with cART, and this is achieved in most of patients with HIV infection. Despite its currently low incidence, however, PML carries a high morbidity and mortality burden. Although cART will reverse immunodeficiency in most of the cases, PML remission may not be achieved in time to avoid death or irreversible brain damage. In the absence of drugs targeting JCV replication, strategies aiming to fasten immune reconstitution, e.g., IL-7 or a therapeutical vaccine, are a potential promising approach in HIV-related PML, which require evaluation in clinical trials.

Cross-References

► **Comorbidity:** [Progressive Multifocal Leukoencephalopathy](#)

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Protease Inhibitor

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Definition

Protease inhibitors (PIs) belong to a class of anti-retroviral medications used to treat HIV-1 infection by acting as competitive inhibitors of aspartyl protease, an enzyme used to cleave precursors of HIV into functional enzymes and structural proteins. These medications are given orally and are metabolized by the liver through cytochrome P450 enzymes. One advantage of using PIs in

combination antiretroviral therapy (cART) is that they have a higher genetic barrier to resistance compared to other classes of medications. Disadvantages include the potential for multiple drug-drug interactions and high frequency of side effects and toxicity.

Introduction

HIV protease inhibitors (PIs) are a class of medications approved for treatment of HIV infection when given as a component of combination antiretroviral therapy (cART). The earliest PIs received Food and Drug Administration (FDA) approval for use in the treatment of HIV infection in 1995; currently, there are nine PIs available on the market (Pokorna et al. 2009). As an advantage over other antiretroviral drug classes, most PIs have a higher genetic barrier to resistance. However, PIs are also plagued by poor oral bioavailability, high pill burden, and frequency of unpleasant side effects (Tsibris and Hirsch 2015). This entry will give an overview of PIs including their drug characteristics, clinical use, side effects and toxicity, and mechanisms of resistance.

Characteristics of Protease Inhibitors

Mechanism of Action

Aspartyl protease is the HIV enzyme necessary to cleave polyprotein precursors of the virus (Gag and Gag-Pol) into mature, functional viral enzymes and structural proteins (Pokorna et al. 2009; Tsibris and Hirsch 2015). As competitive inhibitors of aspartyl protease, PIs prevent the process of HIV “viral maturation,” an essential step in the production of infectious viral particles (Pokorna et al. 2009). In the presence of PIs, therefore, viral particles formed remain non-infectious, preventing the spread of HIV to other cells. This mechanism of action differentiates PIs from all other antiretroviral classes because it occurs post-integration of HIV DNA into the host (human) DNA rather than pre-integration.

Pharmacokinetics

Absorption and Distribution

All PIs currently used in clinical practice are only approved for oral administration. Absorption in the gastrointestinal tract and diffusion through anatomical compartments overall is poor to moderate for the PIs, largely due to their extensive plasma protein binding (90–99%) and their affinity for the efflux pump transporter, p-glycoprotein (Pokorna et al. 2009). Both of these characteristics are thought to play a role in the overall poor penetration of PIs into HIV reservoir sites, including the central nervous system and genital tracts. One notable exception, the PI indinavir, has been found to have good CNS penetration, thought to be due to its lower degree of plasma protein binding (60%) compared to all other PIs.

Metabolism

The PIs are hepatically metabolized by the cytochrome P450 isoenzyme CYP3A4. Commonly, pharmacologic “boosters” that inhibit this metabolic pathway (e.g., ritonavir) are coadministered with PIs to overcome poor absorption and increase systemic drug concentrations. Originally approved as an antiretroviral drug, ritonavir’s utility was limited due to high frequency of side effects, and it is now exclusively used as a boosting agent due to its potent inhibition of CYP3A4. Ritonavir-boosted PIs have led to higher drug exposures and lower pill burden, thereby simplifying dose schedules without decreasing efficacy (Larson et al. 2014). PIs that may be given unboosted (e.g., nelfinavir, atazanavir, and fosamprenavir) are alternatives to first-line therapy due to their higher risk of virologic failure with drug resistance compared to boosted PIs (Shafer and Schapiro 2008). As an inhibitor of multiple CYP enzymes in addition to 3A4, ritonavir can induce many drug-drug interactions, particularly with immunosuppressant medications, antiarrhythmics, antimycobacterials, other antiretrovirals, oral contraceptives, and the statin class of lipid-lowering agents (Pokorna et al. 2009). Cobicistat, a more selective inhibitor of the CYP3A4 enzyme, is a newer pharmacokinetic enhancer without antiretroviral activity. Due

to its lower risk of drug-drug interactions, cobicistat is an attractive alternative to ritonavir for enhancement of PIs in adults with HIV infection (Larson et al. 2014) and is now used to enhance drug concentrations of two commonly used PIs, atazanavir and darunavir, in co-formulated fixed dose drug combinations.

Excretion

Excretion of PIs is predominantly through feces and partially in the urine. Two PIs, indinavir and atazanavir, have been found to have pH-dependent solubility in the urine, leading to increased crystallization and the development of urinary stones at higher pH (Pokorna et al. 2009).

Barriers to Resistance

A major advantage of PIs over other classes of antiretroviral medications is that they generally have a higher genetic barrier to developing resistance. Unlike non-nucleoside reverse transcriptase inhibitors (NNRTIs) or integrase inhibitors, PIs typically require multiple mutations to lose substantial antiviral activity (Tsibris and Hirsch 2015). Mechanisms of resistance will be described in greater detail in section “[Side Effects and Toxicity](#)”.

Side Effects and Toxicity

Although the introduction of the PIs has revolutionized HIV therapy, the dramatic improvement in treatment outcomes associated with their use has been tampered by treatment-limiting toxicities. Side effects observed with the earlier PIs included nausea, vomiting, and diarrhea, in addition to circumoral paresthesia with high-dose ritonavir and nephrolithiasis with indinavir. Laboratory abnormalities such as elevations in serum bilirubin, glucose, triglycerides, and cholesterol were also noted, although these were initially considered not to be clinically significant.

Within two years of their introduction into clinical practice, the US Food and Drug Administration (FDA) received over 80 reports of cases of diabetes mellitus, among HIV-infected patients on PI-based therapy, and subsequently issued a

warning regarding PI use; however, they noted that a causal relationship between PI use and hyperglycemia had not been established. Concurrently, anecdotal reports of increases in abdominal fat, loss of upper and lower extremity fat, and unusual fat accumulation at the base of the neck (dorsocervical fat pad) were emerging.

These observations were initially puzzling as the HIV PIs were designed based on detailed knowledge of the tertiary and quaternary structure of their target molecule, the HIV protease enzyme. These compounds have strong affinity and great degree of specificity for the HIV protease, an enzyme that catalyzes the cleavage of the HIV gag and gag-pol polyproteins in a critical step in the viral replication cycle necessary for maturation and budding of the new virion. Unlike the human proteases, the HIV protease belongs to a group of enzyme known as “aspartyl endopeptidase” that possesses unique specificity for Phe-Pro and Tyr-Pro sequences. Because human proteases do not recognize these sequences, it was thought that blocking this molecule that is vital for HIV replication but unique to the virus would have minimal effects on human cellular processes.

It is now clear from abundance of empiric evidence and more than two decades of clinical experience that the PIs are variably associated with a broad range of metabolic abnormalities. These observations have raised questions about the molecular specificity of these drugs and generated wide speculations about the mechanisms underlying their unwanted effects. For example, to what extent does the virus (apart from the drugs) contribute to the observed metabolic abnormalities? Do HIV disease reversal and correction of the underlying chronic pathologic processes following sustained suppression of viral replication lead to fat gain that may be abnormally distributed? Are the drug-associated metabolic abnormalities that mimic Cushing’s syndrome related to enhanced sensitivity of the body to cortisol? To what extent do genetic or familial factors play a role?

For the purpose of this discussion and to address some of these issues in greater details, we have grouped the PI associated toxicities into

the following commonly observed clinical syndromes: (a) lipodystrophy, (b) hyperlipidemia, (c) glucose intolerance, (d) hepatic injuries, and (e) others.

Lipodystrophy

Lipodystrophy is changes in body fat distribution arising from derangement in fat production, uses, and storage. It was among the most publicized of the PIs’ metabolic abnormalities and was originally referred to as the “Crix belly” because many of the earlier reports were associated with indinavir. Similar body changes have been observed with all FDA-approved PIs although to a lesser extent with the newer agents such as atazanavir and darunavir. Fat redistribution occurs early in therapy, and rates can be as high as 30–50%. Two patterns are recognized: fat loss and fat gain; in general, men tend to lose fat, while women tend to build fat. Subcutaneous fat loss, also referred to as lipatrophy, occurs more commonly when PIs are used in combination with the older nucleoside reverse transcriptase inhibitors such as stavudine, didanosine, or zidovudine. It manifests as fat loss in the face (leading to sunken cheeks, temples, or eyes), buttocks, and arms and legs (resulting in prominence of the peripheral veins). Fat buildup, often referred to as lipohypertrophy or lipoaccumulation or hyperadiposity, is associated with fat accumulations in the abdomen (central obesity), breasts (particularly in women), back of the neck and shoulder (dorsocervical fat pad), and lipomas (fatty growth) in various parts of the body. Lipodystrophy is a major clinical concern and has led some patients to seek liposuction or surgical removal of superficial fat deposits and others to discontinue therapy entirely. While the mechanism underlying this disorder remains unclear, some investigators have proposed a direct drug effect due to a high degree of similarity between a segment of the HIV protease enzyme and a lipoprotein receptor-like protein, which is involved with the transport of circulating lipids. Some of the cases – particularly those involving the dorsocervical fat pad – appeared similar to Cushing’s syndrome; however, serum cortisol levels or dexamethasone suppression tests are often normal in affected patients with buffalo

hump. Tesamorelin, a growth hormone-releasing factor analog, is currently the only FDA-approved drug shown to reduce excess abdominal fat in HIV-infected patients with lipodystrophy.

Hyperlipidemia

Elevation in serum lipids is among the most prevalent laboratory abnormalities seen with the PIs. Prevalence of 8–42% for hypercholesterolemia and 13–80% for hypertriglyceridemia has been reported following PI-based therapy. The association appears to be stronger with ritonavir than for other PIs. Some studies attribute PI-related hyperlipidemia to enhanced very low-density lipoprotein (VLDL) production, whereas others blame this abnormality on impaired lipoprotein clearance. Whether these lipid changes translate into higher rates of coronary artery disease remains controversial; however, increased incidence of cardiovascular disease is reported in patients with HIV infection. Hyperlipidemia in this setting often responds to treatment with lipid-lowering agents although not all of these agents can be safely coadministered with the PIs because of concern for severe drug-drug interaction. A switch from a PI-based therapy to a less lipidogenic antiretroviral regimen has been successful in lowering serum lipids to normal in some cases (Munk 1998).

Glucose Intolerance

Reports of hyperglycemia and overt diabetes increased in the HIV/AIDS population following the introduction of the PI into clinical practice, raising the speculation that this class of drugs induced insulin resistance. Rates of new-onset diabetes ranging from 3% to 7% have been reported with the PIs. The incidence of new-onset diabetes was fourfold higher in HIV-infected men on highly active antiretroviral therapy in the Multicenter AIDS Cohort Study compared with HIV-seronegative men. This effect may be less pronounced with the newer PIs such as darunavir and atazanavir. Standard medical therapy is recommended for PI-induced diabetes. Some patients have been managed by switching to other classes of drug that are less diabetogenic (Hruz et al. 2001).

Hepatic Injuries

Reports of hepatitis or hepatic failure linked to PI therapy are common. Hepatotoxicity occurred at rates of 1–10% in registration trials conducted for the purpose of obtaining FDA approval for the PIs. The prescribing information for all PIs approved by the US FDA includes the following warning: (1) hepatitis, including cases resulting in hepatic failure and death, has been reported in patients taking PIs; and (2) there may be an increased risk for alanine aminotransferase and/or aspartate aminotransferase (ALT/AST) elevations in patients with preexisting liver disease or underlying hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. An increased frequency of AST/ALT monitoring should be considered for these patients (Ogedegbe and Sulkowski 2003).

Others

Toxicities specific to or seen more commonly with certain members of the PI class include atazanavir induced hyperbilirubinemia and bilirubin kidney stones, intracranial bleeds associated with tipranavir, ingrown toenails with indinavir, and skin rashes seen occasionally with fosamprenavir and darunavir. Diarrhea and other gastrointestinal intolerance, though observed with all members of the class at variable rates, appear to be relatively more common with lopinavir/ritonavir.

Mechanisms of Resistance

Multiple protease mutations are often required in order to develop clinically significant HIV-1 resistance to ritonavir-boosted PIs. For example, the accumulation of at least six mutations was associated with reduced virological response in PI-experienced patients receiving ritonavir-boosted lopinavir (Masquelier et al. 2002). Within the protease gene, resistance mutations are classified either as “major,” those selected first when drug is present or substantially reducing susceptibility of the virus to drug, or “minor,” those that typically present later and alone do not have significant impact on phenotype (Wensing et al. 2015). In addition, mutations in the cytoplasmic tail of the HIV-1 *Env* protein or cleavage sites of

the *Gag* protein may emerge before protease mutations and can confer resistance to all PIs.

Major and Minor Mutations

Major mutations at 13 amino acid positions have been shown to confer reduced susceptibility to one or more PIs, including positions 30, 32, 46, 47, 48, 50, 54, 58, 82, 83, 84, 88, and 90 (see Table 1).

Protease Inhibitor, Table 1 Major protease inhibitor mutations causing clinically significant resistance

Amino acid position	Specific mutation ^a	Protease inhibitors affected
30	D30N	Nelfinavir
32	V32I	Lopinavir/ritonavir
46	M46 I/L	Indinavir/ritonavir
47	I47A I47V	Lopinavir/ritonavir Lopinavir/ritonavir Darunavir/ritonavir Tipranavir/ritonavir
48	G48 V	Saquinavir/ritonavir
50	I50L I50V	Atazanavir +/- ritonavir boosting Darunavir/ritonavir Fosamprenavir/ritonavir
54	I54M/L	Darunavir/ritonavir
58	Q58E	Tipranavir/ritonavir
82	V82A/F/ T/S	Indinavir/ritonavir Lopinavir/ritonavir
83	N83D	Tipranavir/ritonavir
84	I84V	Atazanavir +/- ritonavir boosting, darunavir/ritonavir Saquinavir/ritonavir Indinavir/ritonavir Fosamprenavir/ritonavir
88	N88S	Atazanavir +/- ritonavir boosting
90	L90 M	Nelfinavir, saquinavir/ ritonavir

Adapted from table “Mutations in the Protease Gene Associated with Resistance to Protease Inhibitors” in 2015 update of the drug resistance mutations in HIV-1, IAS-USA. *Top Antiv Med.* October/November 2015;23(4):132–41

^aMutations are named by listing the letter abbreviation for the wild-type amino acid, followed by the amino acid position, and then the amino acid substitution conferring resistance. Amino acid abbreviations: *A*, alanine; *C*, cysteine; *D*, aspartate; *E*, glutamate; *F*, phenylalanine; *G*, glycine; *H*, histidine; *I*, isoleucine; *K*, lysine; *L*, leucine; *M*, methionine; *N*, asparagine; *P*, proline; *Q*, glutamine; *R*, arginine; *S*, serine; *T*, threonine; *V*, valine; *W*, tryptophan; *Y*, tyrosine

Minor (or accessory) mutations can contribute to resistance by either compensating for the impaired replication that major mutations cause the virus or reduce susceptibility only in combination with other mutations. For example, minor mutations at positions 10, 20, 36, 63, and 71 cause an upregulation of protease processivity to compensate for the decreased viral fitness associated with major protease mutations. Furthermore, the effect of some mutations differs based on PI; for example, the I50L mutation grants resistance to atazanavir but improves the susceptibility to all other PIs (Shafer and Schapiro 2008). Similarly, for darunavir, one of the most commonly prescribed PIs, two mutations (E35D and V82A) were found to have a positive impact on virological response, possibly explaining the potency of ritonavir-boosted darunavir in PI-resistant HIV (Descamps et al. 2009).

Gag Cleavage Site and Env Cytoplasmic Tail Mutations

Many patients fail PI-containing regimens without any protease mutations. One theory as to why this may occur is that standard resistant assays may ignore other parts of the HIV genome that could contain mutations causing PI resistance. For example, preliminary data has shown that sequence changes in the cytoplasmic tail of the HIV *Env* protein (Rabi et al. 2013) and mutations within the *Gag* protein (Fun et al. 2012) contribute to PI resistance and would not be discovered on standard genotypic PI resistance testing.

Characteristics of the HIV PIs

HIV PIs currently in use in the order they were introduced into clinical practice include saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir/ritonavir, atazanavir, fosamprenavir, tipranavir, and darunavir. Lopinavir is co-formulated with ritonavir (Kaletra[®]); more recently, atazanavir and darunavir were both co-formulated with cobicistat (Evotaz[™] and Prezcobix[®], respectively). Due to toxicities associated with its therapeutic doses and the potential to induce clinically significant drug-drug interaction,



Protease Inhibitor, Table 2 Characteristics of HIV protease inhibitors and boosted doses in treatment naïve patients

Drug ^a	Year approved	^{c,d} Dosing schedule	^e Unique side effects/toxicities
^b Saquinavir	1995	1000 mg twice daily	<ul style="list-style-type: none"> • Buffalo hump • Possible increased bleeding episodes in patients with hemophilia • PR interval prolongation • QT interval prolongation
Ritonavir	1996	100 mg daily or twice daily when used as a booster	<ul style="list-style-type: none"> • Drug-drug interaction • Metabolic abnormalities • Paresthesia (circumoral and extremities) <ul style="list-style-type: none"> • Taste perversion • Possible increased bleeding episodes in patients with hemophilia
^b Indinavir	1996	800 mg twice daily	<ul style="list-style-type: none"> • Kidney stones • Hyperbilirubinemia • Possible increased bleeding episodes in patients with hemophilia
Nelfinavir	1997	1250 mg twice daily	<ul style="list-style-type: none"> • Possible increased bleeding episodes in patients with hemophilia • Serum transaminase elevation
Lopinavir/ritonavir	2000	400/100 mg twice daily	<ul style="list-style-type: none"> • Gastrointestinal side effects • Lipid abnormalities • Pancreatitis • PR interval prolongation • QT interval prolongation
^b Atazanavir	2003	300 mg daily	<ul style="list-style-type: none"> • Hyperbilirubinemia • Rarely kidney stones • PR interval prolongation
^b Fosamprenavir – prodrug of amprenavir	2003	1400 mg daily	<ul style="list-style-type: none"> • Rash • Possible increased bleeding episodes in patients with hemophilia • Kidney stone
^f Tipranavir	2005	500 mg (plus 200 mg of ritonavir) twice daily	<ul style="list-style-type: none"> • Intracranial hemorrhage (rare) • Rash • Possible increased bleeding episodes in patients with hemophilia
Darunavir	2006	800 mg daily	<ul style="list-style-type: none"> • Rash • Hepatic toxicity • Increase in serum creatinine (with cobicistat)

^aThe HIV PIs are metabolized by cytochrome P450 isoenzymes. With the exception of nelfinavir, all can be boosted with either ritonavir or cobicistat

^bAlthough preferable boosted, saquinavir, indinavir, fosamprenavir, and atazanavir can be administered unboosted

^cDosing adjustments for renal insufficiency are not indicated as HIV PIs are metabolized primarily in the liver

^dExcept for nelfinavir, dosing schedules listed in this table assume boosting with either ritonavir or cobicistat in patients that are treatment naïve and receiving initial antiretroviral therapy

^eTo variable degree, the use of drugs in this class is associated with gastrointestinal intolerance and metabolic abnormalities (insulin resistance, dyslipidemia, and/or lipodystrophy)

^fTipranavir is reserved for treatment-experienced patients

ritonavir is now solely used at lower doses for its pharmaco-enhancement properties to boost the systemic exposure of other PIs, and it is no longer used for its antiviral effect. Saquinavir, nelfinavir,

and amprenavir are currently rarely used in clinical practice because of their high pill burden, need for more frequent dosing, and higher rates of side effects and toxicities. Although indinavir has very

good tissue and compartmental drug penetration (including the central nervous system) presumably due to its relatively lower plasma protein binding (60% plasma protein bound), its clinical use has dropped significantly because of toxicities. Tipranavir, the only non-peptidomimetic PI, has activities against HIV strains with multiple PI-resistant mutations and is therefore effective in treatment-experienced patients with prior history of PI exposure; however, its clinical use is limited by toxicities including rare cases of intracranial hemorrhages and drug-drug interactions. Atazanavir and darunavir boosted with ritonavir or cobicistat can be dosed once daily, are better tolerated relative to the other PIs, and are therefore the two more commonly used PIs in cART in many resource-rich countries. Due to cost consideration, lopinavir/ritonavir is the more widely used PI in many resource-limited settings including sub-Saharan African where it is the main second-line agent for patients failing their initial antiretroviral regimen. Lopinavir/ritonavir is also frequently used in HIV-infected pregnant women due to its favorable pharmacokinetic profile in pregnancy. The profiles of individual drugs in the HIV PI class are summarized in Table 2.

Conclusion

By inhibiting the aspartyl protease enzyme, the PIs block viral maturation and prevent continuing productive infection of HIV-susceptible cells. As a class, they possess potent antiviral activities against most strains of the HIV virus and have high genetic barrier to the development of drug-resistant mutations. They are metabolized primarily in the liver by the cytochrome P450 isoenzyme system and as such are amenable to pharmacoenhancement either by ritonavir or cobicistat, thereby simplifying dosing scheduled and significantly improving oral bioavailability, systemic exposure, and clinical efficacy. Treatment-limiting side effects to these drugs are common and include gastrointestinal intolerance and metabolic abnormalities such as insulin resistance, dyslipidemia, and abnormal body fat distribution. Nevertheless, the PIs remain important in HIV

pharmacotherapy, and PI-based regimens are particularly attractive in clinical setting where adherence to therapy is less than optimal and a more forgiving antiretroviral regimen is desired.

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Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense

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Definition

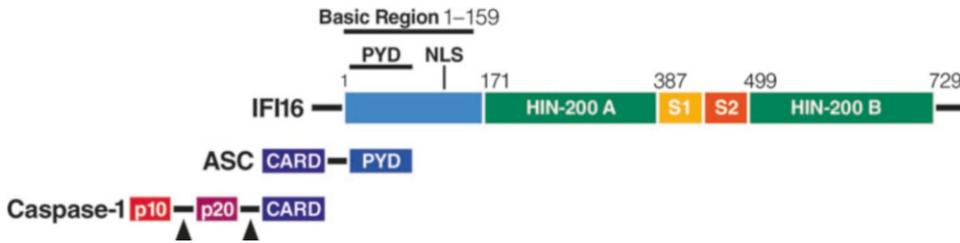
Cell death often occurs via morphological changes, which are typically described in two forms: apoptosis and necrosis. Apoptosis is an active, programmed process that causes cells to destroy themselves without stimulating inflammation. Necrosis is a passive, accidental process induced by environmental stress such as heat, physical force, or lack of oxygen, which causes the cell to uncontrollably release its inflammatory contents. Although apoptosis has received greater attention, because it is a regulated process that occurs during particular infections, both paradigms are widely accepted. However, there are a number of other cell death pathways that have been characterized. For example, in 2001, Dr. Brad Cookson discovered pyroptosis, a novel form of cell death induced by microbial infection with species of *Salmonella* and *Shigella* (Cookson and Brennan 2001). The term pyroptosis was taken from the Greek roots “pyro,” relating to fire or fever, and “ptosis” (pronounce “to-sis”), describing falling, which together describe this proinflammatory form of programmed cell death. This process uniquely depends on caspase-1, which processes the pro-forms of the inflammatory cytokines interleukin (IL)-1 β and IL-18 to their active form. Thus, distinct from apoptosis, which actively inhibits inflammation, pyroptosis involves an intense inflammatory form of programmed cell death that releases the cell’s cytoplasmic contents and proinflammatory cytokines.

Caspase-1 Is Activated in Inflammasomes

Caspases are cysteine-dependent **aspartate**-directed proteases that initiate or execute cellular

programs that cause cell death. They are synthesized as inactive proenzymes (zymogens) containing an N-terminal peptide (prodomain) and two subunits, one large and one small, separated by a linker peptide (Cohen 1997). The activity of caspases is tightly controlled by proteolytic activation. The crystal structures of caspase-3 and caspase-1 revealed that the active forms of caspases are heterotetramers containing two dimers of small and large subunits. These dimers contact each other between the small subunits, while the active site spans both the large and small subunits. Caspases also contain a prodomain that is recognized and bound by specific adapter molecules (such as apoptosis-associated speck-like protein containing a CARD (ASC) in the case of caspase-1), which recruit pro-caspase to the signaling complex (Fig. 1). Caspases are categorized as either proinflammatory or proapoptotic, depending on the outcome of their cell death programs. In humans, the proapoptotic caspases are caspase-3, caspase-7, and caspase-6, while the proinflammatory caspases are caspase-1, caspase-4, and caspase-5. Among the proinflammatory caspases, caspase-1 is the most fully characterized. Its catalytic activity is tightly regulated by signal-dependent recruitment and auto-activation within multiprotein complexes called **inflammasomes**. When activated, caspase-1 is released into the cytoplasm, where it stimulates the processing of proinflammatory cytokines and, ultimately, induces cell death by pyroptosis.

Pyroptosis is a programmed form of cell death that depends on caspase-1 but occurs independently of the caspases involved in immunologically silent apoptosis. Pyroptosis causes plasma membrane rupture, water influx, cellular swelling, osmotic lysis, and release of proinflammatory cellular content, features that are shared with caspase-independent necrosis. Pyroptosis also causes DNA cleavage and nuclear condensation. Because this process does not compromise nuclear integrity, it is distinct from the DNA laddering that occurs with apoptosis (Lamkanfi and Dixit 2010). However, the precise molecular events that regulate pyroptosis and whether pyroptosis plays a pathological role in genetic autoinflammatory diseases are not well defined.



Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense, Fig. 1 IFI16 recruits caspase-1 to assemble a functional inflammasome. IFI16 is composed of an amino-terminal PYD and two carboxy-terminal DNA-binding HIN200 domains (A and B). After binding DNA, IFI16 assembles an inflammasome to which pro-caspase-1 enzymes are recruited via their CARD motifs. The bipartite adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) is then required to bridge the interaction and recruits caspase-1 to the IFI16 inflammasome complex. When the IFI16 inflammasome complex assembles, it promotes the proteolytic autoactivation of nearby caspase-1 (*black triangles*),

which releases the p20 and p10 subunits to form the activated caspase-1 heterotetramer. Next, activated caspase-1 converts its cytoplasmic substrates, pro-IL-1 β and pro-IL-18, into the secreted bioactive cytokines and triggers cell death by pyroptosis. The DNA-binding residues are largely conserved between the AIM2 HIN and IFI16 HIN domains (Jin et al. 2012). However, IFI16 preferentially binds to the stem-rich DNA motifs of HIV reverse transcripts generated by the internal folding of the ssDNA strand (Jakobsen et al. 2013). *HIN-200* hematopoietic interferon-inducible nuclear proteins with a 200-amino acid repeat, *CARD* caspase recruitment domain, *NLS* nuclear localization signal, *PYD* pyrin domain

Despite these gaps in knowledge, pyroptosis has emerged as a key defense mechanism against microbial infections. Researchers believe that pyroptosis stops the replication of intracellular pathogens by simultaneously eliminating infected immune cells and recruiting circulating phagocytes and neutrophils that promote inflammation and destroy surviving bacteria (Lamkanfi and Dixit 2014). Importantly, most reports characterizing inflammasomes have focused on the cells of the myeloid lineage, such as macrophages or dendritic cells; however, cells outside the myeloid compartment can also activate inflammasomes. For example, keratinocyte exposure to skin irritants or ultraviolet B (UVB) irradiation activates NLRP3 inflammasomes.

IFI16 Senses Intracellular DNA That Assembles Inflammasomes

Over the last decade, researchers have greatly advanced the existing knowledge of the molecular mechanisms that activate innate immune responses. For example, they discovered a highly conserved set of cellular sensors (pathogen-related receptors, or PRRs) that detect common viral and microbial motifs known as pathogen-

associated molecular patterns (PAMPs). Viral PAMPs are composed mainly of unique nucleic acids, such as double-stranded (ds)RNA, uncapped single-stranded (ss)RNA, and cytosolic DNA. Despite dramatic advances in how RNA is sensed, much less is known about how cytosolic DNA is sensed. To date, only four soluble (i.e., non-Toll-like receptors) cytosolic DNA sensors have been described. First, **DAI** (i.e., Z-DNA-binding protein (ZBP-1)) is a cytoplasmic DNA sensor that recognizes foreign dsDNA and induces a type-I IFN response that depends on interferon regulatory factor (IRF-3). DAI was the first putative DNA receptor discovered by researchers, who showed that it is required for the type-I IFN response to transfected viral, bacterial, and mammalian DNA in murine L929 cells. DAI is prominently expressed in lymphoid tissues, bone marrow, and small intestine, which are potential sites of interaction with pathogens. Next, the PYHIN protein **IFI16** (IFN- γ -inducible protein 16) and the helicase **DDX41**, which senses intracellular DNA, both induce IFN β by recruiting STING (stimulator of interferon genes), a critical mediator of IFN β responses (Barber 2011). IFN β induces the fourth dsDNA sensor, **AIM2**. AIM2 binds to dsDNA in the cytosol and then assembles and activates an

inflammasome complex. Interestingly, AIM2 has not been implicated in orchestrating innate responses against retroviruses that produce cytosolic DNA intermediates, such as HIV. Notably, the induction of type-I IFN induces additional innate antiviral factors, including the exonuclease **TREX1** and the phosphohydrolase **SAMHD1**. However, these factors do not act as sensors, but rather as host restriction factors that are active against HIV. In fact, a deficiency in TREX1 leads to an enhanced innate immunity response as it promotes the degradation of cytoplasmic DNA species (Yan et al. 2010).

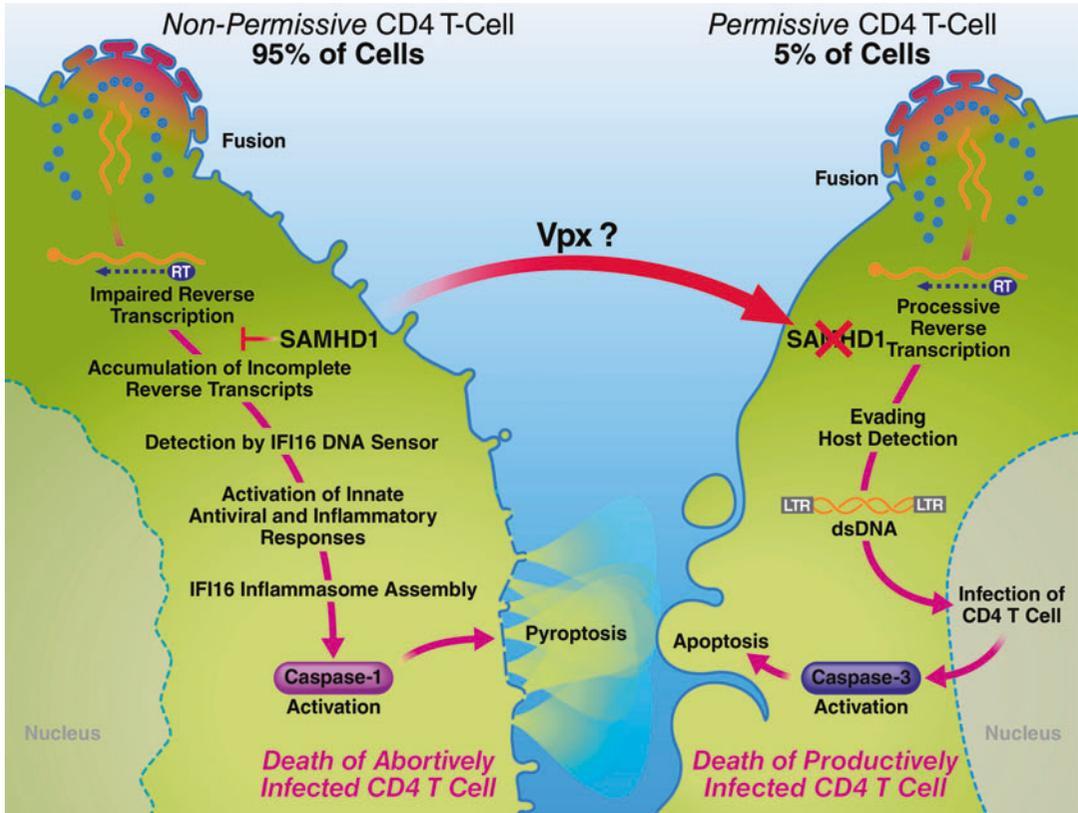
The DNA sensor protein **IFI16** is a PYHIN family member that harbors an N-terminal pyrin domain and two C-terminal HIN domains (Fig. 1). IFI16 is structurally and functionally related to AIM2 and was originally identified as an anti-proliferative protein that responds to DNA damage in the nucleus. In 2010, researchers showed that IFI16 and its mouse homologue p204 are cytosolic dsDNA receptors that induce interferon production. This process requires STING, a nucleotide sensor that resides in the endoplasmic reticulum (ER) and induces type-I IFN production. Importantly, in addition to directly binding and sensing DNA, IFI16 also forms inflammasomes in response to infection by Kaposi sarcoma-associated herpesvirus (Kerur et al. 2011).

Pyroptosis and Apoptosis in HIV Infection: Cell Permissivity Determines the Form of Programmed Cell Death

HIV pathogenesis is characterized by signature processes that deplete CD4 T cells and stimulate chronic inflammation (Deeks 2011). Despite more than three decades of study, the precise mechanisms generating these processes are poorly understood and remain key questions in HIV research (Thomas 2009). To investigate how CD4 T cells die during HIV infection, researchers took advantage of an ex vivo human lymphoid aggregate culture (HLAC) system formed with fresh human tonsil or spleen tissues (Glushakova et al. 1995). This system closely replicates the

conditions encountered by HIV in vivo and thus offers a biologically relevant approach for modeling molecular and cellular events during the HIV infection of human patients. HIV infection of these cultures extensively depletes CD4 T cells, but >95% of the dying cells are **abortively infected** with HIV-1 (Doitsh et al. 2010). Importantly, these cells do not die because of a toxic action of products encoded by HIV. Rather, death occurs as a consequence of a powerful suicidal defensive innate immune response elicited by the cells. Due to the viral nonpermissivity of these quiescent cells, the viral lifecycle attenuates during the chain elongation phase of reverse transcription, giving rise to incomplete cytosolic viral DNA transcripts. Cell death is ultimately triggered after sensing of these cytosolic DNA intermediates by **IFI16** (Monroe et al. 2014), leading to caspase-1 activation in inflammasome and **pyroptosis**, a highly inflammatory form of programmed cell death (Doitsh et al. 2014). These findings mechanistically connected for the first time CD4 T-cell death and chronic inflammation – the two key pathogenic processes of active HIV infection.

While the majority of CD4 T cells in lymphoid tissues are nonpermissive to HIV infection and die via abortive infection and pyroptosis, a small fraction of CD4 T cells become productively infected and die via caspase-3-mediated **apoptosis**. These observations provided the first integrated view of the relative roles of caspase-3-dependent apoptosis and caspase-1-dependent pyroptosis in CD4 T-cell death during HIV infection. They also demonstrated how the permissivity of the host cell determines the specific form of cell death elicited by HIV-1 – productive HIV infection in permissive cells causes a silent death involving caspase-3-dependent apoptosis, while abortive infection of resting, nonpermissive cells causes caspase-1-dependent pyroptosis with high inflammation. Importantly, in human lymphoid tissues such as tonsil and spleen, the activated and permissive subset of cells represents approximately 5% of total CD4 T cells, while nonpermissive quiescent cells represent nearly 95% of the targets encountered by HIV. Thus, contrary to the previous models, caspase-1-mediated pyroptosis



Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense, Fig. 2 Host permissivity determines the specific form of lymphoid CD4 T-cell death elicited by HIV-1. When HIV infects permissive, activated CD4 T cells, cell death occurs silently through caspase-3-dependent apoptosis. Conversely, when HIV abortively infects nonpermissive, quiescent CD4 T cells from the lymphoid tissue, death occurs through caspase-1-

dependent pyroptosis. Blocking SAMHD1 activity by Vpx may relieve the restriction on viral reverse transcription in nonpermissive CD4 T cells, allowing both productive infection and the avoidance of pyroptosis. Thus, Vpx may have evolved to avoid the pathogenic consequences associated with abortive HIV infection by relieving the host restriction in nonpermissive lymphoid CD4 T cells

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predominantly drives death of CD4 T cells in HIV-infected lymphoid tissues (Fig. 2).

Identifying pyroptosis as the predominant mechanism that depletes CD4 T cells during HIV infection provided novel targets, such as caspase-1, for potential therapeutic intervention. Indeed, pyroptosis can be blocked with the caspase-1 inhibitor VX-765, which is safe to use in humans (<http://clinicaltrials.gov/ct2/show/NCT01048255>). This finding raised the possibility that a new class of “anti-AIDS” therapies could target the host rather than the virus. These therapies could be used for a number of applications to treat HIV: (1) a salvage treatment for

individuals with broad-spectrum resistance or limited access to antiretroviral therapy; (2) a treatment to block the chronic inflammation that persists in patients treated for HIV and likely drives the earlier onset of age-related diseases; and (3) a potential component of an HIV cure, if the inflammation associated with persistent pyroptosis maintains the latent reservoir through cytokine dysregulation and increased homeostatic renewal.

In addition to the ex vivo analyses of HLACs, researchers have analyzed caspase-1 activation and IL-1 β secretion in lymph nodes following surgical removal from consenting HIV-infected

volunteers. These volunteers were chronically infected with an R5-tropic strain of HIV-1, were not taking antiretroviral therapy, and displayed high viral loads and low blood counts of CD4 T cells. Immunostaining of these tissues revealed a distinct area of HIV p24^{gag} expression between the mantle zone and germinal centers of the patient's lymph node, where activated CD4 T and B cells proliferate and interact in the follicles. In agreement with the findings from infected HLACs, caspase-1 staining revealed abundant activity in the surrounding paracortical zone (CD3), which is almost entirely composed of resting CD4 T cells, but not in the germinal centers, which consist mostly of B and activated T cells. Similarly, large amounts of bioactive IL-1 β were detected in the paracortical zone, particularly in the extracellular space between T cells, as well as the cell death marker annexin V. In sharp contrast, active caspase-3 localized explicitly to the areas in the germinal center where HIV-1 p24^{gag} expression was detected (Doitsh et al. 2014). These *in vivo* observations supported the findings obtained in infected HLACs, demonstrating that caspase-3 activity occurs in a distinct anatomical region that involves a set of productively infected cells separated from the majority of nonproductive cells undergoing caspase-1 activation, IL-1 β processing, and pyroptosis.

What Causes the Chronic Inflammation in HIV-Infected Subjects?

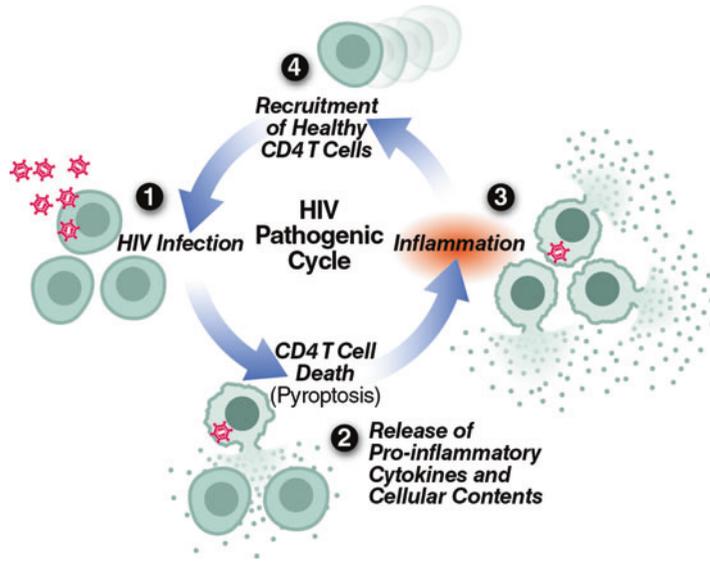
For decades, researchers have recognized that HIV infection is characterized not only by profound immunodeficiency but also by sustained inflammation and immune activation. Substantial evidence implicates chronic inflammation as a critical driver of immune dysfunction, premature appearance of aging-related diseases, and immune deficiency (Deeks 2011). In fact, these data profoundly changed the view of HIV infection, which is now classified as a chronic inflammatory disease. Animal studies also support the relationship between immune activation and progressive cellular immune deficiency. For example, simian immunodeficiency virus (SIV) infection of its

natural nonhuman primate hosts, the sooty mangabey, causes high-level viral replication but limited evidence of disease. This lack of pathogenicity is accompanied by a lack of extensive immune activation and cellular proliferation. Conversely, SIV infection in nonnatural hosts (i.e., rhesus macaque) produces immune activation and AIDS-like disease that closely mimics human HIV infection.

Many potential causes of chronic inflammation exist in HIV disease, including infection with Cytomegalovirus (CMV) and other copathogens, loss of regulatory CD4 T cells, and microbial translocation. Among these potential causes, microbial translocation was proposed as a key contributor that occurs when the barrier function of the gut mucosa breaks down following extensive CD4 T-cell depletion in the gut-associated lymphoid tissue (GALT). However, massive depletion of mucosal CD4 T cells cannot cause progression to AIDS unless significant depletion occurs in the peripheral lymph nodes. Moreover, depletion of mucosal CD4 T cells is also observed during SIV infection of natural hosts who do not progress to AIDS. Thus, further defining the mechanisms that cause immune activation and chronic inflammation remains a key issue in the field. Another more controversial issue pertains to patients who exhibit chronic inflammation despite undergoing long-term and effective combination antiretroviral therapy (cART) and maintain undetectable plasma HIV RNA levels. Some of these patients (defined as “immunological nonresponders”) cannot restore their cellular immunity and their pool of CD4 T-cell lymphocytes. Thus, paradoxically, systemic inflammation in HIV-infected patients may persist in a virus-independent manner.

Pyroptosis Links Chronic Inflammation and CD4 T-cell Death in Lymphoid Tissues

Pyroptosis likely evolved to rapidly clear bacterial infections by removing intracellular replication niches and releasing proinflammatory cytokines and endogenous danger signals that enhance the



Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense, Fig. 3 Pyroptosis links CD4 T-cell death and inflammation and may affect HIV pathogenesis and disease progression. Abortive HIV infection causes caspase-1-mediated pyroptosis of lymphoid CD4 T cells (steps 1 and 2). Dying cells release large amounts of proinflammatory cytokines including

IL-1 β and cellular contents such as 5'-ATP into the extracellular milieu (step 2). These events promote local inflammation (step 3) that mediates the migration of new circulating CD4 T cells into the lymph node (step 4) to establish a vicious cycle of CD4 T-cell death and inflammation

host's defensive responses. However, in pathogenic chronic inflammation, such as with HIV infection, pyroptosis is not a protective response and does not clear the primary infection. Instead, pyroptosis appears to create a vicious pathogenic cycle, where dying CD4 T cells release inflammatory signals that attract more cells into the infected lymphoid tissue to die and produce more inflammation. This cycle of chronic inflammation likely fuels disease progression and tissue injury (Fig. 3).

Investigations of HLACs revealed that healthy, uninfected lymphoid CD4 T cells are naturally primed to mount inflammatory responses and constitutively express high levels of cytoplasmic pro-IL-1 β , as well as the caspase-1 adaptor ASC and NLRP3 inflammasome (Doitsh et al. 2014). It is therefore possible that the release of intracellular 5'-ATP by pyroptotic CD4 T cells may provide a second inflammatory stimulus to induce the activation of caspase-1 by the NLRP3 inflammasome in nearby CD4 T cells that are already primed. Thus, pyroptosis initiated by

HIV may result in an avalanche of new rounds of pyroptosis in primed CD4 T cells by the repeated release of intracellular 5'-ATP in a **virus-independent manner**. Such an "auto-inflammation" scenario could generate persistent rounds of pyroptosis, chronic inflammation, and loss of CD4 T cells even when viral replication is reduced by antiretroviral therapy.

Vpx May Reverse Caspase-1-Mediated Pyroptosis by Enhancing the Permissivity of Host Cells

HIV and SIV carry a unique set of accessory proteins (e.g., Vif, Vpx, Vpr, Vpu, and Nef) that enhance viral replication in the host. Of these accessory proteins, Vpx is the signature gene in HIV-2, SIVsmm (sooty mangabey), and SIVmac (rhesus macaques) viruses. These viruses carry both Vpx and Vpr genes, while HIV-1 carries only Vpr. Why do these HIV-2 and SIV encode these two similar proteins while HIV-1 carries



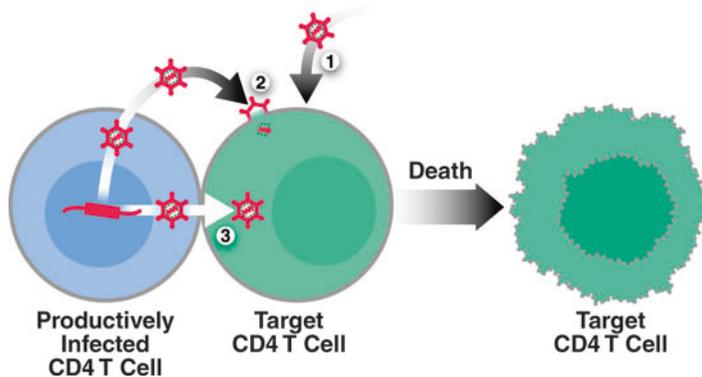
only one? The answer to this question lies in their functional details. A clue may be found in the fact that HIV-2 causes slower declines in CD4 T-cell levels, much longer periods of asymptomatic infection, and lower mortality rates than HIV-1. It is certainly possible that Vpx activity in HIV-2 relieves the SAMHD1-mediated restriction of infection in host target cells, thereby redirecting death from the caspase-1-mediated pyroptotic pathway toward the caspase-3-mediated apoptotic pathway (Fig. 2). The lack of chronic inflammation during apoptosis may underlie the milder clinical course of HIV-2 infection. Thus, Vpx may have not simply evolved to accelerate viral propagation but also to influence the particular cell death pathway utilized and to reduce the pathogenic outcomes of viral infection in the host. These hypotheses are now under active investigation.

The Mode of Viral Spread May Influence HIV Pathogenesis

Retroviruses disseminate between susceptible cells (i.e., cells that support fusion and entry) by cell-free infection or direct cell-to-cell spread. The pathway utilized can greatly affect the infectivity

yield. For HIV, the ratio of infective particles to free virions is low and calculated to be 10^{-3} to 10^{-4} . In contrast, the infectivity of virus-producing cells, as measured in coculture systems, is approximately 10^2 to 10^3 times higher than the infectivity of all the cell-free particles in the culture supernatant from the same infected cells. Although the superior efficiency of cell-to-cell spread, compared to cell-free infection, has been known for 20 years, the contributions of such modes of spread to HIV pathogenesis have never been directly demonstrated. Recent studies in HLACs now reveal that the mode of spread may determine the specific form of CD4 T-cell death elicited by HIV: **cell-free HIV particles mediate direct killing** of permissive CD4 T cells, but the death of nonpermissive cells by **abortive infection occurs only via cell-cell interaction with nearby HIV-producing cells** (Fig. 4). The molecular mechanisms that operate during cell-to-cell spread to kill nonpermissive cells remain unknown.

Because of the relatively low particle infectivity that characterizes retroviruses, cell-free virions might fail to generate an amount of cytoplasmic DNA intermediates sufficient to elicit an innate immune response in abortively infected cells. TREX1, a cellular 3' DNA exonuclease, may



Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense, Fig. 4 Modes of HIV transmission affect the cytopathic outcomes in target cells. HIV can be transmitted into target CD4 T cells by cell-free infection or spinoculation (1), paracrine secretion from a nearby HIV-producing cell (2), or cell-to-cell spread from a neighboring productively infected cell (3). While HIV

transmission modes 1 and 2 promote productive infection and death in *permissive* target cells, mode 3 is required to promote death of *nonpermissive* target CD4 T cells by abortive infection and pyroptosis. Of note, 95% of CD4 T cells reside in lymphoid tissues, are nonpermissive, and die as a result of abortive HIV infection

further antagonize this process by degrading cytoplasmic reverse transcribed DNA products. Indeed, the intrinsic TREX1 activity in the cytoplasm may create a threshold of DNA products necessary either to achieve productive infection in permissive cells or, alternatively, to activate the pyroptotic pathway in abortively infected cells. Cell-to-cell spread across the virological synapse may overcome TREX1 restriction by rapid transfer of large quantities of viral DNA, facilitated by the concomitant clustering of adhesion molecules, lipid rafts, and viral entry receptors on the opposing target cell. These hypotheses are currently being investigated.

Conclusion

The very specific response that is so beneficial during host infection can contribute substantially to the pathogenesis of HIV-1. In this chronic disease, such protective response by the immune system goes into overdrive; the stimulus that triggers the inflammation is not easily cleared, causing inflammation to persist and become a substantial driver of the disease.

A number of experimental methods have been used to acquire results, test hypotheses, and formulate new models of HIV replication and pathogenesis. The differences between these methods greatly influence the conclusions that can be drawn from them. For example, accessory genes are often dispensable in facilitating viral growth in *in vitro* cell culture systems, which causes their loss during long-term propagation. Yet, these genes are strongly maintained and appear critical for viral replication in physiological systems with preserved natural conditions. The use of HLACs prepared from fresh tonsil and spleen tissues has proved to be instrumental in replicating the physiological outcomes of HIV infection, including the pyroptotic death of CD4 T cells, the generation inflammatory responses (i.e., expression and release of IL-1 β by lymphoid CD4 T cells), the requirement for cell-to-cell transfer of HIV-1 to trigger innate suicide response, and the function of Vpr promoting early steps of viral infection.

Interestingly, while cell death by abortive HIV infection is the predominant mechanism for killing CD4 T cells in lymphoid tissues, it is surprisingly not observed in the peripheral blood CD4 T cells. This result is especially surprising in view of the fact that HIV fuses and enters into quiescent blood and lymphoid CD4 T cells at equivalent levels. It is particularly important because peripheral blood cells have traditionally been used as the principal cells for modeling HIV infection and studying HIV pathogenesis. The routine use of blood, instead of lymphoid CD4 T cells, is likely the reason why previous studies entirely missed the pyroptotic cell death pathway through which many abortively infected cells die during HIV infection. Again, these observations highlight how the tissue microenvironment is a key determinant that governs how CD4 T cells die during HIV infection. They also distinguish AIDS as a disease of the lymphoid tissue, not the blood, and underscore striking biological differences between the blood and lymphoid-derived CD4 T cells that have important implications for HIV pathogenesis.

Cross-References

- ▶ [Counteraction of SAMHD1 by Vpx](#)
- ▶ [Inflammasome and HIV](#)
- ▶ [Inhibition of HIV-1 Spread: Cell-Free versus Cell-Cell](#)

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Recombinant Forms of HIV-2

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Definition

Genetic recombination, a mechanism through which large fragments of two viral genomes can be combined, accelerates the evolution of human immunodeficiency viruses (HIVs). Recombination thus has an extremely high potential to create new advantageous viral genotypes for effective transmission, to escape from host immune responses, for better replication capacity, and for the development of drug resistance (Ramirez et al. 2008). This chapter summarizes recent evidence on recombinant forms of HIV type 2 (HIV-2).

HIV-2 recombinant forms can be classified according to HIV-1 nomenclature (Robertson et al. 1999, 2000) into two categories: circulating recombinant form (CRF) and unique recombinant form (URF). The minimum requirement for declaring a new CRF is identification of the virus in at least three cases with no direct linkage,

accompanied by near full-length sequences showing the same mosaic genome structure (Robertson et al. 1999, 2000). Other recombinant forms whose identification does not meet the criteria are called URFs.

To date, eight genetic groups (A to H) have been identified in HIV-2 (Gao et al. 1992, 1994; Chen et al. 1997; Yamaguchi et al. 2000; Damond et al. 2004). Among these eight groups, A and B are the major strains circulating in HIV-2-endemic areas. Therefore, all five isolates of HIV-2 recombinant forms identified to date are chimeras of group A and B strains (Table 1). Among the five isolates, four are classified as a CRF and the remaining one is a URF.

HIV-2 CRF

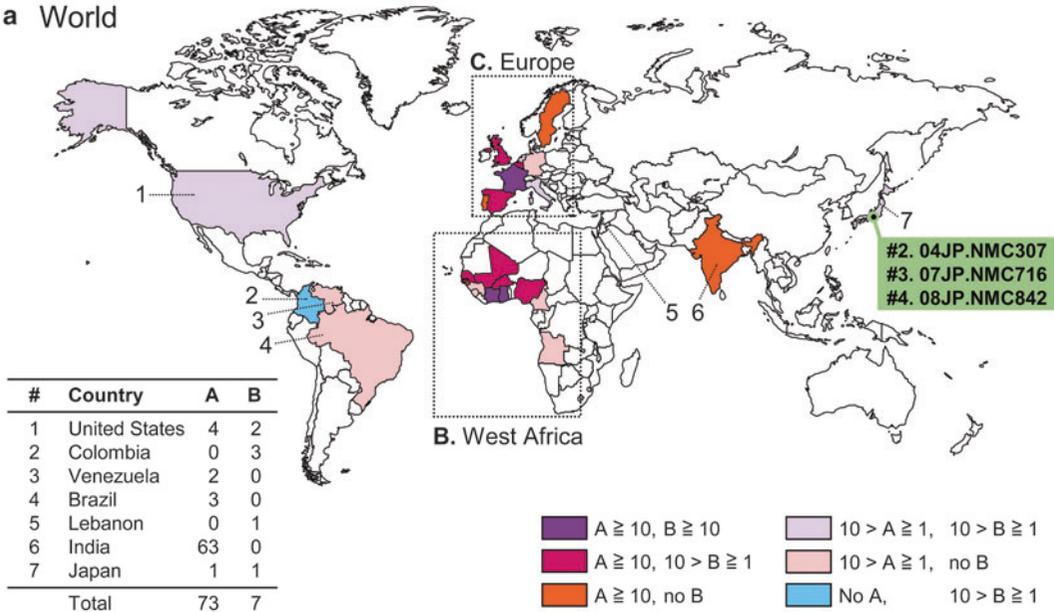
The first HIV-2 recombinant isolate 7312A had been identified through a blood sample obtained in 1990 from a 32-year-old male living in Abidjan, Côte d'Ivoire, inside the original HIV-2-endemic area of West Africa (Gao et al. 1992, 1994; Robertson et al. 1995) (Table 1 and Fig. 1b). Detailed analyses of the complete full-length genomic DNA sequence of 7312A clearly showed a mosaic genome structure consisting of HIV-2 groups A and B, along with four recombination breakpoints (Robertson et al. 1995). Although nearly two decades had passed since the discovery of 7312A, three more isolates were recently identified from epidemiologically

Recombinant Forms of HIV-2, Table 1 Isolates of HIV-2 recombinant forms

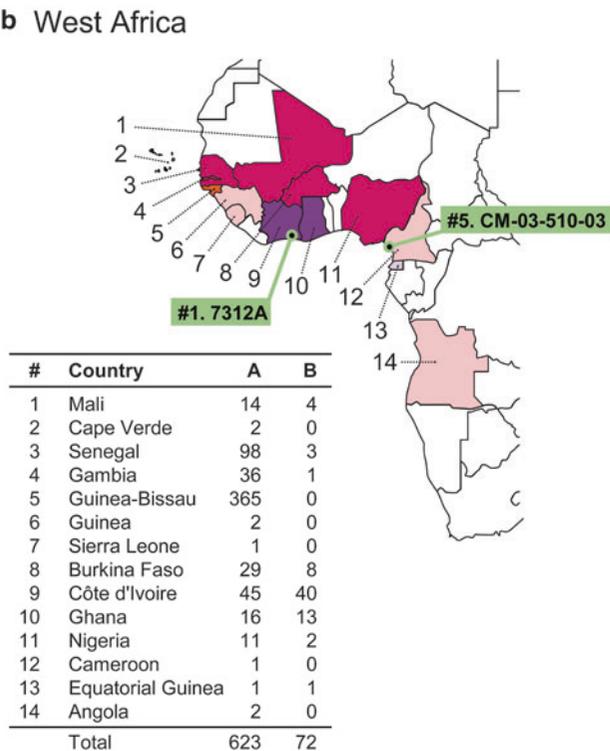
#	Name	HIV-2 genotype	Nucleotide sequence				Sampling year	Sampling point (city and country)	Characteristics of host individual				Stage of HIV infection	References			
			Molecular type	Length (base pairs)	Position in SIVmac239 sequence	Accession number			Nationality	Age (years)	Sex	HIV-1 coinfection			CD4 ⁺ cell count (cells/ μ l)	Plasma HIV-2 viral load (copies/ml)	
1	7312A	CRF01_AB	Complete full-length genomic DNA	10,282	1–10,279	L36874	1990	Abidjan, Côte d'Ivoire	Ivoirian	32	M	Serologically, yes	587	NA	Symptomatic	Gao et al. (1992, 1994), Robertson et al. (1995)	
2	04JP. NMC307	CRF01_AB	Complete full-length genomic DNA	10,255	1–10,279	AB499693	2004	Nagoya, Japan	Nigerian	28	M	No	241	350,000	AIDS	Ibe et al. (2010)	
3	07JP. NMC716	CRF01_AB	Complete full-length genomic DNA	10,255	1–10,279	AB499694	2007	Nagoya, Japan	Nigerian	36	M	No	4	680,000	AIDS	Ibe et al. (2010)	
4	08JP. NMC842	CRF01_AB	Complete full-length genomic DNA	10,250	1–10,279	AB499695	2008	Nagoya, Japan	Japanese	34	F	No	110	25,000	AIDS	Ibe et al. (2010)	
5	CM-03-510-03	Unique recombinant form of AB	Near full-length genomic DNA	9,089	827–10,002	EU028345	2003	Douala, Cameroon	Cameroonian	47	F	No	NA	NA	NA	NA	Yamaguchi et al. (2008)

Abbreviations: *AIDS* acquired immunodeficiency syndrome, *CRF* circulating recombinant form, *F* female, *M* male, *NA* not available

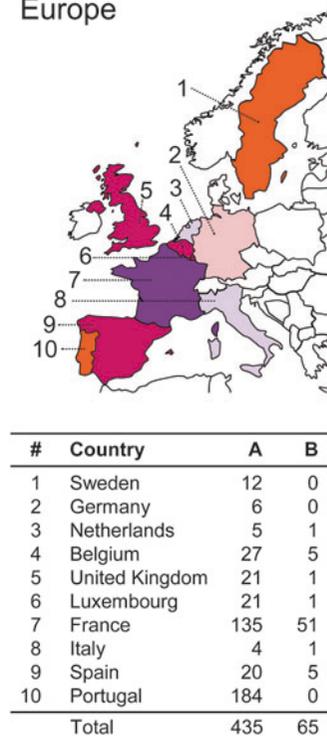
a World



b West Africa



c Europe



Recombinant Forms of HIV-2, Fig. 1 Geographical distribution of HIV-2 isolates registered as group A, B, and recombinant forms. HIV-2 nucleotide sequences ($N = 4,058$) were available with their genotype and sampling country information through the Los Alamos HIV Sequence Database on April 2012. After processing by

using 1 sequence/1 isolate, 1,131 group A and 144 group B isolates were used to make the distribution map. Countries are color-coded according to the number of HIV-2 group A and B isolates. Five isolates of HIV-2 recombinant forms are represented with a *green box*. (a) World, (b) West Africa, and (c) Europe

unlinked patients living in Nagoya, Japan, outside the HIV-2-endemic area (Ibe et al. 2010) (Table 1 and Fig. 1a). These three isolates, 04JP.NMC307, 07JP.NMC716, and 08JP.NMC842, were shown with their complete full-length genomic DNA sequences to have the same mosaic genome structure as that of 7312A, fulfilling the criteria for identifying the first CRF of HIV-2, named CRF01_AB (Ibe et al. 2010).

CRF01_AB has a group B genomic backbone and a chimeric *env* gene from groups A and B (Fig. 2a, b). In particular, the gp120 region contains a group A backbone and a partial group B C2V3 fragment, and the gp41 region contains group A extracellular and transmembrane domains and a group B cytoplasmic domain (Fig. 2c).

All four individuals infected with CRF01_AB were found with symptoms at the time of diagnosis. The host of 7312A presented with lymphadenopathy, cutaneous anergy, and recurrent skin abscesses (Gao et al. 1992). However, the pathogenicity of CRF01_AB was difficult to estimate in this case due to the presence of HIV-1 coinfection (Gao et al. 1994) (Table 1). In contrast, the three hosts of 04JP.NMC307, 07JP.NMC716, and 08JP.NMC842 were identified without HIV-1 infection (Ibe et al. 2010). It is important to note that, despite their young ages (28, 34, and 36 years old), all three cases were at advanced-stage AIDS with opportunistic infections, low CD4⁺ cell counts, and high plasma HIV-2 viral loads (Ibe et al. 2010) (Table 1), suggesting the high pathogenicity of CRF01_AB.

HIV-2 URF

The remaining isolate, CM-03-510-03, had been identified through a blood sample obtained in 2003 from a 47-year-old female living in Douala, Cameroon, in west Central Africa (Yamaguchi et al. 2008) (Table 1 and Fig. 1b). The near full-length genomic DNA sequence of CM-03-510-03 showed a unique mosaic genome structure different from that of CRF01_AB (Yamaguchi et al. 2008).

Like CRF01_AB, CM-03-510-03 has a group B genomic backbone and a chimeric *env* gene of

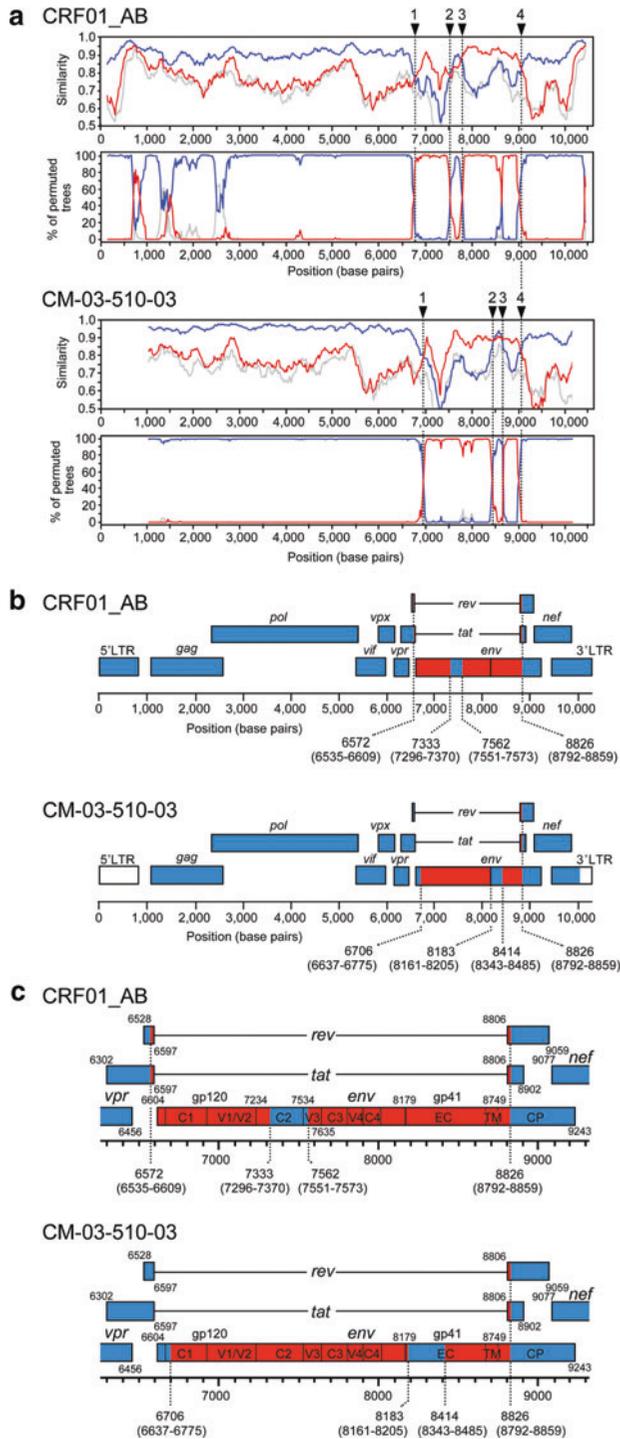
groups A and B (Fig. 2a and b). However, the gp120 region mostly contains group A fragments, and the gp41 region contains a chimeric extracellular domain of groups A and B, a group A transmembrane domain, and a group B cytoplasmic domain (Fig. 2c). As no additional isolate has been identified after the discovery of CM-03-510-03, this strain still remains identified as a URF.

Unfortunately, the host of CM-03-510-03 did not have enough clinical information to estimate the pathogenicity of this URF (Table 1).

Geographical Potential for the Emergence of HIV-2 Recombinant Forms

Coinfection with at least two different HIV-2 groups is essential for the emergence of HIV-2 recombinant forms. Therefore, overlapping areas with circulating HIV-2 groups A and B are the primary candidates for geographical hot spots for HIV-2 recombinant forms. Figure 1 shows the distribution of HIV-2 group A ($n = 1,131$) and B ($n = 144$) isolates available through the Los Alamos HIV Sequence Database in April 2012. Three major HIV-2-endemic areas have been confirmed worldwide: West Africa (also see “► Epidemiology of HIV-2 Infection in West Africa”), Europe (also see “► HIV-2 Infection in Europe, Epidemiology of”), and India in Asia, responsible for 99% (1,258/1,275) of registered HIV-2 isolates (Fig. 1a).

In the original HIV-2-endemic area of West Africa, two countries (Côte d’Ivoire and Ghana) had more than 10 group A and B isolates with nearly equal percentages; Côte d’Ivoire had 53% (45/85) group A and 47% (40/85) group B, and Ghana had 55% (16/29) group A and 45% (13/29) group B (Fig. 1b). Therefore, the southern coastal area of West Africa can be cited as a primary hot spot candidate for the emergence of HIV-2 recombinant forms. In fact, four of five individuals identified with HIV-2 recombinant forms originated from this area. The host of 7312A had been in Côte d’Ivoire, and the hosts of 04JP.NMC307 and 07JP.NMC716 had been in Nigeria before arriving in Japan (Table 1 and Fig. 1a, b). In addition,



Recombinant Forms of HIV-2, Fig. 2 Mosaic genome structures of HIV-2 recombinant forms. (a) Similarity plotting (*top*) and bootscanning (*bottom*) data for each case of HIV-2 CRF01_AB (7312A, 04JP.NMC307, 07JP.NMC716, and 08JP.NMC842) and CM-03-510-03. Plots

for consensus group A (from ALI, BEN, CAM2CG, and UC2 isolates), consensus group B (from D205, EHO, and UC1 isolates), and SIVmac239 are shown in *red*, *blue*, and *gray*, respectively. Both similarity plotting and bootscanning were performed with window and step

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the host of CM-03-510-03 had been in Cameroon (Table 1 and Fig. 1b).

In Europe, several countries had previously developed strong socioeconomic ties with West African countries. Among them, France had abundant numbers of both group A and B isolates: 135 group A (73%) and 51 group B (27%) (Fig. 1c). The large number of group B isolates in France can be explained by a socioeconomic tie with Côte d'Ivoire. Although no HIV-2 recombinant forms have been reported in France to date, the country seems to have a higher potential for the emergence of HIV-2 recombinant forms than other European countries.

In Asia, India is known as an HIV-2-endemic country. However, as all 63 registered isolates belong to group A (Fig. 1a), the country is currently an unlikely candidate for the emergence of HIV-2 recombinant forms.

Future Studies on HIV-2 Recombinant Forms

Understanding the clinical and epidemiological impacts of HIV-2 recombinant forms will require a cohort study to assess the pathogenicity of HIV-2 recombinant forms, along with a parallel comparison of their parent HIV-2 groups A and B. To examine the feasibility of such a study, further molecular epidemiological studies are currently needed to clarify in detail the prevalence and geographical distribution of HIV-2 genotypes, especially in the southern coastal area of West Africa. Of note, the *env* gene is the only region that can specify both types of currently known HIV-2 recombinant forms (Fig. 2b). However, *env* sequencing appears to be not commonly

performed in HIV-2 studies, based on the number of *env* sequences in the public database. In other words, genotyping HIV-2 without the *env* gene risks incorrectly categorizing recombinant forms into group B. Indeed, some HIV-2 *pol* sequences suspected to be from recombinant forms have been deposited as group B sequences in the public database.

Another important future study will be in vitro approaches to the biological significance of recombination in HIV-2 recombinant forms. Research on HIV-1 recombinant forms has identified two hot spots for recombination: (1) the region (600 base pairs) from the first exons of *tat* and *rev* genes to the beginning of the *env* gene and (2) the region (400 base pairs) from the second exons of *tat* and *rev* genes to the end of the *env* gene (Magiorkinis et al. 2003; Fan et al. 2007). These two hot spots have also been identified as strong positive selection sites (Fan et al. 2007), suggesting that recombination breakpoints primarily function to escape from the host immune system. Interestingly, the first and fourth recombination breakpoints of HIV-2 CRF01_AB and CM-03-510-03 are just inside the regions corresponding to the two hot spots in the HIV-1 genome (Fig. 2c). Furthermore, the second and third recombination breakpoints are inside the C2V3 region of gp120 in CRF01_AB and inside the extracellular domain of gp41 in CM-03-510-03 (Fig. 2c), the major determinant sites for anti-envelope host immune responses. Immunological studies are thus needed to assess the ability of HIV-2 recombinant forms to escape from the host immune system. In addition, envelope proteins are essential for HIVs to attach and enter targeted cells at the beginning of the virus life cycle. Thus, virological studies are also needed

Recombinant Forms of HIV-2, Fig. 2 (continued) sizes of 300 and 20 nucleotides, respectively. Bootscanning was performed using the neighbor-joining algorithm with 500 replicates. Each recombination breakpoint is represented with an arrow head. (b and c) Schematic drawings for the genomic structures of HIV-2 CRF01_AB and CM-03-510-03. (b) Whole genomic structures and (c) details around the *env* gene are

represented. Regions belonging to groups A and B are shown in red and blue, respectively. Numbering positions were adjusted to the reference SIVmac239 sequence (Calef et al. 2001; Lin et al. 2007). Each position of recombination breakpoints is represented as the midpoint and range. *C* constant region, *CP* cytoplasmic domain, *EC* extracellular domain, *gp* glycoprotein, *TM* transmembrane domain, *V* variable region

to answer the question of whether unique chimeric envelope proteins of HIV-2 recombinant forms give any replication advantage.

Conclusion

Although few individuals to date have been identified with HIV-2 recombinant forms, ectopic discoveries and identification outside West Africa may alert clinicians, researchers, and public health authorities to the wider spread of the strains worldwide. Therefore, further molecular epidemiological studies will provide more accurate information in the near future on the prevalence and geographical distribution of HIV-2 recombinant forms.

Genetic recombination has an extremely high potential to create quite new viral genotypes by combining large fragments of two viral genomes (Ramirez et al. 2008). Current worldwide efforts to prevent further HIV spread are also important for interrupting the emergence of more pathogenic HIVs by reducing their chance for evolution.

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Retention in Care Interventions

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Definition

Retention in care is broadly defined as consistent primary HIV care visits after HIV diagnosis that leads to the initiation of an antiretroviral (ARV) medication regimen. Importantly, higher retention, as measured by higher rates of appointment attendance, is strongly associated with virological suppression and better health outcomes (Metsch et al. 2008; Mugavero et al. 2012a); individuals newly diagnosed with HIV should expect to live a normal life span and a highly preserved quality of life. This chapter will further define retention, provide an overview of outcomes associated with better retention, highlight interventions to improve retention, provide recommendations for achieving optimal retention based on evidence synthesized from existing research, and close with implications for future research.

Expanding the Definition of Retention in Care

The definition of retention must be expanded to incorporate previous research and reflect how retention has been implemented practically in a clinical setting; the practical usage guidelines have been delineated by the public health agencies and have also been demonstrated in practice.

Retention in care has been defined through best practice measures offered by the Centers for Disease Control and Prevention (CDC) and the Health Resources and Services Administration (HRSA). First, both the US National HIV/AIDS Strategy (NHAS; White House Office of National AIDS Policy 2016) and HRSA (Health Resources and Services Administration 2016) consider retention as the proportions of patients with two

or more medical visits at least 3 months or more apart in a calendar year. To calculate retention using this definition, NHAS and HRSA suggest using dates of CD4 and viral loads reported to surveillance. In that retention rates are commonly reported as a proportion of all patients who ever had any medical visits in a calendar year, specific guidelines are presented to determine this proportion. NHAS (2016) and HRSA (2016) provide specific guidelines to calculate retention rates by defining what proportion of patients were considered “retained” from patients who had a medical appointment; NHAS and HRSA guidelines define retention as the number of patients with two or more CD4 or viral load values at 3 or more months apart in one calendar year divided by the number of patients with one or more laboratory result in the same calendar year.

Research-Based Definitions of Retention in Care

Aside from the HRSA HAB clinic visit performance measure, there are other ways to measure retention; each way has strengths and limitations and is often dictated by who is trying to assess retention, namely, a clinician, an administrator, or a researcher (Mugavero et al. 2012b). Based on findings from previous research, retention has been defined based on kept visit frequency. The most efficacious means of calculating kept visits, however, has varying degrees of predictive validity in being associated with better health outcomes; retention has been measured by missed visits (number or any no show visits over a specified time), appointment adherence (proportion of kept visits from all scheduled visits), visit constancy (proportion of time intervals with at least one kept appointment), and gaps in care (time interval between kept appointments) (Mugavero et al. 2012b).

In clinical practice, the emphasis on calculating retention centers on missed visits and gaps in care in that those data are typically immediately available at the point of care and intuitively can indicate to the clinician disease prognosis. Administrators have a stronger focus on clinic performance, specifically clinic underutilization and revenue. Researchers tend to focus more on appointment adherence and visit constancy in

order to assess retention in a more refined, detailed manner that permits short- and long-term trend analyses. On a population level, engagement in care is defined as the proportion of patients with two or more CD4 or viral loads at three or more months apart in one calendar year (numerator) divided by all PLWHA living in a particular area of interest for that particular analysis in the same calendar year but excluding those whose first labs occurred in the second half of the year (denominator) (Dombrowski et al. 2012).

Some considerations should be noted in calculating retention. Determining kept visits is susceptible to practicality and data accessibility issues, primarily due to how attendance records and historical lab results, indicators of health outcomes in HIV-infected patients, are stored combined with the capabilities of the electronic medical record (EMR) that typically feeds surveillance data to health departments. Additionally, clinic scheduling practices along with computational issues, such as cutoff dates and spans of time in the analysis, can produce variance in retention rates (Mugavero et al. 2012b). Although variability exists between definitions of retention in care, irrespective of the formula used to calculate retention, research has indicated that retention is associated with health outcomes in HIV-infected patients.

Retention in HIV Care and Health Outcomes

Retention in HIV care has been found to be an integral factor of optimal health outcomes in HIV-infected patients; generally, better retention has been associated with better health outcomes due to increased rates of viral suppression and better immunity conferred by higher CD4 counts. Better retention is associated with greater antiretroviral medication adherence (Metsch et al. 2008; White House Office of National AIDS Policy 2016) and lower incidence of morbidity in that the patient's health is consistently monitored. The importance and implications of retention in care is reflected through improved clinical outcomes in patients with high retention.

Increased retention in care has been associated with better health outcomes, including reduced morbidity and mortality, better immunity, and,

importantly, less engagement in risky behaviors that increase HIV transmission (Metsch et al. 2008; Mugavero et al. 2012a). Furthermore, missed regular care visits have been associated with increased antiretroviral drug resistance (Sethi et al. 2003). Given the benefits to retaining patients in HIV care, there are evidence-based interventions that have been shown to improve retention rates.

Interventions to Improve Retention in Care

Retention in HIV care is part of the HIV Continuum of Care in the USA; notably, it is one of the key steps, after linkage to care, where patient numbers decline. In that retention directly impacts health outcomes, numerous interventions to improve retention have been published. The CDC has given providers a list of both best and good practice interventions based on the sustained effect of the interventions on retention rates.

Some interventions have been found to be designated as best practice for improving retention. Enhanced personal contact that establishes a personal relationship between providers and patients increased retention (Gardner et al. 2014). Additionally, interventions involving patient navigators, community and peer outreach, financial incentives, and case management have been found to improve retention (Higa et al. 2012, for a review; McCoy et al. 2013). Focusing on provider-based interventions, alerts for sub-optimal attendance in the EMR as part of a clinical decision support system have been found to be an effective evidence-based intervention to improve retention (Robbins et al. 2012).

Additionally, there are evidence-informed interventions that have shown improved retention and no noteworthy negative effects from pre- to post-intervention but did not have a comparison group. Bilingual care teams, consisting of a nurse practitioner, a Ryan White case manager, and a peer educator, have been found to improve retention in Hispanic/Latino patients (Higa et al. 2012, for a review). HIV-infected minority youth receiving care from adolescent care providers, youth-focused social workers, and case managers demonstrated increased retention (Davila et al. 2013). Better retention was seen in the long term in

out-of-care patients who were targeted to be relinked to care using clinical data and a linkage specialist (Bove et al. 2015). A comprehensive care plan, incorporating home- and field-based patient navigation, coordinated medical and social services, support and coaching for medication adherence, and patient education increased retention (Irvine et al. 2015). Print reminders and verbal reminders used by all clinic staff increase retention (Higa et al. 2012, for a review). Finally, an intervention for African American and Hispanic/Latino young MSM that linked newly diagnosed HIV-infected patients to a physician within 72 h of a positive result that offered ancillary support services such as group meetings, counseling, case management, assistance with appointment scheduling or medical questions by text or phone, and the development of an individual treatment plan designed to reduce that patient's barriers was found to increase retention (Higa et al. 2012, for a review). As with any intervention, their suitability depends partially on the resources available in the setting in which the intervention is deployed.

Recommendations for Retention

Systematic reviews have published guidelines that provide recommendations to optimize retention in HIV-infected patients (IAPAC 2015). Systematic monitoring of retention in care is strongly recommended for all patients, and the electronic medical record (EMR) is used to measure retention, necessitating EMRs that can handle these sorts of data queries. Along these lines, population-level data and HIV clinical monitoring using databases and surveillance systems is recommended.

Second, IAPAC has recommended providers disseminate information and implement communication technologies centered around supporting patient self-care. More specifically, IAPAC recommends using mobile health technology, such as text messaging. Importantly, the IAPAC strongly recommends patient education underscoring the importance of keeping clinic appointments (IAPAC 2015).

Finally, steps to reengage patients with low retention should be proactive. Specifically,

IAPAC recommends targeting patients who frequently miss clinic appointments to the same extent as ensuring new patients are engaged in care. Although resource heavy, IAPAC provides a moderately strong recommendation to provide case management to retain patients in care and to facilitate transportation to clinic visits (IAPAC 2015).

Conclusions

Given the importance of retention on health outcomes in HIV-infected patients along with the varying effectiveness of existing interventions, future research and clinical management should integrate appropriate interventions to optimize retention for specific patient populations. Interventions have differential levels of success depending on the type of patient; efforts taken to improve retention should take these patient characteristics into account.

The International Advisory Panel on HIV Care Continuum Optimization suggests that future retention in care research focuses on the following elements: a validated core set of metrics, ideally sourced from the EMR; comparisons of interventions in high- versus low-resource settings; the impact of technology; the effect of in-person contact; the impact of various forms of appointment reminders; the role of financial incentives; the effect of reducing food instability; reducing transportation barriers; alternative models of HIV care (visit with pharmacist or nurse only); community adherence through peer support; and home-based HIV care (IAPAC 2015). Finally, best practices to improve retention should be widely disseminated to improve population-wide rates of optimal retention in care.

Cross-References

- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Antiretroviral Therapy and Drug Resistance in HIV-2 Infection](#)
- ▶ [Behavioral Interventions for Adherence](#)

- ▶ Behavioral Science Highlights of Evidence and Research
- ▶ Case Management for Linkage
- ▶ Clinical Ethics in HIV/AIDS Prevention, Care, and Research
- ▶ Community Viral Load
- ▶ Comorbidity: Opioids
- ▶ Health Care Workers, Epidemiology of HIV/AIDS
- ▶ HIV Prevention and African Americans
- ▶ HIV Prevention and Asians and Pacific Islanders
- ▶ HIV Prevention and Hispanics
- ▶ HIV Prevention and Women
- ▶ HIV Prevention Efforts Within Substance Use Disorder Treatment Settings
- ▶ HIV Prevention for MSM
- ▶ HIV Prevention for Serodiscordant Couples
- ▶ HIV Prevention for Stimulant Using Men Who have Sex with Men
- ▶ HIV Prevention in Persons 50 and Older
- ▶ HIV Prevention in the Correctional System
- ▶ HIV Prevention in Transgender Persons
- ▶ HIV Prevention in Youth
- ▶ Initial Antiretroviral Regimens
- ▶ Integrase Inhibitors
- ▶ Medication Adherence and HIV-Associated Neurocognitive Disorders (HAND)
- ▶ Multilevel Interventions/Structural Approaches to HIV Prevention
- ▶ Network Interventions
- ▶ Neurocognitive Functioning in HIV-infected Substance Users
- ▶ Non-Nucleoside Reverse Transcriptase Inhibitors for Treatment of HIV Infection
- ▶ Non-Injecting Drug Users, Epidemiology of HIV/AIDS
- ▶ NRTIs
- ▶ Nurse-Delivered Interventions for Adherence
- ▶ Peer-Based Intervention Approaches
- ▶ Positive Health, Dignity, and Prevention (PHDP)
- ▶ Prevention Counseling and Other Strategies in the HIV Care Setting
- ▶ Prevention for People Living with HIV
- ▶ Prevention of Alcohol-Related HIV Risk Behavior
- ▶ Protease Inhibitor
- ▶ Treatment Failure and Resistance
- ▶ Women, Epidemiology of HIV/AIDS

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Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis

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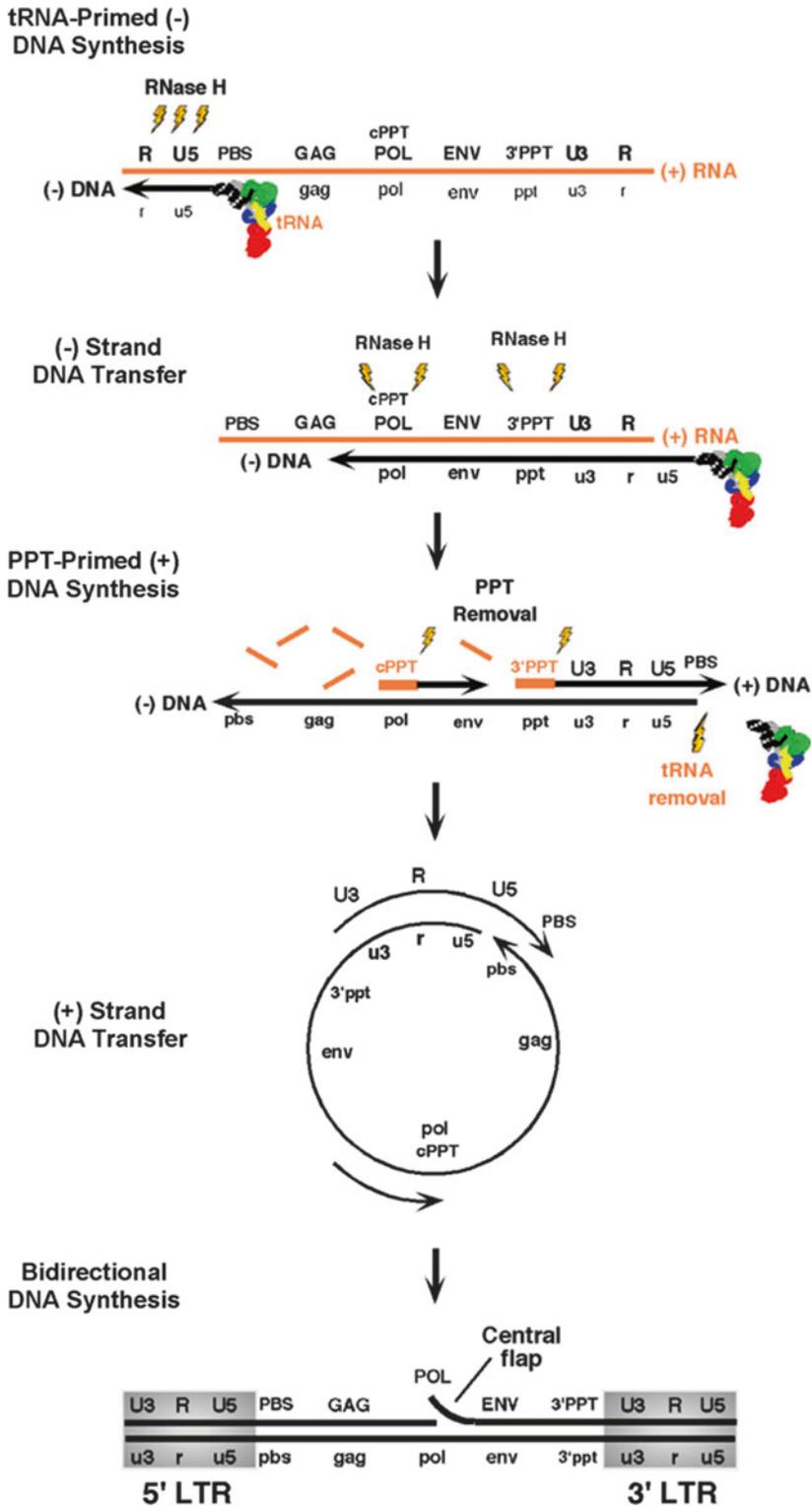
Definition: Reverse Transcription

Reverse transcription, more appropriately described as HIV-1 cDNA synthesis, describes a multi-step process through which the virus-coded reverse transcriptase (RT) converts the single-stranded (+) RNA genome into an integration-competent double-stranded DNA uninterrupted by ribonucleotides (“► [Integration](#)”). It is, however, important to note that while the integrated provirus is flanked by the hallmark long terminal repeat (LTR) elements, these encode sites for initiation and termination of transcription (“► [Transcription \(Initiation, Regulation, Elongation\)](#)”). The (+) RNA genome from which the provirus is synthesized therefore harbors incomplete LTR copies at its 5' and 3' termini, requiring their regeneration through a combination of RNA- and DNA-templated DNA synthesis. Individual steps of HIV-1 DNA synthesis (Telesnitsky and

Goff 1997; Arts and Le Grice 1998) are outlined in Fig. 1 and described in the following sections.

HIV-1 Reverse Transcriptase (RT): Structure and Function

HIV-1 RT is encoded by a single 66 kDa open reading frame of the Gag-Pol precursor polypeptide. However, the biologically relevant enzyme is a heterodimer of 66 and 51 kDa polypeptides, the latter resulting from cleavage of p66 by virus-coded protease cleavage between residues Phe440 and Tyr441 (Fig. 2). Both RT subunits therefore share four similar subdomains, designated (by analogy to a right hand) fingers, palm, thumb, and connection, while p66 retains the C-terminal, 133-residue RNase domain (amino acids 427–560). Despite primary sequence identity, subdomains of the p66 and p51 subunits adopt significantly different folds, i.e., the p66 DNA polymerase domain exhibits an open, extended structure with a large active site cleft, while the equivalent p51 subdomain adopts a closed, compact structure that lacks catalytic activity. DNA polymerase and ribonuclease H (RNase H) activities are encoded by domains at either terminus of the p66 subunit, and proposed roles for p51 include providing a structural support and facilitating p66 loading onto nucleic acid. In contrast to p66, which undergoes large-scale motions, the p51 subunit is essentially rigid. The p66 nucleic acid binding cleft comprises its fingers, palm, and thumb subdomains, and X-ray crystallography has identified numerous contacts with both strands of the duplex, primarily between the sugar-phosphate backbone and highly conserved motifs of the DNA polymerase and RNase H domains (Huang et al. 1998). Helix α -H of the thumb forms extensive contacts with the primer strand in the DNA minor groove. The β 12– β 13 hairpin (Pro227-His235) is designated the DNA polymerase “primer grip” and is proposed to maintain the primer terminus in the appropriate orientation for nucleophilic attack on the incoming dNTP. Contact with the template strand involves the p66 “template grip,” which includes elements of its palm and finger subdomains.



R

Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis, Fig. 1 (continued)

A high-resolution co-crystal structure of an HIV-1 replication complex (i.e., enzyme, nucleic acid, and the incoming dNTP) depicts the template overhang ahead of the DNA polymerase active site bending to interact with the p66 fingers, contacting nucleobases +1, +2, and +3.

The p66 palm houses the DNA polymerase active site, characterized by the Asp110, Asp185, and Asp186 catalytic triad, a common feature of DNA and RNA polymerases. Among nucleic acid polymerase families, palm subdomain architecture is highly conserved, comprising a four- to six-stranded β -sheet flanked on one side by two α -helices. Asp185 and Asp186 are part of the conserved -Tyr-Met-Asp-Asp- active site motif that adopts an unusual β -turn conformation, possibly to promote their positioning for catalysis, while Tyr183 is involved in hydrogen bonding with nucleobase -2. Structures of HIV-1 RT reveal significantly different conformations for the active site motif, indicating a high degree of structural flexibility. The incoming dNTP is tightly coordinated by p66 finger residues Lys65, Arg72, Asp113, and Ala114, and its ribose occupies a pocket lined by Asp113, Tyr115, Phe116, Glu151, and Arg72. dNTP insertion fidelity is critically influenced by interactions of its γ -phosphate with Lys65. Finally, Tyr115 has been coined the “steric gate,” providing the critical discrimination between deoxy- and ribonucleoside triphosphates.

RNase H-mediated hydrolysis of the RNA/DNA reverse transcription intermediate is two-metal catalyzed (metals ions A and B), with a preference for Mg^{2+} . Divalent metal coordination is substrate dependent, i.e., at physiologically relevant concentrations, productive binding occurs only in its presence. The catalytic cycle can be summarized as follows:

- In a “resting” state, metal ions A and B are separated by ~ 4 Å. During catalysis, their

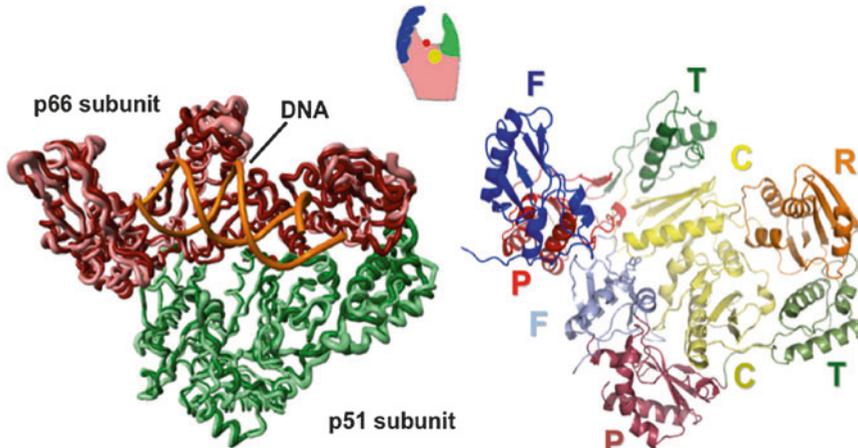
position and separation vary according to the coordination environment.

- Hydrolysis proceeds via an SN2 mechanism, involving a penta-coordinated intermediate, resulting in inversion of configuration at the phosphorus.
- Metal ion A (coordinated by Asp443 and Asp549) coordinates a water molecule, reducing its pKa and aligning this for in-line nucleophilic attack on the phosphodiester backbone.
- In turn, metal ion B, coordinated by Asp443, Glu478, and Asp498, stabilizes the transition state to facilitate the leaving of the 3' oxyanion group.

Crystallographic studies show extensive contacts between RT and the RNA/DNA hybrid several base pairs ahead the RNase H active site. This motif, designated the “RNase H primer grip,” interacts with the DNA primer upstream of the scissile phosphodiester bond of the RNA strand. By interacting with DNA bases of the RNA/DNA hybrid near the active site, the RNase H primer grip is proposed to impose the appropriate trajectory on the RNA strand for catalysis. Two general modes of RNase H-mediated catalysis have been identified, namely, polymerization dependent and polymerization independent. The former cleaves the RNA/DNA hybrid 17–18 nt downstream of the primer 3' terminus and is consistent with the distance spanning the DNA polymerase and RNase H active sites. In contrast, polymerization-independent RNase H activity cleaves the RNA/DNA hybrid 8–10 nt downstream of the primer terminus by an RT relocation mechanism that remains to be fully elucidated. Despite this, polymerization-independent RNase H activity plays a pivotal role in both strand transfer steps of HIV-1 DNA synthesis (see later).

Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis, Fig. 1 Reverse transcriptase-mediated synthesis of double-stranded, integration-competent HIV-1 DNA from the (+) strand RNA genome. RNA and DNA are represented in orange and black, respectively. Note that

for HIV-1 and related lentiviruses, the (+) strand of the DNA duplex is discontinuous, reflecting a second site for initiation of DNA synthesis at the center of the genome. Individual steps of DNA synthesis are described in more detail in the text



Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis, Fig. 2 Structure of the p66/p51 HIV-1 RT heterodimer. *Left:* Ribbon diagram of the RT/dsDNA complex. p66 and p51 subunits are represented in cyan and green,

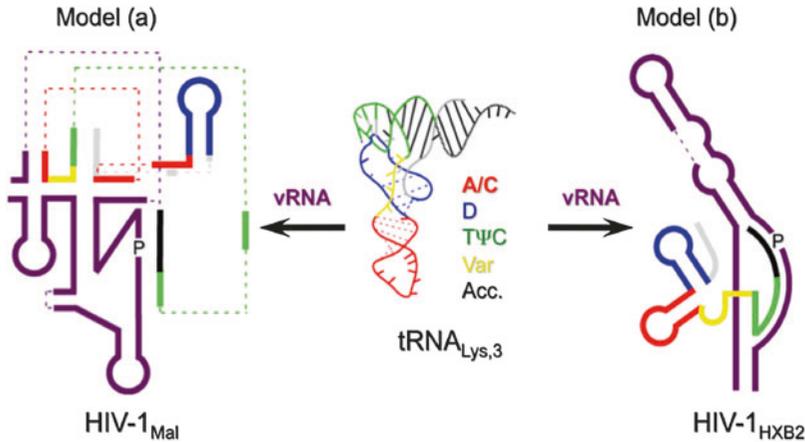
respectively, and DNA in orange. *Right:* Individual subdomains of the p66 and p51 subunits. *F* fingers, *P* palm, *T* thumb, *C* connection, *R* RNase H. *Insert:* Anatomical resemblance of p66 subdomains to a right hand

tRNA-Primed Initiation of (–) DNA Synthesis

Reverse transcription of the HIV-1 (+) RNA genome is initiated from a host-coded tRNA molecule (tRNA^{Lys,3}), whose 18 3'-terminal nucleotides are hybridized to a sequence immediately adjacent to the U5 region of the viral genome designated the primer binding site (PBS) (Wilhelm and Wilhelm 2001; Le Grice 2003). tRNA^{Lys,3} is selectively packaged into the budding virion, i.e., tRNA^{Lys} isoacceptors (tRNA^{Lys,3} and tRNA^{Lys1,2}) constitute ~50–60% of the population of small RNA species in the virion, whereas they represent at most 5–6% of the cellular tRNA population. The viral Gag-Pol polyprotein precursor is necessary for this enrichment, evidenced by data indicating an interaction between its RT coding region and the tRNA molecule.

Since virus particles lacking an RNA genome contain wild-type levels of tRNA^{Lys} isoacceptors, an interaction of the tRNA primer with the PBS of the (+) viral RNA template appears unnecessary to mediate its sequestration. Instead, tRNA^{Lys,3} is packaged into virions complexed with a component of the host translational machinery, namely, lysyl-tRNA synthetase (LysRS). Catalytically inactive LysRS retains the ability to facilitate

packaging of tRNA^{Lys} isoacceptors, indicating that the tRNA does not have to be covalently linked to the synthetase for its enrichment in the virion. LysRS has been shown to interact through an association between a region involved in its contact with HIV-1 Gag, specifically the C-terminal capsid (CA) dimerization region. While this is mostly likely the primary mechanism through which the LysRS/tRNA complex is sequestered into the virus, an interaction between the C-terminal RT domain of the Gag-Pol polyprotein may make an additional contribution. Although virus containing truncated Gag-Pol proteins from which this domain had been removed continued to selectively package their tRNA RNA primer, this was not annealed to the (+) RNA genome, suggesting a conformational change may be required to correctly position tRNA^{Lys,3} on viral RNA subsequent to packaging. Whether the primer is annealed pre- or post-virus assembly and budding remains a matter of speculation. Since tRNA^{Lys,3} is annealed to the RNA genome of viruses whose protease gene has been inactivated, the nucleocapsid (NC) domain of Gag, not the mature NC protein, most likely mediates its hybridization to the PBS. The host-coded RNA helicase A has also been suggested to play an auxiliary role in tRNA annealing by modifying viral RNA conformation.



Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis, Fig. 3 Alternative models of long-range interactions between the HIV-1 $tRNA_{Lys,3}$ replication primer and the 5' region of the viral RNA (vRNA) genome that control

initiation of (–) strand DNA synthesis. Individual tRNA arms are color coded. *A/C* anticodon, *Var* variable loop, *Acc* 3' acceptor stem, *P* primer binding site. Viral RNA is represented in magenta

Establishment of a productive reverse transcription initiation complex occurs subsequent to binding of the p66/p51 RT heterodimer to the tRNA/viral RNA duplex. A combination of chemical and enzymatic probing studies suggests this RNA/protein complex assumes a complex tertiary structure that controls early events of the initiation process. Besides an absolute requirement for the $tRNA^{Lys,3}$ /PBS interaction, several regions in subtype B HIV-1_{HXB2} RNA influence initiation of (–) strand DNA synthesis. One such interaction (Fig. 3a) involves the 3' portion of the $tRNA^{Lys,3}$ anticodon stem and the U5-IR region upstream of the PBS (Isel et al. 2010). Deleting these viral RNA sequences creates a reverse transcription defect in vitro, although virus replication is not significantly impaired. Another important regulatory interaction proposes that a primer activation sequence (PAS) on the viral RNA genome, and present in an extended stem downstream of the PBS, interacts with an “anti-PAS” sequence located in the tRNA TΨC stem (Fig. 3b). In contrast, studies with HIV-1_{Mal}, a subtype A virus, provided an alternative model where an A-rich viral RNA sequence in the U5-IR stem interacts with the U-rich $tRNA^{Lys,3}$ anticodon domain. Although the structures of these complexes are significantly different, both share a common property of controlling early steps of (–) strand DNA synthesis.

Initial steps of tRNA-primed (–) strand DNA synthesis, i.e., addition of the first five dNTPs, are characterized by pausing and premature termination of the replication machinery and are followed by a transition into a productive polymerization mode. Single-molecule spectroscopy with fluorescently labeled enzymes has elegantly illustrated that these premature termination events correlate with multiple inversions of enzyme orientation that have the consequence of positioning the C-terminal RNase H domain over the polymerization site. Productive reverse transcription follows incorporation of the fifth dNTP, which is paralleled by disruption of RNA secondary structural elements immediately ahead of the PBS. This initiation “program,” variations of which can be demonstrated for the related feline immunodeficiency and equine infectious anemia viruses, may provide a means of avoiding premature reverse transcription prior to virus budding. In support of this model, HIV-1 virion-associated $tRNA^{Lys,3}$ has been demonstrated to contain a 2–3 deoxynucleotide extension at its 3' terminus.

(–) Strand DNA Synthesis and Transfer

Subsequent to tRNA-primed DNA synthesis from the PBS, RT copies the U5 and R regions until it

reaches the 5' terminus of the (+) genome, producing the hallmark (−) strand strong-stop DNA. The RNA/DNA hybrid created by this process is hydrolyzed by RNase H activity of both the polymerizing enzyme and additional enzymes that reassociate with the substrate, producing short oligoribonucleotide stretches that can spontaneously dissociate from nascent (−) strand DNA. During RNA-dependent DNA synthesis and RNase H-mediated template hydrolysis, the Gag-coded NC protein aids in unwinding areas of secondary structure and dissociation of larger RNA hydrolysis products.

In the terminal R region, complementarity between nascent (−) DNA and the R region at the 3' end of the viral genome facilitates the first DNA strand transfer event. (−) strand DNA transfer is both intramolecular, i.e., between termini of the same RNA genome, and intermolecular, via two different RNAs. Inter-strand DNA transfer can facilitate recombination between different viral genomes, a process that is essential for maintenance of virus diversity and fitness. (−) strand DNA transfer has been shown to proceed via two distinct mechanisms. In one scenario, transfer takes place between the termini of the full-length (−) strand DNA and the 3' end of the viral RNA genome. An alternative mechanism invokes a strand invasion process, where an internal stretch of single-stranded DNA is accessed by the corresponding complement in the vRNA, followed by branch migration and subsequent completion of (−) strand strong-stop DNA synthesis (Basu et al. 2008).

(+) Strand DNA Synthesis: PPT Primer Utilization and Removal

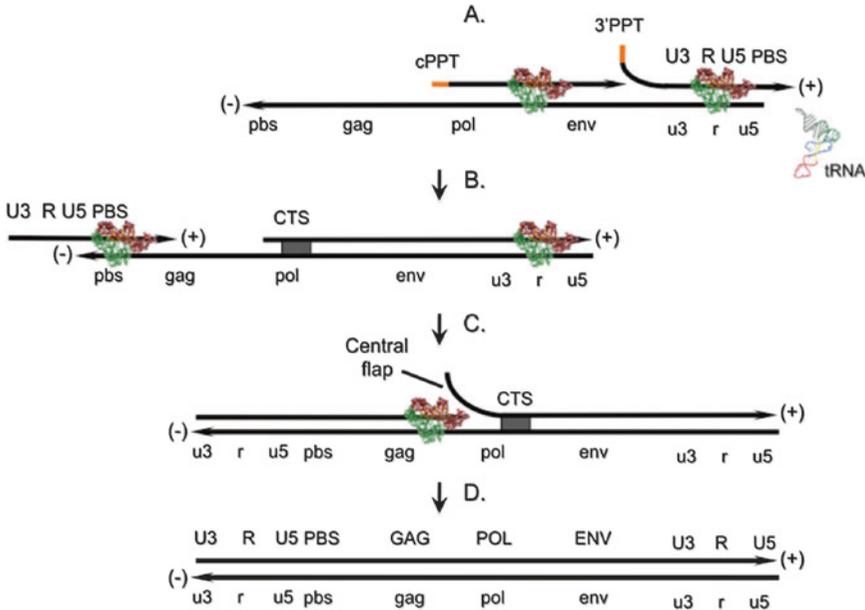
Following (−) strand DNA transfer, RNA-dependent DNA synthesis resumes, accompanied by hydrolysis of (+) RNA of the ensuing RNA/DNA hybrid. However, two polypurine tracts (PPTs), located at the center (cPPT) and in the vicinity of the genome 3' terminus (3' PPT), are refractory to hydrolysis and provide the RNA primers for (+) strand, DNA-dependent DNA synthesis. The cPPT, a unique feature of several

lentiviral genomes, will be discussed in detail later, and therefore only 3' PPT-mediated DNA synthesis is discussed here.

Since 3' PPT-primed (+) strand DNA synthesis defines 5' LTR sequences essential for integration of the full-length double-stranded viral DNA (Fig. 1), its selection from the RNA/DNA replication intermediate and subsequent removal from newly synthesized (+) DNA requires a considerable degree of precision (Rausch and Le Grice 2004). Although the structural basis for selection of the PPT 3' terminus is not completely understood, crystallographic analysis indicates a local and altered pattern of base pairing, an observation that has been supported by chemical footprinting of PPT RNA and its (−) DNA complement. Together, these independent lines of evidence suggest the PPT-containing RNA/DNA hybrid assumes a unique geometry, possibly to promote an “induced” fit with RT to correctly position the scissile phosphodiester bond in the RNase H active site. Initiation of (+) strand DNA synthesis has the consequence of producing a PPT RNA/(+) DNA chimera hybridized to (−) DNA, and the unique architecture of the RNA/DNA junction (demonstrated by NMR spectroscopy) may likewise provide a means of recognition by the RNase H domain that ensures accurate primer removal.

tRNA Primer Removal and (+) Strand DNA Transfer

Prior to second or (+) strand DNA transfer, PPT-primed (+) strand DNA synthesis is templated by both DNA and RNA, namely, U3, R, and U5 DNA, together with 18, 3' nucleotides of the covalently linked tRNA^{Lys,3} primer (Fig. 1). At this point, the replication complex encounters a modified tRNA^{Lys,3} base, 1-methyladenosine 58 (M¹A58), whose modification results in pausing of the replication machinery. Based on the 17–18 bp spatial separation of the DNA polymerase and RNase H active sites of HIV-1 RT demonstrated by X-ray crystallography, pausing at this position places the (−) DNA-tRNA junction in the RNase H active site, facilitating removal of the (−) strand primer.



Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis, Fig. 4 Central termination of HIV-1 (+) strand DNA synthesis and generation of the “central flap.” cPPT and 3' PPT RNA primers are depicted in orange and (+) DNA in

black, respectively. HIV-1 RT is represented by the 2-color cartoon. CTS central termination sequence. Individual steps (a–d) are described in detail in the text

Although the tRNA-DNA junction might be the logical RNase H target site, HIV-1 RT cleaves the penultimate ribonucleotide bond, the rationale for which remains elusive. Polymerization-independent RNase H activity subsequently removes additional ribonucleotides from the tRNA 3' terminus, promoting its dissociation and freeing homologous (+) and (-) strand PBS sequences for second or (+) strand DNA transfer. Confirmation that arrest of DNA synthesis at M^{158A} is critical to (+) strand DNA transfer has been provided in vitro, where substituting tRNA^{Lys,3} with a variant that cannot be methylated at A58 interferes with virus infectivity. Finally, in contrast to (-) strand DNA transfer, which can be either intra- or intermolecular, (+) strand DNA transfer is exclusively intramolecular.

Central Termination of Proviral DNA Synthesis

In principal, (+) strand DNA transfer and ensuing bidirectional DNA-dependent DNA synthesis

should suffice to produce the double-stranded, integration-competent HIV-1 DNA flanked by hallmark LTRs and uninterrupted by ribonucleotides. Surprisingly however, a discrete (+) strand discontinuity was discovered that mapped near the center of the genome and within the integrase (IN) open reading frame (“► Integration”). These observations suggested that *cis*-acting sequences in the IN gene specified (a) a second (+) strand initiation site and (b) a unique mechanism to halt (+) strand DNA synthesis in its immediate vicinity. Since the IN coding region contains a duplication of the 3' PPT sequence (designated the central or cPPT), this provided a rationale for the second initiation site, while the mechanism for terminating (+) strand DNA synthesis was explained by the nearby *central termination sequence* or CTS (Fig. 4).

Describing sequence-specific termination of HIV-1 DNA synthesis mechanistically requires understanding the fate of (+) DNA synthesized simultaneously from the 3' and cPPTs, which is illustrated schematically in Fig. 4. As outlined previously, initiation from the 3' PPT leads to

accumulation of (+) strong-stop DNA containing the PBS sequence necessary for (+) strand DNA transfer. In contrast, cPPT-primed (+) strand synthesis proceeds to the genome 3' terminus (most likely displacing (+) strong-stop DNA in the process), but the absence of a PBS sequence precludes its involvement in strand transfer, thereby establishing one component of the discontinuous (+) strand.

Following (+) strand DNA transfer and resumption of DNA-dependent DNA synthesis, the replication complex ultimately reaches the duplex product of cPPT-primed DNA synthesis. After displacing ~100 nucleotides, RT encounters the CTS in the form of duplex DNA, a prominent feature of which is phased dA:dT tracts. Since dA:dT tracts have been characterized structurally to induce minor groove compression, such successive elements of the HIV genome, in the context of strand displacement synthesis, result “dislocation” of the replication complex and enzyme dissociation. Figure 4 also indicates that the central termination step produces a “flap” of displaced DNA that must be precisely removed and repaired by host-coded enzymes to create the replication-competent DNA provirus. While the function of the central flap remains controversial, several reports indicate that (a) its mutation or deletion is deleterious to virus replication, (b) incorporation of the CTS into lentiviral vectors improves transduction efficiency, and (c) equivalent elements are present in the genomes of equine infectious anemia and feline immunodeficiency virus.

HIV-1 RT Inhibitors and Microbicide Development

Since the approval of AZT in 1987, several nucleoside RT inhibitors (NRTIs) are in clinical use (“► [Clinical Trials: Past, Present and Future](#)”), including lamivudine (3TC), emtricitabine (FTC), abacavir (ABC), didanosine (ddI), and stavudine (d4T). Once phosphorylated to the triphosphate by cellular kinases, NRTIs are incorporated into the growing DNA chain, and the absence of a ribose 3' OH prevents subsequent dNTP incorporation. The related nucleotide RT

inhibitors (NtRTIs), e.g., tenofovir (TFV), function similarly but require only two phosphorylation steps to their active derivative. In contrast, nonnucleoside RT inhibitors (NNRTIs), such as nevirapine and efavirenz, occupy a site at the base of the p66 thumb, “locking” this subdomain in a configuration incompatible with catalysis. Additional mechanistic features of NNRTIs revealed by single-molecule spectroscopy include inducing relaxed contact of RT with nucleic acid (leading to dislocation and “sliding” from the polymerization site) and promoting an inversion of configuration on the PPT. Lastly, indolopyridones represent a new class of RT inhibitors with a unique mechanism that specifically traps the enzyme at a specific stage of translocation.

Despite the requirement of retroviral RNase H for virus infectivity, RNase H inhibitors have not advanced toward clinical trials, possibly reflecting toxicity concerns due to lack of specificity. In particular, inactivating eukaryotic RNase H has been associated with failure to accumulate mitochondrial DNA in model systems. These issues notwithstanding, a considerable body of biochemical and structural data on RNase H inhibitors has accumulated. Chelation of the catalytic divalent metal at the active site is a shared feature of many RNase H inhibitors, which include N-hydroxyimides, hydroxylated tropolones, diketo acids, and pyrimidinol carboxylic acids. Allosteric inhibition of RNase H activity, exemplified by thienopyrimidinones, is also currently under investigation. These compounds occupy a site at the interface between the p51 thumb subdomain and p66 RNase H domain, presumably altering subunit geometry in a manner that is inconsistent with catalysis.

Finally, antiretroviral agents targeting specific enzyme functions of the HIV replication cycle, and reverse transcription in particular, are emerging as promising vaginal and rectal microbicides (“► [Pre-exposure Prophylaxis \(PrEP\)](#)”) (Lewi et al. 2012). Prominent among these is the NtRTI TFV, demonstrated in clinical trials to be safe and well tolerated in a study on HIV-negative women receiving a vaginal gel applied for 24 weeks. Repeated TFV application also produced low

plasma levels and, importantly, failed to select for drug-resistant mutations. NNRTIs such as dapivirine (DPV) also show promising virucidal properties, and long-term constant DPV release has been obtained from a variety of intravaginal rings. However, HIV microbicide development still faces considerable formidable challenges, including conclusive demonstration of efficacy in nonhuman primates, selection of drug-resistant virus in clinical settings (“► [Clinical Trials: Past, Present and Future](#)”), cultural acceptability, and affordability.

Conclusion

HIV-1 DNA synthesis is a multi-step process catalyzed by the viral enzyme reverse transcriptase that creates a double-stranded DNA copy of the plus-sense single-stranded RNA genome. During the course of creating a double-stranded provirus competent for integration into the host chromosome, RT must recognize a variety of different nucleic acid substrates. This multi-functionality offers several stages that can and are being targeted for drug intervention, both therapeutic and prophylactic.

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Risk of Sexual HIV-1 Transmission: Coinfections Associated with Risk

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Definition

For sexual transmission of HIV-1 to occur, a susceptible host has to be exposed to a transmissible virus originating from a transmitter. The transmission risk depends on the frequency and dose of HIV-1 exposure and host susceptibility. The presence of coinfections in the transmitter or in the host influences transmission risk in multiple ways. Coinfections include mucosal coinfections (such as viral and bacterial/protozoal sexually transmitted infections (STIs), altered mucosal microbiota, and genital schistosomiasis) and systemic infections (such as acute malaria and active tuberculosis).

Sexual Transmission of HIV-1

For sexual transmission of HIV-1 to occur, a susceptible host has to be exposed to a transmissible virus originating from a transmitter (Haase 2010). The transmission risk depends on the frequency of exposure and, when exposed, on the plasma and genital HIV-1 viral load in the transmitter. The plasma viral load depends on disease stage and use of combination antiretroviral therapy for HIV

(cART) and can be increased by systemic coinfections. The genital viral load mostly correlates with the plasma viral load, but local genital factors can increase it independently. These include lack of male circumcision and the presence of genital coinfections. Transmission risk also depends on the type of sexual exposure (from highest to lowest risk: receptive anal intercourse, insertive anal intercourse, male-to-female vaginal intercourse, female-to-male vaginal intercourse, and oral intercourse) and host susceptibility.

HIV is not transmitted easily. Even in the case of receptive anal intercourse between men who have sex with men (MSM), only 1 in 70 sex acts results in transmission (Jin et al. 2010). The reason for this is that HIV typically encounters several barriers to infection at the mucosal surfaces of the genital tract, rectum, or oropharynx. The healthy lower female genital tract is lined by multilayered, stratified, and keratinized epithelial cells that form a mechanical barrier and are capable of producing chemokines and antimicrobial compounds. This epithelium is colonized by lactobacilli that produce lactic acid and antimicrobial compounds. Furthermore, it is covered with vaginal exudate and cervical mucus that trap pathogens and distribute the compounds produced by the epithelial cells and lactobacilli. The penis is also protected by epithelium and is bathed in cervicovaginal secretions that are mixed with pre-ejaculate and semen during vaginal sex. Semen has properties that can either enhance or block HIV-1 transmission, but the mixture of cervicovaginal secretions and semen mostly inhibits HIV-1 infectivity. In the rectum, fewer protective barriers exist. The epithelium consists of a fragile single layer of columnar cells, but these cells do produce chemokines and other protective soluble factors. In the oropharynx, transmission mostly occurs via the gingiva and tonsils, which are protected by saliva.

HIV-1 uses a CD4 receptor and either a CCR5 or CXCR4 coreceptor to enter its target cells (mostly lymphocytes and macrophages), but the majority of HIV-1 transmissions involve only a single CCR5-tropic virus (Grivel et al. 2010). HIV-1 can also be captured by dendritic and

Langerhans cells using the cell surface molecules DC-SIGN and other C-type lectins, which is usually followed by transfer of the virus to CD4+ T cells. However, Langerhans cells are capable of degrading HIV-1, but this mostly seems to occur in noninflammatory circumstances when few activated CD4+ T cells are present (de Witte et al. 2007). After HIV-1 has infected its target cells in the mucosa, infected cells are transported to the draining lymph nodes where the virus undergoes rapid replication before being disseminated throughout the body. Innate and adaptive immune responses try to interfere with this sequence of events. The virus is recognized by pathogen-associated molecular pattern (PAMP) receptors on mucosal epithelial cells, which upregulate their production of chemokines, defensins, and other antimicrobial compounds. The complement cascade is activated, more immune cells are recruited, and HIV-1-specific antibodies and cytotoxic T cells are eventually produced. While these defense mechanisms are capable of keeping HIV transmission rates low, they do not always succeed in blocking the virus completely.

From this description of how HIV transmission usually occurs, it becomes clear that host susceptibility increases when the exposed mechanical barrier is weakened or disrupted, protective mucosal fluids are diminished in quantity or altered in consistency or composition, the mucosal microbiome is altered, large numbers of HIV-1 target cells are within reach, and protective innate and adaptive immune responses are insufficient to block the virus. The presence of coinfections in the host can influence all of the above.

Viral STIs: Herpes and Human Papillomaviruses

Herpes simplex virus type 2 (HSV-2) is the etiological agent of genital herpes and the most common cause of genital ulcer disease (GUD). Globally, 16% of the 15- to 49-year-old population is estimated to be living with HSV-2. This amounts to 536 million people, compared to 34 million people living with HIV (Looker

et al. 2008). HSV-2 enters its host via disrupted epithelia, causes blisters at the site of infection due to viral replication in epidermal or dermal cells, and causes a lifelong chronic latent infection of the dorsal root ganglia. Persons infected with HSV-2 alternate between latency and reactivation of viral replication, which causes recurrence of blisters or asymptomatic genital HSV-2 shedding. While the frequency of blister recurrences typically wanes over time, infected persons continue to shed virus for about 25% of days with about half of the shedding episodes lasting less than 12 h (Barnabas and Celum 2012). The HSV-2 viral load produced in these short bursts of viral replication is sufficient for transmission to take place.

Epidemiological studies have shown that prevalent HSV-2 infection (defined as the presence of HSV-2 antibodies in blood) and incident HSV-2 infection increase HIV acquisition risk two- to threefold and sevenfold, respectively, in women (Freeman et al. 2006). Positive HSV-2 serology is also associated with increased HIV acquisition risk in heterosexual men and MSM and with increased HIV-1 RNA in the genital tract of HIV-positive women and men. The mechanisms by which HSV-2 infection might increase HIV-1 transmission include epithelial disruption and recruitment of HIV-1 target cells to the site of HSV-2 replication, but also direct stimulation of HIV-1 replication by HSV-2 in coinfecting individuals (Barnabas and Celum 2012). HSV-2 upregulates HIV-1 replication through different pathways including transactivation of the HIV-1 long terminal repeat. Plasma HIV-1 RNA levels in coinfecting individuals prior to initiation of cART are about 0.2 log₁₀ copies/ml higher than in those without HSV-2 infection, which may translate to higher rates of onward HIV transmission during sexual intercourse and intrapartum. While cART is the most efficient way to reduce plasma HIV-1 RNA levels, acyclovir treatment for HSV-2 also has a modest effect. The mechanism of action is still to be elucidated. In vitro studies suggest that (val)acyclovir may directly inhibit HIV-1 reverse transcriptase. It should be noted that cART in coinfecting patients does not completely eliminate HSV-2 reactivation.

Between 2007 and 2010, three randomized controlled trials (RCTs) of daily oral acyclovir (800 mg) for HIV prevention were conducted (Barnabas and Celum 2012). Two of these showed a reduced incidence of GUD but not of HIV-1 in the study participants. The third one also showed a reduced incidence of GUD as well as a median reduction of 0.25 log₁₀ copies/ml in plasma HIV-1 RNA in index cases, but this was insufficient to lower the HIV transmission rate to their HIV-negative partners. Poor adherence with the acyclovir regimen may be one reason why these RCTs showed no effect. However, since then it has also become clear that HSV-2-infected persons are in an almost continual state of disease reactivation and viral shedding which is not completely suppressed by standard acyclovir treatment. Furthermore, HIV-1 target cells remain present at the site of healed HSV-2 lesions for a surprisingly long time. Current research focuses on development of HSV-2 vaccines and vaginal microbicides that inhibit both HIV-1 and HSV-2. Vaccine candidates to date (most of them using the HSV-2 entry receptor glycoprotein D as the immunogen) have not been successful, and live-attenuated HSV-2 vaccines are currently in early development (Halford et al. 2011). In contrast, 1% tenofovir vaginal gel was shown to reduce HIV-1 acquisition (by 39% over 30 months) and HSV-2 acquisition (by 51% over 30 months) in young South African women in the CAPRISA 004 trial using the so-called BAT24 dosing regimen (first application within 12 h before sex and second application within 12 h after sex) (Abdool Karim et al. 2010). The gel achieved high vaginal levels of tenofovir resulting in direct inhibition of local HSV DNA polymerase. The same gel and BAT24 dosing strategy are currently being retested in another South African RCT (the FACTS 001 trial), which is due to report in 2015. Daily dosing of the gel independent of sex acts was also evaluated in a RCT (the VOICE trial), but this trial was prematurely terminated because of lack of efficacy in reducing HIV-1 acquisition; efficacy against HSV-2 has not yet been determined.

Other highly prevalent herpes viruses may also play a role in HIV-1 transmission but are less well studied in that context. These include HSV type

1 (which mostly causes facial cold sores but can also cause genital herpes), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpesvirus 8 (HHV-8) (Gianella et al. 2013).

Between 1995 and 2009, prior to large-scale introduction of human papillomavirus (HPV) vaccination, the global HPV prevalence in women was estimated to be 12% (Bruni et al. 2010). More than 100 types of mucosal HPV viruses have been identified. High-risk types are associated with cervical, anal, and other anogenital cancers and low-risk types with warts. Worldwide, the high-risk types 16, 18, 31, 52, and 58 are the most common. HPV viruses are sexually transmitted and infect the basal epithelial cells of the anogenital mucosa via microabrasions in the epithelial lining (Veldhuijzen et al. 2010). They evade the immune system by only inducing viremia in terminally differentiated epithelial cells, which are already programmed for apoptosis. Nevertheless, 90% of infections are cleared within 2 years by cell-mediated immune responses. A 2012 systematic review and meta-analysis concluded that HPV infection by both high-risk and low-risk types increases HIV-1 acquisition in women and men about twofold (Houlihan et al. 2012). Several mechanisms of action have been proposed, such as epithelial breaches, increased density of CD4+ CCR5+ T lymphocytes, and a reduced capacity of Langerhans cells to kill HIV-1 in HPV-infected tissues. HPV has also been associated with increased genital HIV-1 RNA in coinfecting women and men.

Two highly effective HPV vaccines were licensed by the US Food and Drug Administration in 2009 (Gardasil and Cervarix, both including high-risk types 16 and 18 and Gardasil also including low-risk types 6 and 11). These vaccines are currently being rolled out in several countries worldwide. A vaccine including HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 is currently completing Phase III efficacy testing. An obvious question is whether HPV vaccination, and particularly vaccination with the 9-valent vaccine, does not only reduce incidence of HPV and cervical cancer but also of HIV-1. This will most likely be investigated in future studies.

Bacterial/Protozoal STIs: Gonorrhea, Chlamydia, Syphilis, and Trichomoniasis

The sexually transmitted bacteria *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) cause endocervical infection in women that can spread to the upper genital tract, sometimes resulting in pelvic inflammatory disease (PID) and/or infertility. NG and CT cause urethritis and balanitis in men. While both infections can cause an inflammatory response with recruitment of HIV-1 target cells, gonorrhea is almost always highly inflammatory, whereas chlamydia is often asymptomatic (Jarvis and Chang 2012). The sexually transmitted spirochete *Treponema pallidum* causes syphilis; primary syphilis is often characterized by GUD and later stages of syphilis by neurological involvement. *Trichomonas vaginalis* (TV) is a sexually transmitted anaerobic, flagellated protozoa. It can infect the ectocervix and vagina in women and urethra in men and is usually asymptomatic or mildly symptomatic. All four STIs are curable with antibiotic treatments, but reinfections are common because sexual partner(s) of index cases are often not treated. The 2005 World Health Organization incidence estimates among women and men aged 15–49 were 88 million new cases per year for gonorrhea, 101 million for chlamydia, 11 million for syphilis, and 248 million for trichomoniasis (World Health Organization 2005). Several epidemiological studies have demonstrated that each infection is associated with increased HIV-1 acquisition in HIV-negative patients and increased genital HIV-1 RNA detection in HIV-positive patients.

NG binds to and invades mucosal epithelial cells using several different surface proteins (Jarvis and Chang 2012). These proteins also activate toll-like receptors (TLR) 2 and 4, thereby initiating cytokine expression and inflammatory cascades, including the recruitment of HIV-1 target cells. NG is also associated with the induction of human defensins that can enhance HIV-1 entry into target cells by promoting HIV-1 attachment. In chronic HIV-1 infection, NG has been associated with transient HIV-1 viremia in plasma and genital secretions. In vitro, NG enhances HIV-1 replication in primary resting CD4+

T lymphocytes, and its surface proteins modulate the degree of this enhancement via TLR-2 activation.

CT serovars D-K infect the genital tract. CT is an obligate intracellular bacterium which alternates between two forms: a metabolically inactive but infectious elementary body (EB) and a metabolically active, noninfectious intracellular reticulate body (RB). EBs enter host cells via receptor-mediated endocytosis; the CT proteins involved in this process have not been completely described. EBs within endosomes then differentiate into RBs that escape host defenses. Like other genital pathogens, CT infection reduces epithelial integrity and induces recruitment of HIV-1 target cells to the genital mucosa. In vitro studies have shown that some CT serovars also increase the expression of HIV-1 primary and coreceptors on epithelial cells, thereby allowing productive infection of epithelial cells by HIV-1. These infections occur via binding of the HIV-1 protein gp41 to the epithelial cell receptor GalCer, instead of the usual binding of HIV-1 protein gp120 to CD4 (Schust et al. 2012).

Syphilis and other GUDs were among the first STIs to be linked to enhanced HIV-1 acquisition and transmission. The explanation was thought to be simple: the genital ulcers caused by these diseases provide a large portal of entry for HIV-1 with plenty of HIV-1 target cells immediately available (Karp et al. 2009). Later on, *T. pallidum* lipoproteins were shown to induce the expression of CCR5 on macrophages in syphilitic lesions and *T. pallidum* itself to increase HIV-1 viral load. The research focus eventually shifted to genital herpes due to its much higher prevalence worldwide.

TV is highly prevalent but was always thought to be a minor STI due to the lack of severe morbidity. However, it has received more attention in recent years due to accumulating epidemiological evidence of its enhancement of both HIV-1 acquisition and transmission (Kissinger and Adamski 2013). TV reduces the mechanical barrier to HIV-1 by causing punctuate mucosal hemorrhages (referred to as “strawberry cervix”) and by breaking down the cervical mucus. TV causes a proinflammatory response but has also been

shown to dampen innate immunity by decreasing secretory leukoprotease inhibitor (SLPI) and other genital innate immune factors. This may allow other genital pathogens to flourish. Perhaps related to this, TV often occurs together with bacterial vaginosis (BV). The two conditions may have synergistic effects on HIV transmission (see further).

Between 1995 and 2007, the results of six RCTs were reported examining the effect of improved management of the abovementioned curable STIs on HIV transmission (Hayes et al. 2010). Disappointingly, only one of these (the Mwanza trial) showed a statistically significant reduction in HIV incidence in the intervention communities. The potential reasons for this have been extensively debated in the literature and include differences in HIV epidemic phases in the study populations (an expanding epidemic in Mwanza and mature epidemics in the other study populations), differences in STI prevalence in the study populations (higher prevalence in Mwanza), the implementation of STI control activities in the control groups (at a minimum, standard STI control practices were allowed to continue), and perhaps the fact that other important genital coinfections (such as viral STIs, BV, and candidiasis) were not included in the interventions. Smaller studies that evaluated the effect of various STI treatments on genital HIV-1 viral load did generally show a reduction in genital viral load after treatment.

Cervicovaginal, Penile, and Oral Microbiota

A healthy cervicovaginal microbiome consists predominantly of lactobacilli, which are thought to restrict growth of pathogenic bacteria and yeasts by keeping the vaginal pH low and by other mechanisms. Several clinical conditions associated with an imbalanced cervicovaginal microbiome have been described, including BV, vaginal candidiasis, and more recently “aerobic vaginitis.” BV has traditionally been associated with anaerobic bacteria, candidiasis with *Candida* yeasts, and aerobic vaginitis with aerobic bacteria, such as streptococci and *Escherichia coli*.

In the last 20 years, several prospective cohort studies have shown significant associations between BV (diagnosed by microscopy) and subsequent HIV acquisition in women. A meta-analysis of individual participant data of 13 African prospective cohort studies showed that intermediate vaginal microbiota (Nugent score 4–6) and BV (Nugent score 7–10) were each associated with HIV acquisition in multivariable models (adjusted hazard ratios of 1.54 and 1.69, respectively) (Low et al. 2011). In addition, four studies have shown a positive association between vaginal candidiasis and HIV acquisition, with adjusted relative risks ranging from 1.8 to 3.3 (reviewed in 19). Taken together, current evidence suggests that any deviation from a normal lactobacilli-dominated vaginal microbiome increases women's susceptibility to HIV. Similarly, studies have shown an association between BV and genital HIV-1 RNA levels, which declined after successful BV treatment (Wang et al. 2001).

In the last decade, new molecular techniques that can characterize the composition of the vaginal microbiome in much more detail (such as next-generation sequencing and DNA microarrays) have become accessible. Published data so far mostly report on smaller, exploratory studies of the cervicovaginal microbiome, but data are accumulating fast. They are showing that the bacterial communities in the vagina are more complex and less stable over time than previously thought. Pioneering work with asymptomatic women of reproductive age in the USA identified five microbiome clusters: four clusters were dominated by *Lactobacillus iners*, *L. crispatus*, *L. gasseri*, and *L. jensenii*, respectively, and the fifth cluster by other BV-associated anaerobic bacteria (Ravel et al. 2011). African studies are finding fewer women with *L. crispatus*-, *L. gasseri*-, or *L. jensenii*-dominated microbiota and more women with *L. iners* or mixed anaerobes. Studies in women with BV worldwide identified multiple BV-associated clusters that were dominated by anaerobic bacteria other than lactobacilli but never a single taxon. No clear patterns have emerged yet. A recent study showed that African sex workers with *L. crispatus*-dominated microbiota were

significantly less likely to be infected with HIV-1, HSV-2, any HPV type, or bacterial/protozoal STIs than women with other types of microbiota, including microbiota dominated by *L. iners*. Furthermore, women with *L. crispatus*- or *L. iners*-dominated microbiota who were already HIV infected were less likely to have detectable HIV-1 RNA levels in their genital tract than women with BV-associated microbiota, even after adjusting for CD4 count. In both cases statistically significant trends were found: the prevalence of STIs or detectable HIV-1 RNA increased with increasing total bacterial load and microbiota biodiversity. Other studies have found that certain types of BV-associated bacteria in particular can have a profound impact on HIV-1 expression in the genital tract.

It has been hypothesized that the effects of vaginal bacterial communities on HIV-1 transmission are due to differences in lactic acid production and vaginal acidity, the production of antimicrobial compounds (such as H₂O₂ and antimicrobial peptides), the production of enzymes (such as sialidases, proteases, and glycosidases) that break down mucins and innate antimicrobial factors, and the extent to which a proinflammatory vaginal environment is induced (potentially resulting in direct damage to the vaginal mucosa and recruitment of HIV-1 target cells) (Mirmonsef et al. 2012). Recently, it has been shown that the bacterial species associated with BV can produce small-chain fatty acids (SCFA; such as butyric acid) that stimulate inflammatory cascades and cell migration and inhibit innate immunity. SCFAs have also been shown to reactivate latent integrated HIV-1 DNA. The wide-ranging effects of individual SCFAs remain to be elucidated.

Studies have also begun to characterize the penile microbiome. The direct influence of the penile microbiome on HIV-1 acquisition in and transmission by men has not yet been determined, but a pioneering study showed that male circumcision reduced the total bacterial load and microbiota biodiversity of the coronal sulcus (Liu et al. 2013). In particular, 12 anaerobic bacterial taxa decreased significantly. Most of these are also frequently found in the microbiota of women with BV, although it should be noted that several

important BV-associated bacteria (such as *Gardnerella*, *Atopobium*, *Megasphaera*, and *Sneathia* species) were not significantly altered in the coronal sulcus by male circumcision. Lactobacilli were found in about a third of men regardless of circumcision status but in low abundance. Male circumcision reduces HIV-1 acquisition by about 60% and also reduces other viral STIs in men and STI transmission to their female partners. The authors hypothesize that the microbiota changes and accompanying reduction of HIV-1 target cells may contribute to the reduction of HIV-1 risk by male circumcision.

Changes of the oral microbiome seem to increase transmission risk during oral sex in a similar fashion as described above for the cervicovaginal microbiome and vaginal sex. The periodontal pathogenic bacteria *Porphyromonas gingivalis*, for example, increases CCR5 expression on oral keratinocytes, induces inflammation, and produces SCFAs (Pretorius et al. 2007). These effects are not generalizable to all oral bacteria.

Schistosoma and Other Helminths

Schistosoma and other helminths influence HIV-1 acquisition and transmission in multiple ways, with the clearest detrimental effects when the infection includes the urogenital tract (as is frequently the case with *Schistosoma haematobium* and sometimes with *S. mansoni*). Cross-sectional studies have shown a positive association between urogenital schistosomiasis and HIV-1 prevalence, but data on other helminth infections and HIV-1 are inconsistent, and longitudinal data are lacking (Webb et al. 2012). Helminths have coexisted with humans for a long time and cause minor pathology, but HIV-1 is a relatively new infection that has disrupted this stable parasitic relationship between helminths and hosts. Helminths induce a T-helper type 2 (Th2)-biased immune response, the extent of which depends on the phase of helminth infection. Regulatory T cells are induced that dampen generalized immune activation (thereby reducing the number of target cells for HIV-1), but most likely also dampen HIV-1-specific immune responses. On the one hand,

schistosomes have been shown to increase CCR5 coreceptor expression and to stimulate CD4+ T cells, which could facilitate HIV-1 entry and replication. On the other hand, competitive binding of helminth antigens to DC-SIGN receptors on dendritic cells in mucosal surfaces presumably hinders HIV-1 entry into these cells. Genital schistosomiasis clearly does increase risk of HIV-1 acquisition: it induces vascularization and granuloma formation around schistosoma eggs in the genital mucosa, thereby impairing the mucosal barrier and increasing the number of HIV-1 target cells. These effects have been shown to last for more than 12 months after successful treatment. HIV-1 transmission has also been shown to occur through lesions in the intestinal mucosa caused by gut-associated helminths.

No clear benefits of antihelminthic treatments on HIV-1 transmission (including mother-to-child transmission) have been shown thus far. In contrast to the hypothesized beneficial effects of all other coinfection treatments, the effects of deworming could be beneficial or detrimental. The gut is regarded as a major site of HIV-1 replication and studies have been done to determine the effect of deworming of HIV-positive patients on HIV-1 viral load. However, about half of the studies found no effect, and the other half were split between beneficial and detrimental effects. Therefore, no consensus has been reached about the potential usefulness of deworming as an HIV prevention tool.

Malaria and Tuberculosis

HIV-1, malaria, and tuberculosis are among the most important global health problems, and they overlap geographically. HIV-1 and malaria seem to interact bidirectionally and synergistically with each other (Alemu et al. 2013). HIV-1 infection and associated immune depression increase the malaria parasite burden, which facilitates malaria transmission. The malaria parasites, on the other hand, induce apoptosis of CD4+ and CD8+ cells during blood stage infection, but they also activate the remaining CD4+ cells; the net effect seems to be more efficient spread of HIV-1 among CD4+

cells and reduced cellular immunity. A systematic review of six studies showed an increase in HIV-1 viral load in acute malaria infection, which partially decreased after antimalaria treatment (Barnabas et al. 2011). In vitro, this enhancement was shown to be mediated by tumor necrosis factor-alpha (TNF- α), which can upregulate HIV-1 replication by acting directly on the HIV-1 long terminal repeat. In vitro studies have also shown mononuclear cell activation by malaria antigens through interferon gamma (IFN- γ), rendering these cells more susceptible to HIV-1 and reactivating integrated HIV-1 DNA.

Mycobacterium tuberculosis (and other pulmonary pathogens) can cause lung-specific expansions of CD4+ CCR5+ T lymphocytes that are highly susceptible to HIV-1, thereby increasing the possibility of HIV-1 transmission via the lower respiratory tract mucosa (Santucci et al. 2004). However, HIV-1 transmissions across the pulmonary tract are exceedingly rare. A systematic review of 19 studies showed that active tuberculosis is associated with an increase in plasma HIV-1 viral load, but tuberculosis treatment did not reduce the viral load (Barnabas et al. 2011). Others have linked *M. tuberculosis* infection with HIV-1 reactivation from latency (and vice versa), thereby increasing onward HIV-1 transmission risk.

Conclusions

Most of the coinfections described in this entry cause significant morbidity and mortality in their own right and deserve to be targeted in public health programs. While this is generally the case for malaria and tuberculosis, STI control is often neglected and has not been successful. While individual RCTs to date have not consistently shown that STI control reduced HIV transmission, the basic concept that STIs increase HIV-1 transmission remains strong and has only strengthened over time. The HIV prevention field is currently moving toward comprehensive HIV prevention approaches to reduce transmission (Vermund and Hayes 2013). While most of the proposed programs to date call for STI control as one of the components, it is not clear which interventions are

likely to have the biggest impact in specific populations and whether screening and treatment of other coinfections (such as genital schistosomiasis and altered mucosal microbiota) should also be included. Furthermore, some of the interventions that are likely to have the biggest impacts are still in development (e.g., HSV-2 and 9-valent HPV vaccines and pre- or probiotics to achieve and maintain a *L. crispatus*-dominated cervicovaginal microbiome). Finally, new approaches to STI screening (rapid point-of-care diagnostic tests, self-sampling, and testing strategies), and improved routine STI surveillance, are also needed. Our understanding of the role of coinfections in HIV-1 transmission is still improving, and this will hopefully eventually result in better tools and better prevention programs.

Cross-References

- ▶ [Antiretroviral Therapy: When to Start](#)
- ▶ [Circumcision and AIDS](#)
- ▶ [Combination Approaches to HIV Prevention](#)
- ▶ [Dendritic Cell Interactions with HIV-1 Envelope Glycoprotein: Implications for Preventing Transmission](#)
- ▶ [HIV Prevention and Women](#)
- ▶ [HIV-1 Transmission Blocking Microbicides](#)
- ▶ [HIV-1 Transmission: Influence of Bodily Secretions](#)
- ▶ [Human Papillomavirus \(HPV\)](#)
- ▶ [Immune Activation and HIV Transmission](#)
- ▶ [Immunopathogenesis of HIV Coinfections](#)
- ▶ [Mucosal Immunity to HIV-1](#)
- ▶ [Preexposure Prophylaxis \(PrEP\)](#)

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Role of Antibodies in HIV Transmission

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Definition

An antibody is a soluble protein that recognizes and binds a specific target, known as its antigen. The region of an antigen bound by an antibody is

known as an epitope. Antibodies are produced and secreted by B cells (► [HIV & SIV, B Cell Responses to](#)) following exposure to their cognate antigens in the presence of appropriate activating signals. Antibodies are members of the immunoglobulin protein superfamily and are made up of four subunits – two heavy chains and two light chains – that come together to form a Y-shaped protein with two functionally distinct regions on opposite ends of the molecule. On one end, the two-headed variable region (the Fab fragment) mediates interaction with the antigen and varies enormously in sequence between antibodies with different antigen specificities. On the other end, the constant region (the Fc fragment) mediates recruitment of other components of the immune system, including innate immune cells and complement. The Fc portion can be one of five main forms, which determine the antibody's isotype (IgA, IgD, IgE, IgG, or IgM). Different isotypes have different binding partners and thus mediate different effector functions. Isotypes also differ in their tissue distributions, as a result of differences in diffusion rates and isotype-specific active transport.

Introduction

Antibodies are key effectors of humoral immunity against pathogens, helping to block transmission, control spread in the body, or clear infection. Following infection, a repertoire of pathogen-specific antibodies of numerous specificities and multiple isotypes is generated. These antibodies bind extracellular pathogens and infected cells, targeting them for destruction. The mechanisms by which this destruction is achieved vary depending on the antibody isotype and epitope and can be divided into two main types: neutralization and non-neutralizing functions. Antibodies that bind extracellular pathogens, and in so doing, sterically block infection, are referred to as neutralizing antibodies. In addition to neutralization, which relies solely on binding of a structurally relevant region of the pathogen to the antibody's variable region, antibodies with bound antigen can mediate other effector

functions by recruiting various cells or molecules through their Fc portion. Antibodies that act through Fc-mediated functions can target infected host cells as well as free pathogens. For example, IgM and some IgG antibodies fix complement, leading to the formation of cytolytic pores by the membrane attack complex. A number of innate immune cells can also be recruited to antibody-antigen complexes, either through fixed complement or through direct interaction of cellular receptors (Fc γ receptors) with the Fc portion of antigen-bound IgG. Upon interaction of their Fc γ receptors (Fc γ Rs) with an antibody-antigen complex, macrophages, neutrophils, dendritic cells, and mast cells phagocytose the coated antigen (by a process known as antibody-dependent cellular phagocytosis, ADCP). Fc γ R engagement on NK cells, macrophages, neutrophils, and eosinophils also results in the release of cytotoxic granules that induce death of antibody-coated infected cells (by antibody-dependent cellular cytotoxicity, ADCC). Fc γ R-bearing cells can also release pro-inflammatory cytokines upon receptor engagement, potentiating other arms of the immune response.

Which of these antibody functions are most important in infection control varies between pathogens, and several mechanisms are likely to play in any given infection. This entry focuses on the roles of antibodies in preventing transmission of HIV. Investigation of these roles is critical to characterizing the immune responses that provide protection from HIV and designing a prophylactic vaccine that elicits such responses.

Most antibodies developed in response to HIV infection target the envelope glycoprotein (Env). Env is expressed on the surface of infected cells during viral budding and is present on the virus particle that is released (cell-free virus), where it facilitates viral entry (► [Life Cycle Overview](#)). Though the antibodies generated in HIV infection mediate a number of effector functions that impact viral replication (described below), they are not able to clear infection. This is largely due to HIV's extremely high rate of evolution and consequent immune evasion (Immune Evasion by HIV). Within a single individual, the virus readily mutates in response to antibody pressure,

generating escape variants resistant to antibody recognition and binding. While escape is sometimes the result of a change within the target epitope, it is also often due to acquisition of additional glycans and conformational changes that shield conserved epitopes. These escape variants in turn elicit a new response – a process that leads to continued evolution of both HIV and the antibody response to it. Given the capacity of HIV to evolve away from immune pressure, the generation of antibodies that can recognize and protect against the vast diversity of HIV variants that an individual might be exposed to is an enormous challenge in vaccine development.

It is important to distinguish the role that antibodies play in established infection – where antibodies are elicited well after significant virus replication and amplification – from their potential role in blocking a new infection, where relatively few virus particles and/or infected cells must be blocked. A number of antibody functions could theoretically be important in blocking HIV transmission: cell-free virions could be bound by neutralizing antibodies that would prevent infection of target cells; infected cells expressing viral proteins could be bound by antibodies and recruit NK cells ([▶ NK cells responses to HIV](#)) to perform ADCC; and antibody-coated infected cells or free virions could recruit macrophages or neutrophils to perform ADCP. Experimentally, a number of different assays can be used to measure antibody inhibition of HIV, and the antibody functions measured vary depending on the assay. Assays may measure neutralization exclusively, ADCC exclusively, or overall antibody-dependent cell-mediated virus inhibition (ADCVI), which accounts for not only ADCC activity but also release of soluble antiviral factors (cytokines and chemokines) that inhibit viral spread. Many of these Fc-mediated functions are dependent upon antibody avidity, which measures the overall strength of the interaction between antibodies and their antigens. Avidity may therefore be an important surrogate measure for such activity (Robinson 2013).

Elucidating which antibody functions are most important in blocking HIV transmission can only be achieved by empirical investigation of the

antibody responses in various models of HIV exposure and protection. This entry will review current knowledge of the role of neutralizing and non-neutralizing antibody functions in preventing HIV transmission. Evidence will be discussed from several settings of transmission in which HIV-specific immune responses exist prior to viral exposure.

HIV-Specific Neutralizing Antibody Function

The expectation that neutralizing antibodies are important in protecting from HIV stems from the fact that almost all successful vaccines for other pathogens elicit strong neutralizing antibody responses. Indeed, HIV-infected individuals generate neutralizing antibody responses targeting Env that persist throughout infection. Although these neutralizing antibodies are not able to clear infection, there is evidence that they do exert a selective pressure on the virus (Overbaugh and Morris 2012). Plasma from an individual can neutralize the individual's own (autologous) viral strain *in vitro* starting as early as a few months after infection, but, as discussed, the virus readily mutates in response to the antibody. Broader responses capable of blocking *in vitro* infection by heterologous viral strains develop in 10–30% of individuals after 2–3 years of infection (Stamatatos 2012).

It has been hypothesized that if neutralizing antibody responses could be elicited by a prophylactic vaccine and be present at or within a few days of HIV exposure, infection could be prevented or halted before establishment of a latent reservoir or outgrowth of escape variants. Evidence from passive immunization studies of non-human primates ([▶ Models for HIV-1 transmission; non-human primates](#)) supports this hypothesis (Lifson and Haigwood 2012). Prophylactic administration of high concentrations of neutralizing antibodies (either human monoclonal antibodies or polyclonal IgG from infected humans or chimpanzees, referred to as HIVIG or CHIVIG, respectively) to nonhuman primates at the time of viral challenge or a few hours later

resulted in dose-dependent protection from infection. While these studies provided proof of concept for the ability of neutralizing antibodies to block HIV or SIV transmission, their interpretation is limited. Nonhuman primate models do not entirely mimic the natural history of human HIV infection, and experimental infections and passive immunizations do not fully replicate the characteristics of human HIV transmission – higher concentrations of antibody, less diverse viral stocks, and closer matching between the antibody specificity and the challenge virus are typically used in nonhuman primate experiments than observed in natural human infection.

A more clinically relevant understanding of the role of neutralizing antibodies in HIV transmission has been afforded by studies of humans naturally exposed to HIV. A number of studies have compared preexisting neutralizing antibody activity in individuals exposed to HIV in various settings who do or do not acquire infection. This section will review the conclusions drawn from such studies.

Mother-to-Child Transmission

Mother-to-child transmission (MTCT) (► [Prevention HIV-1 MTCT](#)) of HIV, which occurs in utero, during delivery, and via breastfeeding, provides one setting in which to study the role of preexisting HIV-specific antibodies in transmission. Maternal IgG crosses the placenta while the infant is in utero and provides protection against a variety of diseases. In HIV-infected mothers, HIV-specific antibodies are passed to their infants and may provide protection while in utero or during delivery. These passively acquired antibodies persist for many months and thus may also guard against infection during the breastfeeding period when infants are continually exposed to the virus. Breastfed infants receive additional HIV-specific IgA and IgG from maternal breast milk, which may coat the virus present in breast milk and thus protect from infection at infant mucosal surfaces.

Early studies showed that neutralizing antibodies exert a selective pressure on viral variants in MTCT. During MTCT, a bottleneck occurs in which only one or a few maternal virus variants

are transmitted to the infant. It has been shown that these infant viruses are generally more resistant to neutralization than their corresponding maternal variants and the most highly neutralization sensitive variants are typically not transmitted (reviewed in Overbaugh and Morris 2012). These findings suggest that variants that are able to escape the neutralizing antibody response are transmitted and support the idea that maternal antibodies may be at least partially protective.

However, when comparing infants who become infected to those who do not, the direct impact of maternal and infant neutralizing antibodies on MTCT is unclear (reviewed in Overbaugh and Morris 2012). A number of studies have suggested that mothers with higher neutralizing antibody activity are less likely to transmit than those with lower activity. Other studies, however, have only seen a protective effect during certain stages of pregnancy and infancy or not at all. Similarly, although studies specifically focused on infant passively acquired antibodies are limited, the largest study of this type observed no protective effect mediated by these antibodies (Lynch et al. 2011). The inconsistency in these results may be partially explained by differences in methodology, discussed below, but highlight the current debate in the field and need for more studies.

Similar to passive immunization studies in macaques, HIV-specific antibodies have been tested as preventative therapies for MTCT. In two different clinical trials, polyclonal HIVIG was administered to pregnant HIV-infected women and their infants at birth (Mofenson 2011). Similar infection rates were seen among infants who received HIVIG and control groups in both studies. However, all study participants were also given antiretrovirals, resulting in overall low infection rates, which may have limited ability to detect an additional protective effect of HIVIG. In addition, HIVIG has only moderate neutralization activity against diverse HIV viruses, whereas some more recently identified broadly neutralizing antibodies have been shown to neutralize the vast majority of circulating variants (Stamatatos 2012). It remains unknown whether these broadly neutralizing antibodies may provide additional

protection over that of antiretrovirals and could be used as a successful immunotherapy to prevent MTCT.

HIV-Infected Individuals Exposed to Superinfection

Another setting in which HIV exposure occurs in the presence of an immune response to HIV is in infected individuals who are continually exposed to the virus. These individuals are at risk of acquiring another HIV infection, a process referred to as superinfection. Superinfection has been reported at relatively high frequencies in cohorts of individuals at risk of transmission through sexual contact and intravenous drug use (reviewed in Waters and Smit 2012). Comparison of the immune responses of individuals who acquire superinfection with those who do not despite continued exposure has offered insight into whether a strong neutralizing antibody response protects from infection. Three studies have compared plasma neutralizing activity of superinfected individuals prior to superinfection to that of singly infected individuals at a matched time after initial infection (Smith et al. 2006; Blish et al. 2008; Basu et al. 2012). The findings differed, with two studies reporting weaker neutralizing antibody responses in superinfected individuals, while the other reported no difference. Similar to MTCT studies, these conflicting results in the setting of superinfection may be due to experimental differences, outlined below. Thus, the ability of neutralizing antibody responses to protect from superinfection remains unclear and additional studies are needed.

Highly Exposed, Persistently Seronegative Individuals

Another setting that indicates a potential role for neutralizing antibodies is individuals who remain HIV negative despite repeated exposure to the virus (highly exposed, persistently seronegative). A number of studies have shown that individuals with repeated HIV-1 exposure more frequently develop mucosal HIV-specific neutralizing IgA than controls (reviewed in Hirbod and Broliden 2007). This association has been shown in

multiple populations, including serodiscordant couples and female sex workers, and suggests a potential protective effect of mucosal neutralizing IgA antibodies. At this time, however, these intriguing results are still somewhat controversial as there is limited precedent for the development of pathogen-specific antibodies in the absence of infection.

HIV Vaccine Trials

Data from human vaccine trials have yet to show support for a role for neutralizing antibodies in preventing transmission. While most vaccines that incorporate HIV Env have been shown to elicit neutralizing antibodies, these antibodies are often not very potent or effective against diverse HIV isolates (Mascola and Montefiori 2010). The elicited antibodies typically target variable regions of Env, rather than conserved epitopes that are the target of broadly neutralizing antibodies.

Of the phase IIb/III human vaccine trials to date, the RV144 trial, completed in 2009, is the only one to show a statistically significant decline in the rate of HIV infection among vaccinated recipients, and this effect was modest (~31%) (Rerks-Ngarm et al. 2009). In this trial of a canarypox vector prime and gp120 protein boost vaccine, HIV-specific neutralizing antibody levels were low and did not differ between vaccinated individuals who became infected and those who remained uninfected (Haynes et al. 2012). However, there was some evidence of a role for non-neutralizing antibodies, as discussed below.

HIV-Specific Non-neutralizing Antibody Function

In the last few years, there has been increased interest in non-neutralizing antibody functions in HIV infection. Most studies to date have focused on ADCC. Like neutralizing antibodies, antibodies capable of inducing ADCC have been detected in HIV-infected individuals. These antibodies arise earlier in infection than neutralizing antibodies, usually within days or weeks. Interest

in non-neutralizing antibody functions in HIV protection stems from the known role of such antibodies in protective vaccines against other pathogens, such as *Haemophilus influenza* and *Streptococcus pneumonia* (Robinson 2013). An important role for non-neutralizing antibody function in blocking HIV transmission is supported by evidence from passive antibody transfer and vaccine studies in nonhuman primates (Robinson 2013). In one passive transfer study (Hessell et al. 2007), a broadly neutralizing monoclonal antibody known to protect macaques from SHIV challenge was engineered to have reduced Fc γ R binding. Macaques given the form lacking Fc-mediated effector functions were more susceptible to infection than those given the original antibody, despite no difference in neutralizing activity between the two forms. Recent vaccine studies reporting partial protection of macaques from SIV acquisition have shown a correlation between the number of low-dose SIV challenges required for infection and the avidity, but not neutralizing activity, of the antibody response mounted (Lai et al. 2011; Barouch et al. 2012). These findings in nonhuman primates have promoted the idea that a protective HIV vaccine will need to elicit non-neutralizing antibodies, and prompted investigation of non-neutralizing functions in studies of HIV infection in humans. Evidence of non-neutralizing antibodies, predominantly ADCC, as correlates of protection in transmission settings is presented below.

Mother-to-Child Transmission

Limited data exist on the role of non-neutralizing antibodies in mother-to-child transmission. Early studies suggested no association between antibodies capable of mediating ADCC in mothers or their infants and risk of infant infection, though some did observe a correlation with delayed progression in infected infants, suggesting a protective effect of ADCC (reviewed in Farquhar and John-Stewart 2003; Huber and Trkola 2007). Additionally, in a recent study of breast milk-derived antibodies from HIV-infected mothers, ADCC was found to correlate with protection from infection (Mabuka et al. 2012). In this

small study, mothers who transmitted the virus to their infants had lower ADCC activity than those whose infants remained uninfected. This study was the first to examine the impact of HIV-specific ADCC activity in maternal breast milk and requires further follow-up to determine if the association is observed in larger cohorts and, if so, the mechanism of protection.

HIV Vaccine Trials

Perhaps some of the best evidence for non-neutralizing antibodies in protection comes from recent human vaccine trials. While the RV144 trial showed no evidence of protection due to neutralizing antibody activity, protection in vaccinated individuals correlated with binding of IgG antibodies to the V1V2 loop of HIV-1 Env and inversely correlated with Env-specific IgA (Haynes et al. 2012). The increased risk of infection seen with Env-specific monomeric IgA is thought to be due to IgA competing with IgG for binding rather than antibody-dependent enhancement of infection. Such competition limits non-neutralizing effector functions mediated by the Fc portion of IgG as the Fc portion of IgA is displayed instead. Indeed, in a secondary analysis of vaccinated individuals with low IgA, ADCC activity was correlated with reduced risk of infection, supporting the hypothesis that IgG binding to V1V2 mediated protection through non-neutralizing antibody function.

An additional level of support for non-neutralizing antibodies in protection is from one of the first large-scale HIV vaccine trials, Vax004. Although this vaccine was not efficacious, higher ADCVI activity and antibody avidity were associated with lower infection risk among vaccinated individuals (Forthal et al. 2007). The combination of results from RV144 and Vax004 suggests a role for Fc-mediated effector functions in protection. However, given that the Vax004 trial showed no vaccine efficacy and the associations observed in the RV144 trial were detected in a hypothesis generating study design (a design that does not account for multiple statistical comparisons), these findings represent promising leads for future studies, rather than definitive correlates.

The Contribution of Methodological Differences to Study Findings

The role neutralizing and non-neutralizing antibodies play in protection from HIV infection remains unclear, despite years of study. This is in part because the results of studies in exposed human populations have often been conflicting, as highlighted above. A number of technical differences may contribute to such conflicting data and are important considerations when designing new studies and drawing conclusions from previous research.

First, differences in cohort and sample size may limit conclusions and comparisons across studies. For example, the three studies of neutralizing antibodies and superinfection were all relatively small (ranging from 3 to 12 superinfected individuals). With such small sample sizes, the power to detect any statistical significance is reduced.

Differences in the virus(es) against which antibody activity is assayed may also confound results and limit comparisons across studies. Older studies, including the studies of ADCC in MTCT discussed above, typically used lab-adapted viruses such as HIV III_B and RF. Newer work has revealed that these lab-adapted viruses do not mimic the majority of transmitted viruses, particularly with respect to co-receptor usage and epitope masking. Primary isolates, obtained directly from infected individuals rather than passaged in the lab, are now more commonly used. Additionally, while some studies test antibodies against autologous viruses, others quantify activity against a panel of heterologous variants. Both approaches provide valuable information, but differ in the questions they answer and their interpretations. For example, in studies of MTCT, infant antibody responses against heterologous viruses may not be relevant to the biological effect of these antibodies against autologous maternal virus. On the other hand, in settings of sexual exposure to diverse viruses, such as human vaccine studies and individuals at risk of superinfection, antibody activity against heterologous viruses will likely be a more relevant measure of protection. Furthermore, the degree of

sequence similarity between immunogens (the antigens that provoke immune responses) and challenge viruses in these contexts will influence interpretation of results and comparison across studies.

Another major consideration is the timing of sampling of the virus and/or antibody. As described above, the virus continually evolves to evade host immune responses. Thus, antibodies are often capable of neutralizing historical, but not contemporaneous, variants. This detail is important when measuring antibody responses against autologous virus – matching sampling time points of viruses and antibodies is important to obtain a complete, relevant picture of the humoral response at the time of transmission or protection. Similarly, timing of virus exposure relative to the development of the antibody response may influence results. For example, it is difficult to compare studies of individuals superinfected very early after initial infection when the neutralizing response has not yet fully developed with studies of individuals superinfected late; or likewise infants infected early after birth when passively transferred antibodies circulate at high concentrations with those infected later in life when they have begun to decay.

Conclusion

Antibodies are central components of the immune response to HIV and will likely be essential in a protective vaccine-induced response. A key question in the HIV antibody field has therefore been what antibody specificity and effector functions correlate with protection from infection. The studies described in this entry suggest that both neutralizing and non-neutralizing antibodies have the potential to provide protection, but which responses do so in natural transmission remains unclear.

More studies are needed to continue to characterize all antibody-mediated effector functions in HIV transmission and their potential role in protection. For example, the roles of antibodies that mediate phagocytosis, complement fixing, and viral trapping in mucus need to be clarified.

Some preliminary studies have reported that these functions are associated with reduction in viral load, which would reduce infectiousness and thus may help prevent transmission, but the direct impact of preexisting antibodies with these effector functions has not been determined. Another feature of the antibody response that is garnering greater attention is tissue distribution. The majority of HIV transmission events occur at mucosal surfaces (► [Mucosal Immunity](#) and ► [Infection in the female genital](#); ► [transmission blocks to HIV-1](#)), but for technical reasons, studies to date have mostly focused on plasma samples. Consequently, local responses at the site of virus exposure might not have been adequately assessed. Finally, the lack of protective effect seen in the majority of vaccine studies to date may be due to inadequate stimulation of the antibody response by the immunogens used. Research is therefore needed to determine how immunogens can be designed to generate high-titer, long-lived responses that mediate protection against transmitted variants (► [HIV-1 Transmission blocking vaccines; how feasible are they?](#), ► [HIV-1 transmission blocking vaccines; new designed immunogens](#)). The studies presented in this entry and future research into other antibody effector functions and their elicitation will help us understand how to harness the humoral immune response to ultimately prevent HIV infection.

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Role of Dendritic Cells in HIV-2 Pathogenesis

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Definition

Dendritic cells (DC) are fundamental components of the response to infection, and it has become clear that these cells play a key role in HIV/AIDS pathogenesis.

This chapter focuses on how DC may contribute to the immunopathogenesis of HIV-2 infection, a relatively “benign” form of HIV/AIDS. The current situation regarding the global HIV epidemic will be addressed first, along with a brief review of the known differences and similarities between HIV-1 and HIV-2 disease. Then, the role of various DC subsets that may functionally contribute to these differences will be discussed.

HIV: The Current Situation

The human immunodeficiency virus (HIV) pandemic still represents one of the major healthcare problems to be faced in the twenty-first century. Approximately 33 million people are estimated to be living with HIV infection, with the latest

reported figure for AIDS deaths of 1.8 million (www.unaids.org/globalreport), with African countries bearing an inordinate share of the burden. The introduction of effective antiretroviral therapy (ART), the development of drug provision, and worldwide health education programs, although bringing marked benefits, are still unable to significantly reduce viral transmission and, thus, control the rate of new infections (2.6 million in 2009 as compared to 3 million in 2001) and HIV prevalence (remaining approximately 0.8% among adults).

Achieving a “functional cure” relies on a better understanding of HIV/AIDS pathogenesis. As HIV-2 infection can be viewed as a naturally occurring attenuated form of HIV/AIDS, it offers a unique opportunity to reveal important clues regarding the determinants of a protective host response to HIV (Grossman et al. 2002).

HIV-1 and HIV-2

HIV is a member of the *Lentivirus* genus, belonging to the *Retroviridae* family of viruses, all of which feature single-stranded ribonucleic acid (ssRNA) as their genetic material. There are currently two viruses associated with HIV infection: HIV-1, discovered in 1983, is the one responsible for the pandemic, while HIV-2 (► [HIV2: Identification of the second AIDS virus, a historical perspective](#)), isolated in 1986, is associated with geographically confined epidemics, mainly in West Africa (► [The epidemiology of HIV-2 infection in West Africa](#)), but also in India and in Europe (► [Epidemiology of HIV-2 infection in Europe](#)), particularly in Portugal, due to the latter connections with its African ex-colonies (de Silva et al. 2008).

The overall gross structure (► [HIV-2: viral structure, sequence variation and pathogenesis](#)) of the viruses is very similar (Reeves and Doms 2002). A protein core, constructed from the viral capsid protein, encapsulates two copies of the unspliced ssRNA genome, which is stabilized as a ribonucleoprotein complex with the virally encoded nucleocapsid protein. In addition to the diploid viral genome (► [Molecular biology of](#)

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HIV-2), the capsid contains six virally encoded molecules: the enzymes protease, reverse transcriptase and integrase, and the accessory proteins Nef, Vif, and Vpr/Vpx (HIV-1 and HIV-2 specific, respectively). The protein core is surrounded by a host-cell-derived lipid bilayer, or envelope (► [The HIV-2 envelope: structure, diversity and evolution](#)). This envelope is characteristically modified by the insertion of two viral glycoproteins which heterodimerize and subsequently associate to form trimeric complexes, sometimes referred to as “spikes,” which extend beyond the envelope’s surface and mediate virus-cell interactions.

HIV-2 Infection: Why Is It Less Severe?

It is well-documented that HIV-2 infection results in a markedly different disease course (► [Natural history and clinical features of HIV-2 infection](#)), with limited impact on the mortality of the majority of infected adults (de Silva et al. 2008). Two main features set HIV-2 apart from HIV-1 infection. The first one is the much slower rate at which CD4+ T-cell depletion, the hallmark of HIV/AIDS, occurs; the second is the typically undetectable to low levels of circulating virus (► [HIV-2 diagnosis and viral load measurements](#)) (plasma viremia) that are observed throughout disease in the majority of infected patients (Table 1).

At first sight, such differences could suggest an impaired ability of HIV-2 to replicate (► [HIV-2: viral replication](#)) and/or destroy its primary

cellular target, the CD4+ T cell. However, both HIV-2 and HIV-1 have been shown to have comparable in vitro replication and cytopathicity (Sousa et al. 2002).

Assuming that HIV-2 can replicate and destroy cells at similar levels as HIV-1, what other factors could then account for the distinct rates of disease progression?

One straightforward explanation would be that the immune response to HIV-2 infection (► [The antibody response to HIV-2](#) and ► [The cellular immune response to HIV-2 infection](#)) is in some way more effective. Thus, in HIV-2-infected individuals, the overall immune response may be somehow more appropriate, and, possibly, more sustainable (de Silva et al. 2008). DC likely play a role in these processes.

Natural History of HIV-2 Infection

In order to understand the potential role of DC in HIV-2 disease, it is first necessary to discuss the natural history of HIV infection. Whether resulting from HIV-1 or HIV-2 transmission, the ensuing infection is typically subdivided into three phases: an acute period, associated with the dissemination of the virus and establishment of the infection, followed by a chronic phase that ultimately results in the collapse of the infected individual’s immune system, and the consequent development of acquired immune deficiency syndrome (AIDS) and death (Fig. 1).

There is a great deal of interest in the events that occur in the hours and days following HIV exposure, particularly in the context of the genital mucosa, given the relevance of heterosexual transmission to the pandemic. Resident DC in the tissues have been shown to capture HIV and transport the virus to regional lymph nodes contributing to both the dissemination of the infection and promotion of the initial, HIV-specific T-cell response (Wu and KewalRamani 2006). It is possible that disparities between HIV-1 and HIV-2 infection in terms of dissemination of the virus and establishment of infection could play a role in determining the different rates of subsequent disease progression. Acute HIV-1 infection may be

Role of Dendritic Cells in HIV-2 Pathogenesis,

Table 1 Comparison of disease parameters of HIV-1 and HIV-2 infection

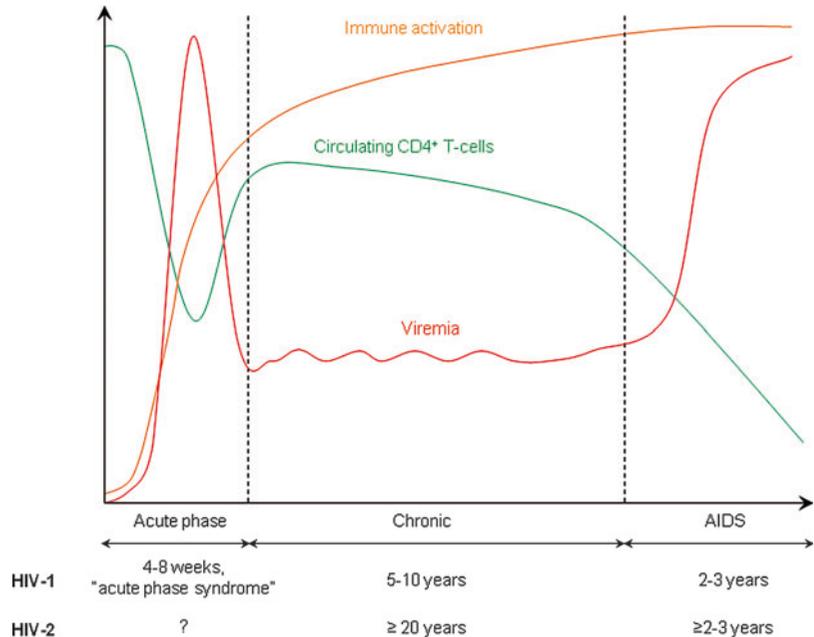
	HIV-1	HIV-2
Rate of CD4+ T-cell decline ^a	−49 cells/μl per year	−9 cells/μl per year
Time to AIDS (years)	≤ 10	≥ 20
Viremia (HIV RNA copies/ml)	10 ⁴ –10 ⁶	Undetectable to 10 ⁴
Transmission	Common	Rare
Geographical distribution	Global	Restricted (West Africa)

^aDrylewicz et al. 2008



Role of Dendritic Cells in HIV-2 Pathogenesis,

Fig. 1 Natural history of HIV-2 and HIV-1 infection



associated with flu-like symptoms. There are no clinical reports of HIV-2-associated "acute-phase syndrome." Thus, it would appear that this initial disease phase is asymptomatic in HIV-2-infected individuals. It is also possible that this apparent lack of an "acute-phase syndrome" is indicative of differences in the establishment of infection and/or the subsequent degree of virus dissemination.

Chronic/asymptomatic HIV infection is characterized by a gradual increase in plasma viral load and concomitant decrease in CD4+ T cells over time. Importantly, despite the lack of major clinical manifestations, this disease stage is characterized by a progressive immune dysregulation. As previously mentioned, the rate of CD4+ T-cell loss is much slower in HIV-2- than HIV-1-infected individuals, typically resulting in this phase lasting more than 20 years in HIV-2+ patients (Drylewicz et al. 2008). Nevertheless, when HIV-1- and HIV-2-infected individuals are matched for the degree of CD4+ T-cell depletion, they feature similar levels of immune activation (► [HIV-2 infection: the role of immune activation in pathogenesis](#)), a well-established hallmark of HIV disease which has been shown to better

correlate with disease progression than plasma viral load (Sousa et al. 2002). Although, a progressive increase in viremia also occurs during the chronic phase in HIV-2-infected individuals, it is typically low throughout the disease. Notably, more than half of the patients reaching the AIDS phase have undetectable plasma viral load (Drylewicz et al. 2008).

The clinical spectrum (► [Natural history and clinical features of HIV-2 infection](#)) of the AIDS stage is similar in both infections. Notably, immunological recovery upon antiretroviral therapy (ART) (► [Immunological response to ART](#)) is usually poorer in HIV-2 than in HIV-1-infected patients (Drylewicz et al. 2008), which may explain some reports of worse outcomes of opportunistic infections during HIV-2 infection. Hence, although HIV-2-infected individuals with low CD4+ T-cell numbers may remain asymptomatic for long periods, when opportunistic infections develop their prognosis is frequently poor (de Silva et al. 2008). Thus, there is no reason to believe, once this final stage of disease has been reached, that DC, like many other cells of the immune system, are not severely impaired in their ability to function normally.

Dendritic Cells: A Brief Overview

Dendritic cells (DC) constitute a large and heterogeneous leukocyte population, of differing origins and functional abilities. They are considered the main orchestrators of the immune system. Briefly, they serve a dual purpose. Firstly, as part of the innate immune system (Innate immunity in HIV-2 infection), they respond directly to pathogens by producing a variety of pro-inflammatory effector molecules, such as cytokines, interferons (IFN), and chemokines, which can, in turn, enhance the function of other innate immune cells, such as natural killer cells (► [Natural killer cells and innate immunity in HIV-2 infection](#)). Secondly, they provide a vital link between innate and adaptive immunity, through their induction of T-cell responses and modulation of their quality. Both of these functions result, in part, from the ability of DC to recognize molecular patterns which are common to a variety of pathogens.

It is possible to distinguish several DC subpopulations, based on a combination of localization, migratory properties, molecular markers, and functional abilities. This chapter will focus on two main subsets found in humans that have been implicated in HIV infection: myeloid (Piguet

and Steinman 2007) and plasmacytoid DC (Fitzgerald-Bocarsly and Jacobs 2010).

Myeloid DC (mDC)

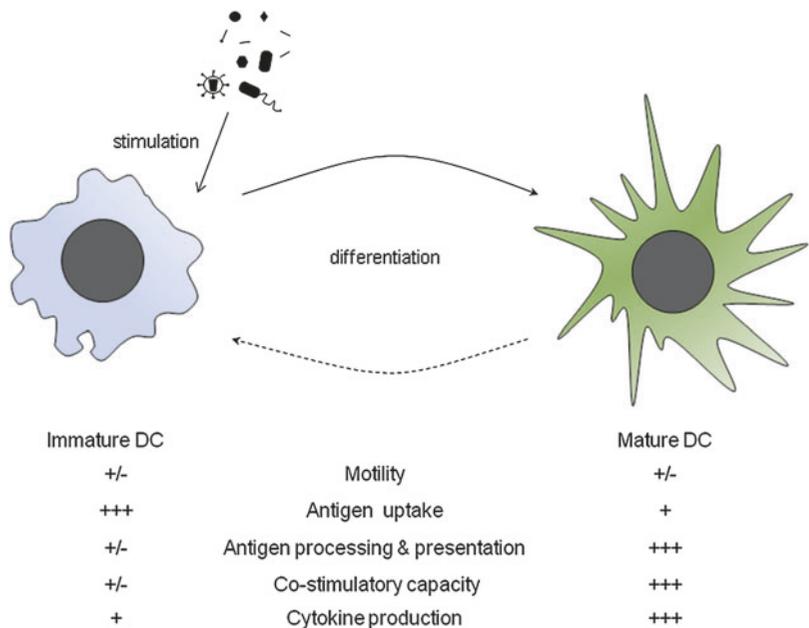
mDC are thought to be mainly produced in the bone marrow but can also result from differentiation of monocytes. A population of mDC can be found in the peripheral blood and has been the main target of investigation in HIV/AIDS (Piguet and Steinman 2007). These cells, also referred to as “conventional DC,” represent “professional” antigen-presenting cells (APC) and are characterized by their ability to recognize foreign pathogens and ingest, process, and subsequently present peptides to cells of the adaptive immune system.

Tissue-resident mDC, located in all body tissues, constitute “peripheral sentinels,” which are able to survey their environment for the presence of foreign molecules. Upon interaction with such molecules, these immature DC become activated and engage a coordinated program of differentiation (Fig. 2).

These functional and phenotypic alterations ultimately allow them to efficiently home, via the afferent lymphatics, to the draining lymph nodes. There they serve as APC, presenting

Role of Dendritic Cells in HIV-2 Pathogenesis,

Fig. 2 Schematic overview of myeloid dendritic cell maturation



antigens sampled in the tissues to T cells circulating through the lymphoid tissues. Within the secondary lymphoid organs, mDC are also thought to contribute to the homeostasis of lymphocyte subsets.

Plasmacytoid DC (pDC)

These cells are thought to be of lymphoid origin, although there may be some degree of developmental plasticity. They are mainly restricted to peripheral blood and lymph nodes but can, nevertheless, be recruited to sites of inflammation. pDC are the major producers of type I interferons (IFN), a family of proteins with multiple antiviral functions. This is considered their primary function in response to pathogen exposure. Nevertheless, they can also serve as APC, although at a lower efficiency than mDC.

Orchestrating DC Activation Through HIV Pattern Recognition

Despite the fairly specialized function of each DC subset, it is important to note that some degree of functional overlap exists between mDC and pDC. Thus, while the former can produce a range of pro-inflammatory mediators, on exposure to pathogens, the latter are able to differentiate into APC, albeit less efficient ones than those differentiated from their mDC counterparts, upon receiving the appropriate signals. Of note, the regulation of the immune function also depends on the flexibility with which each cell responds to distinct stimuli and on the interaction between DC subsets.

DC, like other cells, expresses a specialized, but heterogeneous, group of receptors, referred to as pattern-recognition receptors (PRR). These germ-line encoded receptors are able to recognize structurally repetitive elements associated with a variety of pathogens, the so-called pathogen-associated molecular patterns (PAMP). PAMP are associated with either genomic and/or structural elements of a pathogen, which can be recognized by PRR expressed on the surface and/or in the cytosol of DC.

Surface-expressed PRR include members of the toll-like receptor (TLR) and C-type lectin receptor (CLR) families. The latter consist of membrane-associated receptors able to bind to

carbohydrates/sugars, such as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN). Both virally encoded glycoproteins, gp120 in HIV-1 and gp105 in HIV-2, that extend beyond the surface of the viral envelope (► [The HIV-2 envelope: structure, diversity and evolution](#)) and attach to the viral receptor CD4 and chemokine co-receptors, are able to bind a variety of CLR, particularly DC-SIGN (Reeves and Doms 2002). Thus, the fact that both pDC and mDC express DC-SIGN is of critical importance for their contribution to HIV-2 as well as HIV-1 immunopathogenesis.

Some TLR family members, such as TLR3, TLR7, and TLR9, are located within the endosomal compartment of DC, whereas cytosolic PRR include the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) and retinoic acid-inducible gene-I (RIG-I)-like receptor (RLRs) families. Of note, TLR expression patterns are distinct and complementary in the two aforementioned DC subsets. pDC selectively express TLR7 and 9, which recognize single-strand RNA molecules and un-methylated DNA, respectively (Fitzgerald-Bocarsly and Jacobs 2010). mDC, on the other hand, preferentially express TLR1-6 and TLR8, a characteristic that allows them to recognize distinct stimuli (Piguet and Steinman 2007). Additionally, both subsets express members of the CLR family, as well as a various members of the cytosolic nucleic acid-sensing PRR.

When HIV interacts with a cell, it can engage a variety of PRR. Which particular receptors are involved and the eventual outcome are determined partly by the subcellular localization this interaction occurs in, and by the cell-type involved. Both HIV-1 and HIV-2 genomes consist of ssRNA, which can bind directly to TLR7. This has a particular bearing on determining the virus' interaction with pDC (Fitzgerald-Bocarsly and Jacobs 2010).

It is important to note that neither interactions between HIV-derived PAMP and endosomal TLR nor CLR require viral replication. However, should productive infection of either of these DC subsets occur, then the replication intermediates

and/or end products would themselves be able to activate various cytosolic PRR resulting in the activation and subsequent maturation of these cells.

HIV-2 Infection of mDC and pDC

Several reports indicate that both DC subsets express CD4 (► [CD4, Receptors](#)), the primary receptor utilized by HIV-1 and HIV-2, together with various members of the chemokine receptor family (► [CXCR4, Co-receptors](#) and CCR5) that serve as co-receptors for these viruses, and thus, should be potential targets for infection (Wu and KewalRamani 2006).

In the case of HIV-1, both ex vivo-isolated pDC and mDC have been shown to be able to be infected with lab-adapted viral strains. However, HIV-1 replication is generally markedly less productive in DC as compared to that in CD4+ T cells (Wu and KewalRamani 2006).

There is only very limited data regarding the ability of HIV-2 to infect and replicate in mDC and pDC. Peripheral blood mDC and pDC have been shown to be less susceptible to ex vivo infection with HIV-2 than HIV-1, independently of whether lab-adapted strains or viruses isolated from HIV-2 infected individuals were used (Duvall et al. 2007). Others have shown that HIV-2 could infect, replicate, and subsequently produce virions in monocyte-derived DC in vitro, the commonly used in vitro model of mDC, although with kinetics distinct from HIV-1 (Marchant et al. 2006).

Does Vpx Make a Difference?

A large amount of effort has been made to understand the reduced ability of HIV-1 to replicate in pDC, mDC, and other myeloid cells such as monocytes and macrophages (► [Macrophages in HIV immunopathogenesis](#)). It has been recently proposed that the restriction of HIV-1 replication in these cells is due to SAM- and HD-domain-containing protein 1 (SAMHD1) (Laguette et al. 2011). Intriguingly, this molecule is targeted by Vpx, a protein only encoded by HIV-2 and a

subset of simian immunodeficiency viruses (SIV), but not HIV-1 (Reeves and Doms 2002).

Thus, HIV-2 should be able to circumvent the SAMHD1-imposed restriction to viral replication (► [Interactions between HIV-2 and host restriction factors](#)) in DC via the action of Vpx. However, it is possible that other HIV-2 accessory proteins are unable to fully antagonize additional pathways that are likely activated upon viral infection and limit HIV-2 replication in DC, resulting in the failure to produce infectious virus. This does not rule out the possibility that HIV-2 remains able to establish a pool of latently infected DC.

On the other hand, it has been suggested that the putative Vpx-mediated increase in permissiveness of myeloid cells to HIV-2 infection may result in an augmented DC processing and presentation of HIV-2 antigens and, in this way, promote the induction of HIV-2-specific T-cell responses.

mDC in HIV-2 Infection: Contribution to Viral Dissemination and Promotion of Specific T-Cell Responses

The ability of HIV to be taken up by mDC allows the virus to exploit their migratory potential and, thus, use them as “Trojan horses,” allowing delivery of the virus to the draining lymph nodes (Wu and KewalRamani 2006). Once there, it can be transmitted to CD4+ T cells via a process called “trans-infection,” consequently establishing the lymph node as the main site of viral production.

Initial in vivo and in vitro studies suggested that intraepithelial and submucosal mDC were among the first cellular targets of HIV/SIV after intravaginal infection. Although, more recently, CD4+ T cells have been suggested to be the initial HIV targets, mDC are still thought to play an important role in the amplification of viral replication and subsequent viral dissemination to lymphoid tissues. Thus, it is generally accepted that mDC play a crucial role in sexual HIV transmission (► [HIV-2 transmission](#)). It is likely that these mechanisms also underlie HIV-2 infection, although there are no studies formally addressing this.

HIV capture by mDC may be mediated by several receptors, of which DC-SIGN is the best studied. This molecule has been shown to be equally capable of interacting with HIV-2 and HIV-1, suggesting that both viruses are similarly disseminated by and/or transferred to susceptible T cells (Reeves and Doms 2002). In agreement, both viruses preferentially infect HIV-specific CD4⁺ T cells, a subset known to be particularly vulnerable to DC-mediated trans-infection, despite mDC's limited ability to transfer HIV-2 to autologous CD4⁺ T cells (Duvall et al. 2007). Moreover, the total number of infected T cells has been shown to be similar in HIV-2- and HIV-1-infected patients, in spite of the low-to-undetectable HIV-2 viremia, suggesting that cell-to-cell transmission may play a significant role in HIV-2 infection. This process occurs when a CD4⁺ T cell encounters an HIV-loaded mDC. The virus is recruited to the site of cell interaction, together with CD4 on the T cell, and this strong cell adhesion facilitates HIV infection. This "infectious synapse" may be particularly critical in the establishment of HIV-2 reservoirs.

HIV-1 infection is associated with quantitative and qualitative alterations in mDC, which may contribute to the overall dysregulation of the immune system. A progressive decline in the number of peripheral blood mDC and increased differentiation/maturation are characteristic of HIV-1 infection and are linked to the generalized state of chronic immune activation (Piguet and Steinman 2007).

The limited data available suggest that mDC disturbances during HIV-2 infection mimic those observed in the context of HIV-1 disease. mDC depletion and increased activation during HIV-1 disease seem to be intimately related to the degree of CD4⁺ T-cell depletion and, more importantly, the increased levels of immune activation. As this parameter is known to be comparable in HIV-2+ and HIV-1+ individuals with the same degree of CD4⁺ T-cell depletion (Sousa et al. 2002), it is plausible that similar mDC disturbances are observed during HIV-2 infection, even though the kinetics involved may be much slower.

Of note, in contrast to what has been reported for HIV-1, HIV-2 envelope proteins per se do not

appear to enhance DC differentiation and maturation (Cavaleiro et al. 2009a). This may help restrain immune activation (► [Chronic immune activation](#)) in the context of HIV-2 infection and to a better maintenance of mDC function.

pDC in HIV-2 Infection: Low IFN- α Production In Vivo

pDC are depleted in the peripheral blood of HIV-2 infected patients to levels similar to those observed in HIV-1+ individuals (Cavaleiro et al. 2009b). This depletion occurs throughout infection and is likely associated with pDC homing to secondary lymph nodes, although impairments in their production and/or destruction by the virus are yet to be addressed. Moreover, the differentiation state of circulating pDC is remarkably similar in HIV-2 and HIV-1 infected individuals despite the differences in viremia. Importantly, a clear up-regulation of the inhibitory molecule, programmed death ligand 1 (PD-L1), also occurs on pDC in HIV-2 infection, which may play a role in restraining the rate of increase of immune activation. Activated pDC that have migrated to lymphoid tissues are known to be key modulators of immune responses. One of the implicated mechanisms involves tryptophan catabolism via induction of indoleamine 2, 3-dioxygenase (IDO). This has been linked with the generation of CD4⁺ regulatory T cells and, in this way, the suppression of immune responses. Notably, HIV-2+ patients feature a relative expansion of regulatory T cells (► [Regulatory T cells](#)) in the peripheral blood during disease progression similar to that found in HIV-1 (Foxall et al. 2011).

Importantly, IFN- α production, the hallmark of pDC function, is significantly different in HIV-2 and HIV-1 infection despite the aforementioned similarities regarding pDC depletion and differentiation state. It is known that the contribution of pDC to HIV-1 immunopathogenesis stems from their status as "natural interferon-producing cells" (Fitzgerald-Bocarsly and Jacobs 2010). This capacity results from two features that are unique to these cells: the presence of TLR7 and TLR9 in the endosomal compartment and the constitutive

expression of a key factor in the activation of the interferon (IFN) response, interferon response factor 7 (IRF7). Hence, upon endosomal TLR stimulation, pDC are able to quickly produce large amounts of type I IFN, inducing an antiviral program that impacts upon HIV replication. IFN are known to induce the expression of a range of genes, collectively termed interferon-stimulated genes (ISG), such as *MxA*, that target various phases of the HIV life-cycle.

HIV-1 infection is associated with chronic IFN- α production that likely contributes to immune activation. HIV-2-infected patients, in contrast with HIV-1-infected patients, feature no increase in the expression levels of *MxA*, a known interferon-inducible gene, providing evidence of a lower in vivo activation of pDC in the context of HIV-2 infection (Cavaleiro et al. 2009b). The lower levels of in vivo IFN- α production in HIV-2 infection may be beneficial, contributing to the slower rate of progression.

Why HIV-2 would be less able to induce an interferon response in pDC than HIV-1 remains unknown. Both viruses have ssRNA able to stimulate TLR7, and both can enter the endosomal pathway of these cells, via CLR- or CD4-mediated endocytosis. An intriguing possibility may be related to differences in viral accessory proteins. HIV-1 encodes Vpu that is known to target the ISG product tetherin, whereas HIV-2, lacking this gene, relies instead on Env to perform this function (Evans et al. 2010). HIV-1 Vpu and HIV-2 Env differ in their ability to target tetherin, which serves the dual function of inhibiting the release of enveloped viruses from the cell surface of infected cells and regulating the IFN-pathway via a negative-feedback mechanism. Thus, it is possible that the limited ability of HIV-2 Env to target tetherin may result in a preserved capacity to down-modulate the IFN-pathway and ultimately lead to its better preservation.

Chronic in vivo stimulation of pDC has been suggested to result in the induction of a refractory state, where these cells' ability to produce IFN- α upon in vitro stimulation with the appropriate ligands is severely impaired. In agreement with reduced in vivo IFN activity, pDC from HIV-2+

patients have a relatively preserved ability to produce IFN- α ex vivo. Nevertheless, this ability is impaired in patients with detectable viremia (Cavaleiro et al. 2009b). These data support the idea that viremia, even at the low levels observed in HIV-2-infected patients, has a major impact on pDC.

Conclusion

DC play a central role in HIV immunopathogenesis and could potentially contribute to the slower course of HIV-2 infection. The HIV-2 accessory protein, Vpx, has been shown to counteract an intrinsic host-factor receptor, SAMHD1, thus, possibly, facilitating DC infection and presentation of HIV-2 antigens. Despite the major imbalances in circulating pDC that were found in HIV-2+-infected patients, there is evidence of lack of significant increase in IFN- α production in chronic HIV-2 infection, contrasting with HIV-1 infection. Given the major role of IFN- α in the activation of several components of the immune system, it is possible that limiting its in vivo production may help slow the stepwise increase of immune hyperactivation that is linked to AIDS progression.

Overall, despite the potential importance of HIV-2 to the understanding of HIV immunopathogenesis, many areas of the immune response to this infection remain to be explored in detail. The understanding of the role of DC in this naturally occurring model of attenuated HIV infection may significantly aid in the identification of key pathways of the immune response to HIV that could, in turn, lead to the identification of novel therapeutic targets or help advance areas of existing research such as vaccine development.

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Role of Histone Deacetylases 1 and Yin Yang 1 Protein in Proviral Latency

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Definition

HDAC1 is a member of the HDAC family of proteins, whereas YY1 is a ubiquitously expressed transcriptional factor with both repressive and activating functions. Cooperatively, these two proteins have been shown to repress transcription from the viral LTR promoter and have been proposed to contribute to the state of post-integration latency.

Introduction

After the integration into the host cell chromatin, the HIV-1 genome can be reversibly silenced through multiple mechanisms, establishing a state of post-integration latency. Although different posttranscriptional mechanisms participate in maintaining the latent state, it is usually a block at the transcriptional level that gives a major contribution to the establishment of latency (Lucic and Giacca 2014).

The establishment of latency, according to a now prevailing theory, foresees the infection of activated CD4⁺ T cells prior to their natural reversion to a quiescent memory state; this is also compatible with the finding that latent HIV-1 resides predominantly in the memory subset of resting CD4⁺ T cells (Siliciano and Greene 2011). During the reversion from an activated into a quiescent state, the availability of certain cellular factors becomes limited, and they can no longer bind to the viral long terminal repeat (LTR) promoter, while at the same time, transcriptional repressors present in the cell may have a

facilitated access to the viral LTR promoter (Lusic and Giacca 2014; Siliciano and Greene 2011).

Control of HIV-1 Gene Expression

The regulation of HIV-1 transcription is a complex event of significant pathological relevance, which recapitulates general concepts of cellular transcription with some peculiarities. In CD4⁺ T cells, a rapid and effective replication of the HIV-1 genome occurs through a concerted action of cellular transcriptional activators, which bind the 5' LTR of the viral genome and the viral Tat protein, which binds the trans-activation-responsive region (TAR) mRNA sequence. HIV-1 expression is initiated from the 5' LTR region, at the single transcription start site (TSS). The HIV-1 promoter and adjacent regulatory elements in the U3 region of the LTR are both involved in the recruitment of RNA polymerase 2 (RNA Pol 2). The TAR sequence, found within the R region in the 5' end of all viral transcripts, contains important regulatory motifs, and so does the U5 region (Ott et al. 2011). Basal transcription from the viral promoter is controlled by the Sp1 protein, whose binding sites are located upstream of the TATA element in the "core" promoter. Other transcription factors (TFs), mostly inducible by cell activation with cytokines or similar stimuli, bind the enhancer region that lies upstream of the core promoter. Activator protein-1 (AP-1), nuclear factor of activated T cells (NFAT), and cytokine-inducible nuclear factor κ B (NF- κ B) are among the best-characterized TFs that regulate HIV-1 transcription. However, multiple TFs are associated with repression of viral transcription, such as Yin Yang 1 (YY1), the p50 subunit of NF- κ B, chicken ovalbumin upstream promoter transcription factor (COUP-TF)-interacting protein-2 (CTIP2), and specificity protein 1 (Sp1).

A Role of Chromatin in Transcriptional Silencing of the Provirus

When analyzed *in vitro*, as naked DNA, HIV-1 5' LTR exhibits very strong promoter activity, but as

any other eukaryotic promoter, it is embedded into chromatin that imposes a significant level of control of gene expression (Colin and Van Lint 2009). Nuclease digestion of intact nuclei of infected cells under basal conditions showed the presence of at least three precisely positioned nucleosomes, called nuc-0, nuc-1, and nuc-2 and their intervening nucleosome-free regions (Verdin et al. 1993). As with other promoters, both activation and silencing of the HIV 5' LTR are governed by the acetylation status of histones in the nucleosomes. In a simplified model, chromatin is accessible for transcription factors when histones positioned at the gene promoters are acetylated; conversely, chromatin compaction is achieved by histone deacetylases (HDACs) through deacetylation of specific lysines within the N-terminal tails of histones. This promotes high-affinity binding between the histones and DNA backbone and reduces the accessibility of underlying DNA to transcriptional activators. Nucleosome remodeling (especially of nuc-1) is an early event in the reactivation of latent HIV-1, and histone acetylation induced by treatment with an HDAC inhibitor might lead to activation of latent HIV-1 (Shirakawa et al. 2013).

Histone Deacetylases (HDACs)

Based on their homology to the yeast prototype deacetylases, HDACs are grouped into four classes: class I and IV show homology to reduced potassium dependency 3 (RPD3) and class II to HDAC1, while class III HDACs have homology to the Sir2 yeast deacetylase (Shirakawa et al. 2013).

Class I HDACs include HDAC1, HDAC2, HDAC3, and HDAC8 and represent a subset of mostly nuclear and ubiquitous enzymes. Different members of this family have been shown to be recruited to the HIV-1 promoter and to participate in transcriptional silencing of the provirus. HDAC1 has initially been brought in connection with the proviral latency when its recruitment to the 5' LTR promoter by Yin Yang 1/late SV40 factor (LSF) complex was described (Coull et al. 2000). Multiple cellular factors that associate with the viral promoter during proviral latency

also recruit HDAC1 to the viral promoter. HDAC1 partners identified during HIV-1 LTR transcriptional silencing include the NF- κ B p50 homodimer, CTIP2, C-promoter-binding factor-1 (CBF-1), Sp1, and c-Myc (Shirakawa et al. 2013). HDAC1 comprises of the N-terminally positioned catalytic domain and the HDAC association domain (HAD) important for the homodimerization, while the C-terminal part encodes for the nuclear localization domain (NLS). Class I HDACs have generally been considered transcriptional silencers; however, several recent studies challenged this view by showing that histone deacetylases play a role in the activation of certain genes and that class I HDACs have preferences for active genes (Moser et al. 2014). In support of their complex regulatory functions is the fact that these enzymes also acetylate nonhistone proteins and are hence implicated in different aspects of cellular metabolism aside from transcription. In fact, HDAC1 has also been shown to be involved in integration, where in complex with KRAB-associated protein-1 (KAP-1) HDAC1 deacetylates HIV integrase (IN) and restricts integration (Allouch et al. 2011). Despite multiple studies and a number of different interactors, a specific transcription factor crucial for HDAC1 recruitment to the HIV-1 promoter during silencing has not been revealed to date.

Yin Yang 1

Yin Yang 1 protein is a fascinating example of the inherent complexity of the regulation of gene expression in eukaryotes. As one of the most intriguing players in gene regulation, YY1 has found its part in a plethora of cellular processes, such as regulation of B-cell development, malignant transformations, apoptosis, gene dosage, and viral latency. The first reports about the factor, today widely known as YY1, came 30 years ago when specific binding of the YY1 protein in the intronic enhancer of the immunoglobulin heavy-chain (IgH) locus was reported. YY1 was cloned and characterized as a zinc finger protein initially known as NF-E1 that binds to a DNA sequence within the negative-acting segment during B-cell

development. Soon after, the protein was shown to cause not only transcriptional repression but was demonstrated to act as a transcriptional activator as well. Based on its dual property to repress and activate transcription depending on the context of *cis* elements and interacting proteins, this transcription factor was named YY1 for “yin and yang 1.” Further studies supported both positive and negative roles of this transcription factor. The complex role that YY1 has inside the cell is also demonstrated by the finding that it is crucial for embryonic development: homozygous mutation of the *yy1* gene results in peri-implantation lethality. YY1 is implicated in a wide number of processes such as in cardiac and skeletal lineage development and in the cell growth control, as well as in disease pathways such as dystrophic muscle disease (see the recent review from Atchison (2014)).

The YY1 gene is highly conserved between different species, showing a remarkable similarity (greater than 98%) to its mouse homologue NF- δ . After several attempts to position human YY1 gene to its chromosomal location, YY1 was finally mapped to the telomere region of chromosome 14 by fluorescence in situ hybridization (FISH). YY1 gene consists of five highly conserved exons and is alternatively spliced into eight different isoforms whose functions still remain elusive. Although an estimated molecular weight of the protein, based on 414 amino acids, is 44 kDa, YY1 migrates at a molecular weight of 68 kDa in SDS–polyacrylamide gels which is probably due to its protein structure. The hallmark of Yin Yang 1 protein is four zinc finger motifs located in its carboxyl terminus (amino acids 298–414) which are responsible for the recognition of a consensus DNA sequence. Of interest, in addition to its DNA binding activity, YY1 also binds Xist RNA through different sequence motifs during X chromosome inactivation process. Functionally, part of the C-terminally located zinc fingers (amino acids 333–397) and a more centrally positioned region (amino acids 170–200) harbor transcriptional repression activity. In contrast, residues involved in transcriptional activation are positioned closer to the amino terminus (Atchison 2014). A region

defined between residues 201 and 226 forms the recruitment of polycomb (REPO) domain which is implicated in polycomb group repression. More recently, a higher oligomer form of YY1 was purified, suggested to facilitate sequence independent association with DNA and protein–protein interactions during homologous recombination-mediated DNA repair (Atchison 2014).

A Role for HDAC1:YY1 in Proviral Latency

Repression of HIV-1 transcription has been ascribed to multiple TFs that directly bind the viral LTR promoter. Almost all of these factors, including Yin Yang 1, the p50 subunit of NF- κ B, COUP-TF-interacting protein-2, CBF1, Sp1, and c-Myc, recruit HDACs to the viral promoter to attenuate viral transcription (Shirakawa et al. 2013). Initial studies identified YY1 as a cellular partner that binds the AAV P5 promoter, contributing to the silent state of this promoter. Since then, YY1 was identified as a critical host cell transcription factor for numerous viral promoters. Interestingly, in almost all cases it was connected to the phenomenon of viral latency (Shi et al. 1997), proposing that this TF might be a general regulator of the latent phenotype in different viruses. One such example shows that YY1 represses the BZLF1 gene, the gene product of which is necessary for the latent Epstein-Barr virus (EBV) to enter the lytic state in B cells. YY1 also represses HIV-1 transcription, and in fact, it was one of the first host cell transcription factors demonstrated to be involved in repression of the HIV-1 LTR. Studies in HeLa cells by David Margolis and his group showed that YY1 binds to the position -16 to $+27$ of the HIV-1 LTR ($+1$ marks the start site of transcription) and that over-expression of YY1 inhibits viral transcription and virion production (Margolis et al. 1994). By using nuclear extracts from the physiologically more relevant CEM cells (a T-cell line), they showed later that YY1 acts together with the transcription factor late simian virus 40 (LSF)/leader binding protein-1 (LBP-1) to form a transcriptionally repressive complex (Romerio et al. 1997). This YY1 and LSF

complex copurified with HDAC1, while deletion of a glycine/alanine-rich domain of YY1 responsible for YY1 and HDAC interaction diminished the ability of YY1 to silence the HIV-1 LTR. A similar effect on the LTR was observed after treatment with deacetylase inhibitor trichostatin A, reinforcing the role of HDAC1 in transcriptional silencing of HIV-1 (Coull et al. 2000).

In addition to LSF-mediated interaction near the core promoter, YY1 was recently shown to bind an upstream binding site called RBEIII (-120 to -140) in a highly conserved RBF-2 (USF1/2-TFII-I) region of the LTR enhancer in Jurkat cells. The association of YY1 with this upstream sequence in the LTR promoter was confirmed by ChIP experiments in unstimulated Jurkat cells but unfortunately the contribution of this site to YY1-mediated repression of the LTR promoter remained unclear (Bernhard et al. 2013).

More recent work of Margolis and colleagues showed that upon YY1 and c-Myc depletion in Jurkat 2D10 and J89 cell clones, HDAC1, HDAC2, and HDAC3 occupancy of the LTR remains unchanged (Barton and Margolis 2013). Surprisingly, levels of viral mRNA increased upon YY1 removal, but remained the same upon c-Myc silencing contradictory to the previous reports. Moreover, expression of latent HIV-1 drastically increased in combination with an HDAC inhibitor vorinostat (or SAHA) treatment, with higher transcriptional induction than upon HDAC inhibition alone (Barton and Margolis 2013). These findings underline the existence of multiple repressor mechanisms involved in transcriptional silencing of the provirus. Indeed, HDACs were shown to participate in different repressive complexes at the viral promoter which in turn require selective targeting to effectively activate quiescent proviruses from latency (Shirakawa et al. 2013).

Conclusions and Future Directions

Association between YY1 and HDAC1 represents the basis of the first epigenetic mechanism found to repress the LTR promoter during HIV-1 latency. The highly heterogenic interactome of YY1,

together with a number of reports that suggest a more global role in long-distance interactions across the eukaryotic genome, is putting this factor in the limelight of HIV-1 latency regulation. In this context, one could envisage a mechanism according to which YY1 contributes to the latent phenotype by establishing long-range interactions between the LTR and the chromosomal regions either on the same or on different chromosomes. In fact, such mechanism was previously reported to take part in proviral silencing in at least one fraction of a latent Jurkat cellular clone J-Lat A1 (Dieudonne et al. 2009). In support of this hypothesis, the requirement for YY1 in the long-distance DNA interactions and loop formation was proposed based on several lines of evidence. In particular, YY1 interaction with PcG proteins and its recruitment to DNA as well as interactions with cohesin, condensin, and lamin proteins could all contribute to the formation of long-distance contacts that lead to contraction and silencing of the provirus (Atchison 2014, #51). HDAC1 could also participate in such interactions, possibly together with other chromatin modifiers and scaffolding proteins.

Despite the described mechanisms, the role of YY1 in the establishment and maintenance of latency in primary CD4⁺ cells still remains to be determined (Barton et al. 2013, Bernhard et al. 2013, Coull et al. 2000).

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Role of LEDGF/p75 in Cell Biology and Disease Pathogenesis

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Definition

LEDGF/p75 is a chromatin-associated cellular protein with roles in embryonic development, transcriptional regulation, and cell survival that

has been implicated in autoimmunity, carcinogenesis, and HIV replication. A key feature is its ability to act as a molecular adapter to tether proteins to the chromatin. Recent studies have uncovered its role in leukemia, cell survival, and DNA repair. Biophysical characterization of its dynamic interaction with chromatin sheds light on how a single protein can exert so many functions. The function of LEDGF/p75 in HIV replication has been further elaborated, and the insight in the interaction between LEDGF/p75 and IN has resulted in a new class of inhibitors (LEDGINs) blocking HIV replication.

Introduction

LEDGF/p75 has been identified on four different occasions in association with transcriptional regulation, cell survival, autoimmunity, or virology (Ge et al. 1998; Singh et al. 2000; Ochs et al. 2000; Cherepanov et al. 2003). LEDGF/p75, together with LEDGF/p52, co-purified with the general transcriptional coactivator positive cofactor 4 (PC4) (Ge et al. 1998) and was termed p75, due to its apparent mass of 75 kDa. Since both proteins enhanced activity of the general transcription machinery, they were hence referred to as transcriptional coactivators p75 and p52. Later, a cDNA clone coding for a protein identical to p75 was isolated from a lens epithelium cell (LEC) library (Singh et al. 2000), and the protein was coined lens epithelium-derived growth factor or LEDGF/p75. In 2000, screening of a cDNA library with human serum reactive against the nuclear autoantigen dense fine speckled protein of 70 kDa (DFS70) revealed DFS70 to be identical to LEDGF/p75 (Ochs et al. 2000). Finally, LEDGF/p75 was reported as a binding partner of HIV-1 IN by isolating complexes of ectopically expressed IN from the nuclei of HEK293T cells (Cherepanov et al. 2003).

LEDGF/p75 is a ubiquitously expressed nuclear protein that associates with mitotic chromatin. The protein interacts with various nuclear proteins and is generally regarded as a molecular adapter. It binds chromatin with its N-terminal

chromatin/DNA-interacting domains and interacts with proteins via its C-terminal region, thereby tethering these interacting proteins to the chromatin.

The *PSIP1* Gene and the Genomic Organization

The 530 amino acid (aa) protein LEDGF/p75 is encoded by *PSIP1* (PC4 and SFRS1 interacting protein), a gene located at chromosome 9p22.2 which also gives rise to a smaller splice variant encoding the 333 aa protein LEDGF/p52. LEDGF/p52 shares a region of 325 residues with LEDGF/p75 at the N-terminus but contains only 8 additional C-terminal amino acids.

LEDGF/p75 is a member of the hepatoma-derived growth factor (HDGF)-related protein (HRP) family. HRPs are characterized by a conserved N-terminal region, also known as HATH (homologous to amino terminus of hepatoma-derived growth factor) or PWWP domain. This 90- to 135-amino acid domain is found in a variety of nuclear proteins. Seven human HRP family members have been described: HDGF, HRP-1, HRP-2, HRP-3, HRP-4, LEDGF/p75, and LEDGF/p52. Recently, the transcriptional control of *PSIP1* itself has been scrutinized. Expression is probably initiated from different transcription start sites (TSS) in different cell types, an important source of regulatory diversity, and regulated mainly by the transcriptional protein specificity protein 1 (Sp1) next to other regulatory proteins such as TGF-beta1. A region within the promoter has been suggested to regulate the ratio of LEDGF/p75 over LEDGF/p52 expression. Sp1 was shown to bind to multiple (5) sites in the promoter region of LEDGF/p75 and to differentially regulate expression. Also a TGF-beta1-inhibitory-element-like sequence and other regulatory element responsive sequences were proposed. TGF-beta1 has been shown to decrease the LEDGF/p75 promoter activity. Oxidative stress, hyperthermia, TNF- α , heat shock protein (Hsp) 70-2, and gonadotropins have all been reported to induce LEDGF/p75 expression.

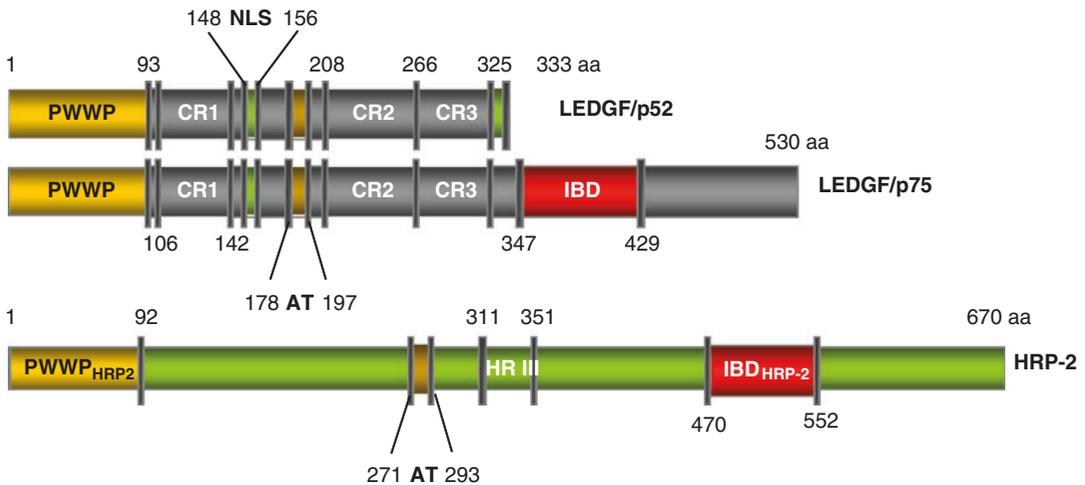
Structure and Distribution of LEDGF/p75

Domains in LEDGF/p75

LEDGF/p75 contains the following functional domains: (1) a PWWP domain (aa 1–93) in the N-terminal part of LEDGF/p75 that functions as a chromatin interaction domain; (2) a functional nuclear localization signal (NLS) [23]; (3) two AT-hook-like motifs; (4) four charged regions (CR1–4) of which recently CR2–4 were described to contain a supercoiled DNA recognition domain (SRD, aa 200–336); (5) a C-terminal integrase-binding domain (IBD) of approximately 80 amino acids (aa 347–429) that is responsible for the interaction with HIV integrase (IN) but is absent in the LEDGF/p52 splice variant (Fig. 1).

The N-terminal PWWP domain, named for a conserved Pro-Trp-Trp-Pro motif, is present in all HRP family members. PWWP domains are members of the Tudor domain “royal family” consisting of Tudor, chromodomain, malignant brain tumor, and PWWP domains. The PWWP domains are currently regarded as chromatin-interacting domains capable of binding methyl-lysine residues on histones and DNA, probably without sequence specificity. They are present in diverse chromatin-associated proteins involved in DNA repair, histone modification, transcriptional

regulation, and DNA methylation. NMR structures and more recently crystal structures of related PWWP domains demonstrate the presence of a five-stranded antiparallel beta-barrel core responsible for the interaction between methyl-lysine residues on histones and two C-terminally located alpha-helices. An insertional third motif between the second and third beta-strand is not present in the PWWP domain of HRPs. The PWWP domain of LEDGF/p75 (aa 1–93) alone interacts with chromatin, not with naked DNA. Detailed mapping of the chromatin-binding profile of LEDGF/p75 using DamID technology in the highly annotated ENCODE region revealed an association with histone modification marks associated with active transcription such as H3 or H4 acetylation, H3K4me1, RNA polymerase II and a disfavoring of promoter regions, and H3K27me3, a determinant of heterochromatin. Recent work indicated that H3K36me3, H3K4me1, and to a lesser extent H3K36me2 and H3K36me1, associated with active chromatin, efficiently co-immunoprecipitated with LEDGF/p75, while H3K9me3 and H3K27me3, markers of inactive chromatin, did not (Daugaard et al. 2012). Pradeepa et al. also suggested a direct interaction of the PWWP domain with H3K36me3, although this was only



Role of LEDGF/p75 in Cell Biology and Disease Pathogenesis, Fig. 1 Structure of LEDGF/p52, LEDGF/p75, and HRP-2. Cartoon representation of LEDGF/p52, LEDGF/p75, and HRP-2: PWWP domain (*PWWP*),

charged regions 1–3 (*CR1–3*), nuclear localization signal (*NLS*), AT-hook-like sequence (*AT*), homology region III (*HR III*), and integrase-binding domain (*IBD*) are indicated

demonstrated for the PWWP domain of LEDGF/p52 and not for the identical domain in LEDGF/p75 (Pradeepa et al. 2012). The PWWP domain in LEDGF/p75 is also important for its nuclear distribution. A PWWP-truncated protein (Δ N93-LEDGF/p75) lost its interaction with condensed mitotic chromatin and was no longer excluded from nucleoli, contrary to LEDGF/p75. Since LEDGF/p75 has been demonstrated to tether and target HIV integration, the integration pattern itself provides valuable clues of the interaction of LEDGF/p75 with the chromatin. HIV-1 integration favors genomic regions enriched with histone modifications associated with active transcription (such as H3K4me1, H3K9me1, H3K36me3). Notably, when the PWWP domain of LEDGF/p75 was swapped with other PWWP domains from HRPs and the HIV integration pattern near histone marks was compared, the PWWP of HDGF increased integration near H3K36me3, while the PWWP of HRP-2 increased the likelihood of integrating near other marks associated with active transcription (i.e., H2BK5me1, H3K4me3, H3K9me1, H3K27me1, H3K36me3). However, not all LEDGF/p75 chromatin-binding sites correlated with the HIV-1 integration pattern. Next to the PWWP domain, a tripartite motif consisting of a functional NLS, GRKRKA EKQ (aa 148–156), and two AT-hook-like domains (aa 178–198) capable of binding DNA *in vitro* can be distinguished. NLS deletion leads to cytoplasmic localization of newly synthesized LEDGF/p75. In dividing cells, however, LEDGF/p75 is trapped to the mitotic chromatin, rendering the NLS dispensable for its nuclear localization. In line, mutations of the NLS region reduced but did not abolish the interaction of LEDGF/p75 with naked DNA.

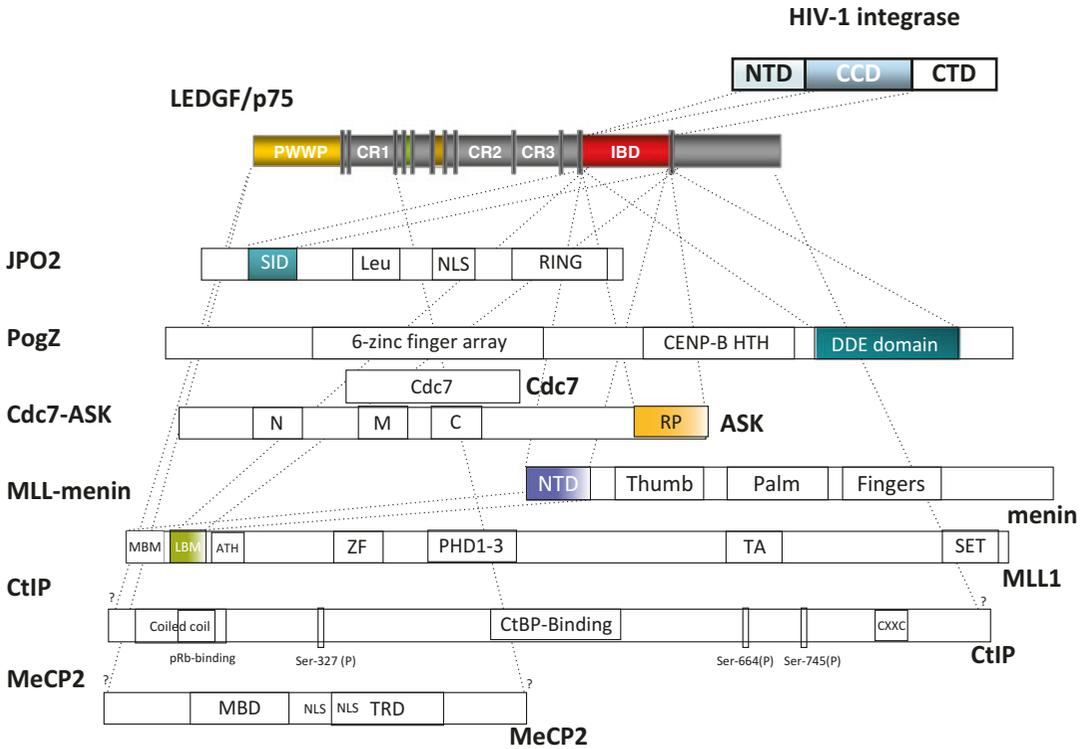
Four relatively charged regions (CR1–4) are interspersed between the different domains; CR2 and CR3/4 to a lesser extent were recently shown to bind supercoiled DNA. This so-called supercoiled DNA recognition domain (SRD) in LEDGF/p75 (aa 200–336) was also capable of binding to relaxed DNA, consistent with observations that LEDGF/p75_{226–471} has minor DNA-binding properties and LEDGF/p75_{326–471}, containing the IBD, has none.

The C-terminus of LEDGF/p75 contains the IBD (aa 347–429), capable of interacting with HIV integrase (IN). The IBD is formed by a compact right-handed bundle composed of five α -helices [based on a NMR solution structure of the IBD and a co-crystal structure of the IBD with the catalytic core domain (CCD) of IN (Cherepanov et al. 2005)] that is topologically similar to a pair of HEAT repeats. Binding IN is obviously not the natural function of this domain as it has been shown to bind other cellular partner proteins as well: c-Myc-interacting protein JPO2, mixed-lineage leukemia (MLL)/menin complex (Yokoyama and Cleary 2008), a domesticated transposase PogZ (pogo transposable element-derived protein with zinc finger), and Cdc7 activator of S-phase kinase (Cdc7/Ask). Thus, the IBD probably functions as an adapter, associating divergent factors and enabling them to be tethered to chromatin through interactions with the N-terminus of LEDGF/p75 (Fig. 2).

After translation, LEDGF/p75 can be a substrate of posttranslational modifications such as phosphorylation and SUMOylation by SUMO1 (small ubiquitin-like modifier 1). LEDGF/p75 phosphorylated at Ser206 by Cdc7/Ask was recovered during the S-phase. A fraction of endogenous LEDGF/p75 is SUMOylated, and four SUMOylation sites, Lys-75, Lys-250, Lys-254, and Lys-364, were identified, of which only Lys-364 was located in a conserved consensus motif. SUMOylation does not affect the cellular localization of LEDGF/p75 but negatively regulates its half-life and transcriptional activity. Mutating Lys-364 to arginine abolished SUMOylation and increased the capacity of LEDGF/p75 to bind naked DNA in EMSA.

Cellular Localization of LEDGF/p75

LEDGF/p75 is a nuclear protein that binds to chromatin during mitosis. Initial studies suggested that GFP-LEDGF/p75 was secreted into the culture medium of lens epithelial cells and that temperature influenced the location of LEDGF/p75 in these cells, with LEDGF/p75 in the cytoplasm at 4 °C to 28 °C and in the nucleus at 37 °C. Next, exogenous GSP-LEDGF/p75 was reported to be taken up by lens epithelial cells



Role of LEDGF/p75 in Cell Biology and Disease Pathogenesis, Fig. 2 LEDGF/p75 interacts with HIV integrase and various cellular proteins. Cartoon representation of the different interaction partners of LEDGF/p75: HIV-1 integrase, JPO2, PogZ, Cdc7/Ask, MLL-menin, CtIP, and MeCP2. Dashed lines indicate the interacting domains. Abbreviations: N-terminal domain (NTD), catalytic core domain (CCD), C-terminal domain (CTD), PWWP domain (PWWP), charged region 1–3 (CR1–3), integrase-binding domain (IBD), specific interaction domain (SID), putative leucine zipper (Leu), nuclear

localization signal (NLS), RING-finger-like zinc-binding motif (RING), helix-turn-helix (HTH), N-motif (N), M-motif (M), and C-motif (C), C-terminal regulatory peptide (RP), menin-binding motif (MBM), LEDGF-binding motif (LBM), ZF_CXXC motif (ZF), PHD fingers (PHD1–3), SET domain (SET), retinoblastoma protein-binding motif (pRb-binding), C-terminal-binding protein binding motif (CtBP-binding), CXXC motif (CXXC), methyl-CpG-binding domain (MBD), transcriptional repression domain (TRD)

where it is transported through the cytoplasm into the nucleus. These observations could not be reproduced independently. Recently, in a series of biophysical experiments in cell culture, Hendrix et al. proposed a chromatin scan-and-lock model for LEDGF/p75 in the nucleus (Hendrix et al. 2011). LEDGF/p75 is for 98% of the time bound to chromatin and moves in the remaining 2% to other sites on the chromatin. In the chromatin-bound fraction, a slow moving, scanning state, and a smaller more tightly bound or locked state could be distinguished. A comparable phenomenon, a scanning/hopping mechanism, was previously described for

transcription factors. In the scan-and-lock model, the N-terminal PWWP domain plays a pivotal role. Mutating the PWWP domain (R74D) considerably increased the nuclear mobility of LEDGF/p75, suggesting a decreased chromatin interaction. However, it did not completely abrogate the binding of LEDGF/p75 to chromatin as suggested by the remaining slower-moving and presumably chromatin-bound fraction of the mutant LEDGF/p75 compared to freely diffusing eGFP. The PWWP domain in LEDGF/p75 was proposed to constitute a “protein lock,” docking LEDGF/p75 and its potential cargo at specific sites on the chromatin.

Cellular Binding Partners of LEDGF/p75

Several cellular interaction partners of LEDGF/p75 have been identified. JPO2 and PogZ have been identified by Y2H and the interaction with JPO2 was independently confirmed by co-IP. Although, like integrase, they each interact with the IBD domain of LEDGF/p75, the interaction differs considerably. The interaction of JPO2 with the IBD is not inhibited significantly by mutating the Ile-365, Asp-366, and Phe-406 residues in the IBD that abrogate the interaction with IN. JPO2, also known as R1, RAM2, or CDC7L, is a c-Myc interactor and potentiates c-Myc transforming capacity in medulloblastoma cells. JPO2 is a repressor of human monoamine oxidase A and B expression. Contrary to JPO2, PogZ shares more interacting IBD amino acids with IN and mutation of Ile-365 or Phe-406 abrogates its interaction with the IBD. The Asp-366 mutation in the IBD is however exclusively linked to a loss of interaction with lentiviral integrases. PogZ binds heterochromatin protein 1, essential in the formation of heterochromatin and mitotic progression, through its zinc-finger-like motif and destabilizes the heterochromatin protein 1-chromatin interaction enabling normal mitotic progression.

LEDGF/p75 has also been implicated in oncogenesis and acts as a chromosomal tether for the mixed-lineage leukemia (MLL) histone methyltransferase protein complex through menin (Yokoyama and Cleary 2008) via interaction with its IBD. LEDGF/p75 is required for both MLL-dependent transcription and leukemic transformation.

LEDGF/p75 interacts with cell division cycle 7-related protein kinase (Cdc7) activator of S-phase kinase (ASK) through its IBD. Cdc7/ASK phosphorylates LEDGF/p75 *in vitro*, with Ser-206 being the major target, and LEDGF/p75 stimulates the enzymatic activity of Cdc7/ASK *in vitro* by relieving autoinhibition of Cdc7/ASK, imposed by the C-terminus of ASK.

MeCP2, a methylation-associated transcriptional modulator, is a common interaction partner of both LEDGF/p75 and LEDGF/p52. The interaction was demonstrated *in vitro* and in various cancer cell lines using a wide array of techniques (transcription factor protein arrays, pull-down,

AlphaScreen assays, co-IP, nuclear co-localization studies using confocal microscopy) and was mapped to the N-terminal region, particularly the PWWP-CR1 domains, shared by both LEDGF/p75 and LEDGF/p52.

Endogenous LEDGF/p75 co-immunoprecipitated with C-terminal-binding protein interacting protein (CtIP), a component of the homologous recombination repair machinery (Daugaard et al. 2012).

Physiological Roles of LEDGF/p75

LEDGF/p75 as a Transcriptional Regulator Affecting Stress Response

Initial work attributed a role to LEDGF/p75 in transcriptional regulation after its co-purification with the transcriptional coactivator PC4 (Ge et al. 1998). Addition of the protein to the culture medium of LECs stimulated their growth and prolonged cell survival (Singh et al. 2000). LEDGF/p75 was shown to be a stress response protein promoting cell survival in multiple cell lines (i.e., LECs, mouse keratinocytes, monkey kidney cos7 cells, human fibroblasts, retinal pigment epithelial cells, HepG2 cells) in the presence of environmental stress stimuli such as hyperthermia, serum starvation, or UVB radiation. LEDGF/p75 is proposed to activate stress-related genes in response to environmental stress stimuli, and LEDGF/p75 expression itself is upregulated upon oxidative and thermal stress or via TNF- α -induced oxidative stress. Genes known to be transactivated by LEDGF/p75 are Hsp27, peroxiredoxin-6 (also known as antioxidant protein 2), involucrin, alcohol dehydrogenase, aldehyde dehydrogenase, α B-crystallin, gamma-glutamylcysteine synthetase, vascular endothelial growth factor C (VEGF-C), and interleukin 6 (IL-6). In a "human oxidative stress and antioxidant defense" qPCR array in PC-3 cells, only 5 out of 84 genes were differentially regulated by LEDGF/p75 (albumin, cytoglobin, phosphoinositide-binding protein commonly known as IPCEF-1, superoxide dismutase 3, and thyroid peroxidase), and Hsp70-2 depletion resulted in LEDGF/p75 depletion in HeLa cells

but did not differentially alter the expression of the previously reported target genes of LEDGF/p75 (including Hsp27, α B-crystallin, peroxiredoxin-6). Recent studies indicated that LEDGF/p75 binding to chromatin is not necessarily restricted to STRE or HSE in the genome but predominantly associated with active chromatin markers. It remains, however, possible that under conditions of stress, LEDGF/p75 interacts with specific transcription factors and tethers them to active chromatin regions, thereby promoting expression of specific stress and antioxidant genes. However, potent LEDGF/p75 knockdown (KD) or over-expression in HeLa and various T-cell lines including MT4, SupT1, and PM1 cells, or even LEDGF/p75 knockout (KO) in pre-B Nalm-6 cell lines used to study the role of LEDGF/p75 in HIV replication, did not alter growth kinetics of the cells, although introduction of incidental compensatory mechanisms or clonal selection cannot be excluded. LEDGF/p75 depletion in HEK293T cells was reported to hamper cell growth and viability. Homozygous *PSIP1* KO mice lacking the expression of the C-terminal part of LEDGF/p52 and LEDGF/p75 had expected birth rates but exhibited a higher incidence of early postnatal death. Most of these *PSIP1* KO mice had a failure to nurse early after birth, for yet unknown reasons, whereas a reduced cell survival or stress responsiveness remains speculative.

A Role of LEDGF/p75 in Embryonic Development

The importance of *PSIP1* for embryonic development was demonstrated by the disruption of *PSIP1* using a gene trap screen in embryonic stem cells in mice. The gene trap resulted in fusion proteins that retained a part of the N-terminus of LEDGF/p52 or LEDGF/p75 (aa 1–152 in case the gene trap was inserted between exon 6 and 7 or aa 1–208 in case insertion occurred between exon 8 and 9) but abolished expression of the remaining part of LEDGF/p52 or LEDGF/p75, the latter containing the C-terminally located IBD. Although the number of littermates was not decreased, the majority of homozygous *PSIP1* knockout mice died early after birth due to a

failure to nurse. Mice that did survive also demonstrated a low fertility, tendency for blepharitis, and diverse motor and/or behavioral defects including reduced grip strength and locomotor activity. Almost all mice demonstrated craniofacial and skeletal abnormalities such as brachycephaly and small rib cages. The homeotic skeletal transformations suggested a role for *PSIP1* in homeobox (Hox) gene expression. LEDGF/p75 KD in HEK293T cells resulted in the upregulation of 358 and downregulation of 479 genes, among which Hoxa5, Hoxa6, Hoxa9, Hoxa10, and Hoxa13. Next, LEDGF/p75 has been shown to be essential for constitutive Hoxa9 gene expression via MLL-fusion proteins and possibly regulates Hox gene expression under physiological conditions via interaction with normal menin/MLL proteins (Yokoyama and Cleary 2008). In the *PSIP1* knockout MEFs, no obvious effect on Hox gene expression could be noticed. Notably, heterozygous *PSIP1* knockout mice were indistinguishable from wild type. The study in *PSIP1* KO mice, however, did not allow to differentiate the impact of LEDGF/p75 and LEDGF/p52 since both were deleted.

Role of LEDGF/p75 in Disease Pathogenesis

A Role of LEDGF/p75 in Tumor Cell Survival

Based on recent publications, LEDGF/p75 should be regarded as an oncoprotein that is over-expressed in a considerable number of cancer cells, providing a survival advantage to rapidly proliferating cells and promoting resistance against chemotherapy. In addition, Nup98-LEDGF/p75 fusions have been reported in chronic myeloid leukemia (CML), acute myeloid leukemia (AML), and myelodysplastic syndrome, and LEDGF/p75 tethers mixed-lineage leukemia (MLL)-fusion proteins resulting in a malignant transformation (Yokoyama and Cleary 2008). LEDGF/p75 expression was reported to be elevated in several tumor cell lines, consistent with earlier data in prostate cancer, breast cancer, bladder cancer, and blasts from chemotherapy-resistant acute myeloid leukemia.

Recently, LEDGF/p75 has been shown to be a component of the effective homologous recombination DNA-repair machinery. Repair of DNA double-strand breaks via the homologous recombination repair pathway is restricted to S and G2 phases and involves the recruitment of DNA-repair factors such as BRCA1, the Mre11–Rad50–Nbs1 (MRN) complex, and C-terminal-binding protein-interacting protein (CtIP) to the site of damage. These factors cooperate to catalyze DNA resection of DNA double-strand breaks, an initial 5′–3′ DNA resection of the broken DNA ends generating 3′ single-stranded DNA overhangs. LEDGF/p75 depletion reduced the repair of DNA double-strand breaks by the homologous recombination repair pathway and hampered the recruitment of CtIP to the site of damage. Endogenous LEDGF/p75 also co-immunoprecipitated with endogenous CtIP, an interaction that was enhanced by DNA damage. However, LEDGF/p75 is not an absolute requirement for a functional homologous recombination-mediated repair, as suggested by the milder phenotype of *PSIP1* KO mice compared to mice depleted for known components of the homologous recombination pathway (e.g., CtIP, Rad51, or RPA KO mice). LEDGF/p75, binding to specific methyl-lysine histones associated with active transcription, probably enhances the recruitment of CtIP to the site of DNA damage in LEDGF/p75-decorated parts of the chromatin. In this context, depletion of LEDGF/p75 abolished the ability to co-immunoprecipitate H3K36me3 via CtIP. The observations provide an additional explanation how LEDGF/p75 can protect cancer cells from chemotherapy (e.g., anthracycline-induced DNA damage) or ionizing irradiation. These findings however probably also relate to the physiological role of LEDGF/p75.

LEDGF/p75 is cleaved during apoptosis by caspase-3 and caspase-7 at three sites (DEVPD³⁰↓G, WEID⁸⁵↓N, and DAQD⁴⁸⁶↓G) generating fragments of 65 and 58 kDa. Interestingly, while full length LEDGF/p75 promoted tumor cell survival, overexpression of caspase cleavage fragments (LEDGF/p75_{1–486}) abrogated this effect or resulted even in a proapoptotic effect (LEDGF/p75_{86–486})[75]. Overexpression of the

smaller splice variant LEDGF/p52 (or its caspase-mediated variant with a truncated PWWP domain) induced apoptosis in several tumor cell lines, suggesting a regulation of the survival/apoptosis balance via alternative splicing. In turn, transient expression of LEDGF/p751–326 increased the viability of mutant rhodopsin expressing human retinal pigment epithelial cells, although this study lacked essential controls.

LEDGF/p75 as a Molecular Tether for MLL Fusions in Leukemia

Increasing evidence suggests that LEDGF/p75 is involved in the pathogenesis of acute leukemia. A significant fraction of acute leukemia is associated with translocations involving the mixed-lineage leukemia (MLL) gene. Over 50 different MLL translocation partner genes have been described, all retaining the N-terminal region of MLL. The most prevalent MLL alterations are t(4;11), t(9;11), and t(11;19), resulting in MLL-AF4, MLL-AF9, and MLL–ENL fusions, respectively. The MLL gene encodes for MLL-1, a H3K4 methyltransferase that acts as a regulator of *hox* genes (such as *Hoxa9*, *Hoxc6*, and *Hoxc8*) and is essential for adult hematopoiesis and body plan formation. C-terminal fusion partners AF4, AF9, and ENL orchestrate aberrant transcriptional elongation. Expression of MLL-fusion genes in hematopoietic progenitor cells blocks normal differentiation and provides a malignant self-renewal capacity. LEDGF/p75 has been shown to bind MLL-fusion proteins through interaction of its IBD with a surface formed by MLL1 and the adaptor protein menin. Menin is the gene product of *MEN1* (multiple endocrine neoplasia 1) and an essential oncogenic cofactor for malignant transformation by MLL fusions in vitro and in vivo. LEDGF/p75 tethers the menin/MLL or menin/MLL-fusion complex to the chromatin, the latter resulting in constitutive activation of *Hox* genes and malignant transformation. Knockdown of LEDGF/p75 revealed that LEDGF/p75 is required for both MLL-dependent transcription regulation in nonpathogenic conditions and leukemic transformation by MLL fusions. The transforming properties of the tripartite complex of

LEDGF/p75 and menin/MLL fusion could be bypassed by fusing the PWWP domain of LEDGF/p75 to the N-terminus of a MLL-fusion protein. Conversely, mutating the evolutionary conserved Trp-21 to alanine in the fused PWWP domain disrupted the transformation potential. A crystal structure revealed the LEDGF/p75 IBD alpha-helix E being sandwiched between MLL1 and menin and provides valuable information for future therapeutic strategies targeting this interaction.

LEDGF/p75 as an Autoantigen

The presence of antibodies against LEDGF/p75 has been reported in a variety of diseases as well as in healthy individuals, and its exact role in pathogenesis or its diagnostic value remains uncertain. Initially immunohistochemistry using sera from Japanese patients with atopic dermatitis and interstitial cystitis revealed a dense fine speckled pattern during interphase in combination with chromatin staining during mitosis. The responsible antigen was termed dense fine speckles 70 kd (DFS70) and turned out to be identical to LEDGF/p75 (Ochs et al. 2000). Since then, anti-LEDGF/p75 (or anti-DFS70) antibodies have been recovered in patients with atopic dermatitis [14%, 29.7% (Ochs et al. 2000), or 71.4%, Japanese patients], interstitial cystitis [9% (Ochs et al. 2000)], asthma [16%, patients from the USA (Ochs et al. 2000)], psoriasis (Ochs et al. 2000), Sjögren (Ochs et al. 2000), Behçet, sarcoidosis, chronic fatigue syndrome (16%, Japanese patients), alopecia areata (20%, Japanese patients), and patients with prostate cancer (18.4%).

A Role of LEDGF/p75 in HIV Replication

The function of LEDGF/p75 has been studied most in the context of HIV. LEDGF/p75 has been shown to be a cofactor of HIV and more specifically of HIV integrase. For successful replication, HIV and other retroviruses rely on the virally encoded IN enzyme to orchestrate insertion of their reverse transcribed genomes into the host cell DNA. The active site of IN catalyzes two

distinct reactions. First, IN removes a conserved dinucleotide from the 3' ends of the viral DNA, a process termed 3'-processing. This reaction takes place in the cytoplasm of the host cell in the context of a large nucleoprotein complex, termed the preintegration complex (PIC). The second reaction takes place in the nucleus and involves a pair of coordinated transesterification reactions that cuts both strands of target DNA and joins them to the exposed reactive 3' hydroxyl groups of the invariant CA dinucleotides of the viral DNA molecule, a process termed the strand transfer reaction. Following the concerted strand transfer step, host enzymes presumably repair the remaining gap. During its replication cycle, HIV is dependent on cellular proteins, so-called cofactors. LEDGF/p75 is such a cofactor of HIV replication that tethers the viral preintegration complex toward the host cell chromatin thereby promoting integration and determining the HIV integration site distribution pattern. Although initial work led to contentious results, from a hindsight due to limitations of emerging RNAi technology, recent work using a human somatic LEDGF/p75-specific knockout cell line clearly established the crucial role of LEDGF/p75 in HIV replication (Cherepanov et al. 2003, 2005; Hendrix et al. 2011; Gijssbers et al. 2010; Schrijvers et al. 2012; Llano et al. 2006; Christ et al. 2010; Ciuffi et al. 2005). The interaction between LEDGF/p75 and HIV IN has been well characterized (Cherepanov et al. 2003, 2005) and enabled the generation of a new class of antiretroviral agents that block HIV replication by docking at the LEDGF/p75 binding pocket in HIV IN (Christ et al. 2010). In this entry we first focus on the characterization of the LEDGF/p75-IN interaction, continue with its role in HIV replication and lentiviral targeting, and conclude with its potential as an antiviral target.

The HIV Integrase-LEDGF/p75 Interaction

Cherepanov et al. first reported on LEDGF/p75 as a binding partner of HIV-1 IN by isolating complexes of ectopically expressed IN from nuclei of HEK293T cells (Cherepanov et al. 2003). The interaction of IN-LEDGF/p75 was confirmed by *in vitro* pull-down assays using recombinant

proteins and was shown to be specific for *Lentivirinae* and not a general characteristic of all retroviral integrases. Co-localization studies of different HIV-1 eGFP-IN deletion mutants in the absence or presence of LEDGF/p75 revealed that both the N-terminal zinc-binding domain and the core domain of HIV-1 IN are involved in the interaction with LEDGF/p75. The core domain proved to be the main determinant for the interaction since overexpression of LEDGF/p75 was able to restore nuclear/chromosomal localization of the IN core domain but not that of the N-terminus. Information about the interacting amino acids in the IN-LEDGF/p75 interface came from a crystal structure of the HIV-1 IN CCD in complex with the IBD (Cherepanov et al. 2005) and a later crystal structure comprising the HIV-2 IN NTD-CCD in complex with the IBD and was supported by several mutagenesis studies. Critical interacting residues of the IBD, Ile-365, Asp-366, Phe-406, and Val-408 are located on two interhelical loops of the IBD and contact two regions in a pocket formed by an IN CCD dimer. In the first interhelical loop ($\alpha 1/2$ connector), Asp-366 makes hydrogen bonds with the main amino groups of IN residues Glu-170 and His-171, and LEDGF/p75 Ile-365 makes van der Waals interactions with Met-178, Leu-102, Ala-128, Ala-129, Trp-131, and Trp-132. In the second interhelical loop ($\alpha 4/5$ connector), Phe-406 and Val-408 pack against a hydrophobic patch on IN formed by Ala-128 and Trp-131. A second crystal structure containing the NTD and CCD of HIV-2 IN in complex with the IBD elucidated additional charge-charge interactions between the IN NTD, i.e., Glu-6, Glu-10, Glu-13, and the IBD, i.e., Lys-401, Lys-402, Arg-404, and Arg-405. Of note, although all lentiviral integrases can interact with LEDGF/p75, not all key amino acids in HIV-1 integrase, such as Ala-128, Trp-131, and Gln-168, are conserved between primate and non-primate lentiviral integrases.

LEDGF/p75 functions as a molecular tether, binding chromatin with its N-terminal region and IN with its C-terminally located IBD. Several lines of evidence support this theory. Integrase is known to localize predominantly in the nucleus

and the distribution of nuclear IN perfectly matches with that of LEDGF/p75. Knockdown of LEDGF/p75 using RNAi abolished the nuclear localization of HIV-1 IN as well as its association with chromosomes in cells transiently transfected with IN fused to eGFP. Recent work indicated that IN affects the dynamics of LEDGF/p75 in cells. When LEDGF/p75 was co-expressed with IN, a possible binding partner, the complex moved 75-fold slower compared to LEDGF/p75 alone (Hendrix et al. 2011). Vice versa, depletion of LEDGF/p75 significantly increased the mobility of IN. The reduction of LEDGF/p75 mobility was only sixfold when PWWP-mutated LEDGF/p75 (R74D), retaining wild type affinity for IN, was co-expressed with IN, suggesting that increased viscosity of the complex could not explain these observations. An allosteric effect of IN on the chromatin-binding properties of LEDGF/p75 was deemed unlikely, since the IBD and chromatin-binding domains of LEDGF/p75 are located in distinct structural parts of the protein. Instead the PWWP domain in LEDGF/p75 probably constitutes a “protein lock” that strongly tethers IN to chromatin. McNeely et al. corroborated the tethering function of LEDGF/p75 in an in vitro assay. While the interaction of IN and DNA was much weaker compared to the interaction of LEDGF/p75 and DNA, adding LEDGF/p75 increased the apparent affinity of IN to DNA 10- to 32-fold by tethering of IN to DNA. The interaction was outcompeted by LEDGF/p75₃₂₆₋₅₃₀ but not by LEDGF/p52 or LEDGF/p75_{D366N}, incapable of interacting with IN. LEDGF/p75 with mutated NLS and AT-hook-like domains, preserving binding to IN but reducing DNA binding, likewise did not result in a stimulation of the IN-DNA interaction. Finally, LEDGF/p75 affects the lentiviral integration site distribution and changing the N-terminal chromatin-binding domains with other chromatin-interacting proteins enables retargeting of integration (see section “[The Role of LEDGF/p75 in Targeting Lentiviral Integration](#)”), again alluding to the tethering role of LEDGF/p75.

LEDGF/p75 has been shown to be an allosteric stimulator of HIV IN catalytic activity and stimulates HIV-1 integration into chromatinized templates. LEDGF/p75 stimulates both the

3'-processing and strand transfer. LEDGF/p75 was shown to stimulate half-site (insertion of only one viral DNA into template DNA) but not full-site concerted integration (insertion of both viral DNA ends in a template DNA) *in vitro*. This discrepancy between half- and full-site integration stimulation was not seen with another LEDGF/p75-dependent lentiviral IN, EIAV IN, and proved to be dependent on the concentration and molar ratios of LEDGF/p75 and IN used in these experiments. McNeely et al. observed a stimulation of overall IN activity by LEDGF/p75 as measured using ELISA, yet this activity most pronounced upon preincubation of IN with donor DNA. LEDGF/p75 also stabilizes IN subunit to subunit interactions and promotes IN tetramer formation. Using FRET, two distinct IN conformations were observed upon preincubation of IN with DNA or LEDGF/p75, respectively. These findings suggest that premature binding of LEDGF/p75 to IN precludes the formation of a functional IN-viral DNA complex. How these findings relate to the *in vivo* situation and when LEDGF/p75 is exactly engaged in the viral PIC remains unanswered.

Earlier work indicated that the presence of LEDGF/p75 increased the stability of ectopically expressed IN and protected IN from ubiquitination and proteasomal degradation. LEDGF/p75 disabled efficient immunoprecipitation of C-terminally tagged IN from whole-cell extracts 8 h after transduction with a HIV-derived viral vector, suggesting that the C-terminus of IN in the PIC is masked in the presence of LEDGF/p75.

The Role of LEDGF/p75 in HIV-1 Replication

Depletion of LEDGF/p75 significantly hampers HIV-derived lentiviral vector transduction and HIV replication in different cell lines (Schrijvers et al. 2012; Llano et al. 2006) as well as in primary CD4⁺ T cells or macrophages. In hindsight, the initial controversy surrounding the potential role of LEDGF/p75 in HIV replication, with RNAi-mediated knockdown of LEDGF/p75 failing to affect HIV replication, was caused by unknown limitations and misinterpretation of the RNAi technology. These observations, however, also support the notion that minute amounts of LEDGF/p75 are

already sufficient for HIV replication and explain why LEDGF/p75 was not withheld in different RNAi screens for host factors of HIV, despite its currently established role in HIV replication.

In early LEDGF/p75 KD experiments, a reproducible two to fourfold reduction in HIV-1 replication was shown. The residual HIV-1 replication observed in LEDGF/p75 KD cells was attributed to the remaining chromatin-bound LEDGF/p75. Luciferase reporter gene expression was more than tenfold reduced in *PSIP1* KO mouse embryonic fibroblasts (MEFs) compared to wild type levels. Reintroduction of human LEDGF/p75 in these cells alleviated the block in transduction, whereas LEDGF/p75 with a D336N mutation, defective for interaction with integrase, or LEDGF/p52 did not. Deletion mutants of LEDGF/p75 demonstrated that both the N-terminal domains, containing the PWWP, AT-hook, and NLS region, and the C-terminal region containing the IBD were required to rescue the observed block. Deletion mutants of LEDGF/p75 lacking the PWWP domain rescued HIV transduction and/or replication only up to 17% of wild type levels, while AT-hook-like-domain deletions or mutations alone had no effect. One report suggested the PWWP domain to be dispensable for single-round HIV-fluc transduction. A similar transduction block was observed in *PSIP1* KO MEFs generated using a gene trap. Although the domains in LEDGF/p75 important for its function in HIV-1 replication are 100% conserved in the murine orthologue, HIV is not a murine virus, and studies in MEFs were restricted to single-round VSV-G-pseudotyped lentiviral vectors. Recently a human somatic LEDGF/p75 knockout cell line was generated enabling the study of multiple-round HIV replication in a human context (Schrijvers et al. 2012). Here, the exons encoding the C-terminal region of LEDGF/p75 were specifically knocked out via homologous recombination in Nalm-6 cells, leaving the smaller splice variant LEDGF/p52 unaltered. HIV replication was significantly hampered in the absence of LEDGF/p75 and could only be observed using laboratory-adapted HIV strains. In the absence of LEDGF/p75, HRP-2, the only known other cellular protein harboring a PWWP

domain and a functional IBD besides LEDGF/p75, was shown to mediate HIV replication. Previous attempts to demonstrate a role for HRP-2 in HIV replication were probably obscured by the remaining LEDGF/p75 or premature RNAi technology. The observations explain the residual replication observed in potent RNAi-mediated LEDGF/p75 KD cell lines and probably as well the residual transduction observed in *PSIP1* KO MEFs. Since clinical HIV isolates demonstrated futile to no replication in the absence of LEDGF/p75 (Schrijvers et al. 2012), in vivo LEDGF/p75 is probably essential, although relative expression levels of LEDGF/p75 and HRP-2 in relevant cell lines remain unknown.

The block opposed by LEDGF/p75 depletion on HIV-derived vector transduction or HIV replication was pinpointed using qPCR analysis of HIV-1-derived DNA intermediates. Equal amounts of late reverse transcripts but lower amounts of integrated copies, ranging from a two to tenfold reduction, were observed. The number of 2-LTR circles, suggested to rise in the nucleus upon abortive integration and to decrease in case of a block in nuclear import, was 1.5- to 2.5-fold increased at 24 h postinfection upon RNAi-mediated LEDGF/p75 KD or was equal to modestly increased in case of LEDGF/p75 or *PSIP1* KO. Cytoplasmic PICs isolated from *PSIP1* KO MEFs also demonstrated equal IN enzymatic activity in vitro, suggesting a role of LEDGF/p75 downstream of PIC formation. Together these data suggested a block at the integration step, after reverse transcription and nuclear import. LEDGF/p75 KD did not affect production or infectivity of progeny virus in initial experiments. These observations have yet to be confirmed in more potent LEDGF/p75 KO cell lines. Of note, introducing IN W131A, Q168A, or A128T/E170G mutations, abolishing or reducing the interaction with LEDGF/p75, in HIV impaired reverse transcription and integration capacity, suggesting a role of LEDGF/p75 upstream of integration. In this context, functional PICs that were precipitated from the cytoplasm using anti-LEDGF/p75 antibodies and wild-type IN, but not IN Q168A, could be immunoprecipitated with anti-LEDGF/p75

antibodies using whole-cell extracts 8 h post-infection, although these observations were not confirmed in another study. Together these RNAi, knockout, and mutagenesis experiments thus point to a crucial role of LEDGF/p75 in HIV replication with LEDGF/p75 tethering the viral PIC toward the host cell chromatin thereby promoting integration.

Different studies have shown that genomic variation of *PSIP1* and LEDGF/p75 expression levels affects HIV acquisition and progression to AIDS. The link between several single-nucleotide polymorphisms (SNPs) in *Psip1*, few of them resulting in non-synonymous mutations, and disease outcome has been recently evaluated (Madlala et al. 2011). Madlala et al. mapped four known synonymous and one non-synonymous SNP resulting in the polymorphism Q472L in *PSIP1*, in the prospectively followed acutely HIV-infected, chronically HIV-infected, or uninfected South African people. The noncoding SNP (rs2277191) was associated with a higher likelihood of HIV-1 acquisition and more rapid disease progression, and another noncoding SNP (rs12339417) with slower CD4⁺ T cell decline and lower mRNA levels of LEDGF/p75. Recently, two non-synonymous SNPs, resulting in S436I and T473S mutations in LEDGF/p75, were described in HIV-infected long-term non-progressors ($N = 2$ out of 149), while these mutations could not be retrieved in a population of HIV progressors ($N = 92$) or healthy controls ($N = 1,500$, only T473S was evaluated), suggesting a possible role of these mutations in disease progression. However, a causal relationship or an effect of these mutations on the cellular functions of LEDGF/p75 has not yet been established. Expression levels of LEDGF/p75 in PBMCs also matter. In the prospectively followed South African CAPRISA cohort, higher LEDGF/p75 mRNA levels were associated with an increased likelihood of HIV infection (Madlala et al. 2011), and LEDGF/p75 protein levels in CD4⁺ lymphocytes were lower in HIV-exposed but seronegative individuals compared to healthy controls in a Senegalese cohort, although differences were only subtle. LEDGF/p75 was previously reported to be upregulated in HIV-infected

cells in vitro, and PBMCs from untreated HIV-1-infected individuals in vivo had higher expression levels of LEDGF/p75. Contrary, Madlala et al. found lower expression levels of LEDGF/p75 in PBMCs from seropositive compared with seronegative individuals in South African cohorts, and longitudinal data suggested a downregulation of LEDGF/p75 expression in seroconverters (Madlala et al. 2011).

The Role of LEDGF/p75 in Targeting Lentiviral Integration

LEDGF/p75 is clearly required for HIV replication. Interestingly this tethering factor also determines the integration site distribution. HIV-1 integration was demonstrated to occur preferentially in the body of actively transcribed transcription units in contrary to the gamma-retroviral integration pattern in which a less pronounced preference toward transcriptionally active regions and a preference to integrate near transcription start sites and CpG islands were observed and avian sarcoma leukocytosis virus (ASLV) or human T-cell leukemia virus (HTLV) is displaying a much weaker preference for these features. Integration site preferences of other lentiviruses, such as EIAV and SIV (SIVmne), correspond well with those observed for HIV-1.

Knockdown or knockout of LEDGF/p75 alters this integration pattern (Ciuffi et al. 2005), supporting the hypothesis that LEDGF/p75 targets HIV-1 integration toward transcriptionally active genes. Interestingly, LEDGF/p75 KD already affects the HIV integration site distribution pattern even in the absence of inhibition of HIV transduction (Ciuffi et al. 2005), and the effect in integration site selection becomes more outspoken upon KO of LEDGF/p75 (Schrijvers et al. 2012). LEDGF/p75 depletion reduced integration in active genes and increased integration near CpG islands, although still not in a range observed with the *Gammaretrovirus* MLV. Recently, the PWWP domain was demonstrated to be critical for targeting integration to regions enriched in specific histone posttranslational modifications. Interestingly, although integration events shifted toward a random distribution, random HIV integration was not observed even in LEDGF/p75

KO cells, suggesting alternative targeting mechanisms to play a role in the absence of LEDGF/p75. Transportin-SR2, a nuclear import factor for HIV, and RANBP2 also affected the integration site distribution pattern, suggesting a role for nuclear trafficking in target site selection.

Chromatin-binding sites of LEDGF/p75, mapped by DamID technology in the ENCODE region, revealed a chromatin-binding profile of LEDGF/p75 reminiscent to that of HIV-1 integration. Correlation of binding sites of LEDGF/p75 with known genomic features revealed a high correlation with markers of active chromatin. LEDGF/p75 binds active genes and disfavors promoter regions, although not all LEDGF/p75-binding sites correlated with HIV-1 integration.

Next, LEDGF/p75 hybrids in which the N-terminus was replaced by an alternative chromatin interaction domain, such as the heterochromatin-binding element CBX1, have been shown to retarget HIV-1 integration out of transcription units and toward heterochromatic regions (Gijsbers et al. 2010). These observations were independently confirmed using various other chromatin-binding domains. These studies therefore provide an in vitro proof of principle for redirecting retroviral integration, leading to the generation of safer lentiviral vectors that can selectively be targeted to regions of choice in the chromosome.

The LEDGF/p75–HIV Integrase Interaction as a Target for Antiretroviral Therapy

The LEDGF/p75–IN interaction can be targeted to tackle HIV using various approaches. First, overexpression of the IBD of LEDGF/p75 was shown to have a dominant negative effect on HIV-1 replication and was further exploited as a potential gene-therapeutic approach for HIV/AIDS. Next, small molecules targeting IN at the pocket formed by the IN dimer and responsible for the interaction with LEDGF/p75 were developed. They display potent antiviral activity (Christ et al. 2010). These compounds form a new class of antiretrovirals, are considered as allosteric HIV–integrase inhibitors, and termed LEDGINs (Christ et al. 2010). They are currently tested in phase 1 clinical trials. Lastly, also LEDGF/p75

itself can be targeted. In proof-of-concept studies, peptides were shown to block the LEDGF/p75-IN interaction and hamper HIV replication.

LEDGF/p52

Despite the shared N-terminal 325 amino acids, LEDGF/p52 seems to have distinct cellular functions compared with LEDGF/p75. In contrast to LEDGF/p75, the smaller LEDGF/p52 has been attributed a stronger and more general transcriptional coactivator activity (Ge et al. 1998). LEDGF/p52 interacts with the essential splicing factor ASF/SF2 (Ge et al. 1998) and was proposed to modulate alternative splicing. LEDGF/p52 is, contrary to LEDGF/p75, not implicated in HIV replication nor repair of double-strand breaks. While LEDGF/p75 overexpression seems to increase cell survival, overexpression of LEDGF/p52 or its caspase-mediated variant with a truncated PWWP domain induced apoptosis in several tumor cell lines.

HRP-2

HRP-2 (also known as HDGF2) is a 670 aa basic protein encoded by *Hdgfp2*, located on chromosome 19p13.3. It is structurally highly related to LEDGF/p75. Like LEDGF/p75, it contains a PWWP domain and AT-hook-like sequences at its N-terminus, next to a C-terminal IBD (48% identical and more than 70% similar to the IBD of LEDGF/p75). The remaining part of HRP-2 is estimated to be unstructured. HRP-2 is a nuclear protein, but has a more homogenous distribution when compared to the speckled pattern observed with LEDGF/p75, and does not bind to mitotic chromatin.

Fluorescence polarization, NMR titration experiments, and crystal structures suggest that the PWWP domain of HRP-2 also binds methyl-lysine residues on histones. HRP-2 bound best to peptides derived from H3K36me2 and weakly to H3K36me3, H3K79me2, H3K79me3, H4K20me2, and H4K20me3. In another study where the PWWP of LEDGF/p75 was changed for that of

HRP-2, an increased likelihood of integration near marks associated with active transcription, i.e., H2BK5me1, H3K4me3, H3K9me1, H3K27me1, and H3K36me3, was observed.

Little is known of the cellular role of HRP-2. Most observations were made in parallel with studies evaluating the role of LEDGF/p75. Interestingly, HRP-2 was shown to bind as well to the cellular LEDGF/p75-interacting proteins JPO2, PogZ, and Cdc7/ASK, although the relative contribution of HRP-2 in these interactions remains unknown. Much alike LEDGF/p75, the IBD of HRP-2 was sufficient to bind and stimulate the enzymatic activity of Cdc7/Ask. HRP-2 was also attributed a crucial role in the recruitment of CtIP to DNA DSBs, paralleling observations with LEDGF/p75.

HRP-2 also binds HIV IN via its IBD. HRP-2 overexpression relocated HIV-1 IN from the cytoplasm to the nucleus in LEDGF/p75 depleted cells. Although HRP-2 was investigated previously as a potential alternative for LEDGF/p75, no effect in multiple-round HIV-1 replication was observed after HRP-2 KD alone or in combination with LEDGF/p75 KD. However, these observations may have been obscured by the remaining LEDGF/p75 after incomplete RNAi-mediated KD. Recently, both single-round transduction and multiple-round replication were shown to be additionally hampered upon HRP-2 KD in LEDGF/p75 KO cells (Schrijvers et al. 2012). HIV-1 engages HRP-2 as an alternative for LEDGF/p75, but this low affinity IN binding partner can only substitute for LEDGF/p75 after depletion of the latter, suggesting a dominant role for LEDGF/p75 over HRP-2. The inferior role for HRP-2 compared with LEDGF/p75 is probably due to its lower affinity for HIV-1 IN. Considerably less IN could be co-immunoprecipitated by HRP-2 than LEDGF/p75; flag-LEDGF/p75 but not flag-HRP-2 co-immunoprecipitated IN from cell lysates, and AlphaScreen technology estimated that the IBD of HRP-2 has a 13-fold lower affinity for HIV-1 IN compared to the IBD of LEDGF/p75. The fact that HRP-2 does not bind to mitotic chromatin is probably irrelevant for HIV replication since LEDGF/p75 depletion for instance also affects HIV replication in nondividing cells.

Conclusion

LEDGF/p75 is a nuclear protein that acts as a molecular adapter to tether proteins to the chromatin. Besides a crucial role in tethering the HIV PIC to the chromatin, recent studies have uncovered a role for LEDGF/p75 in leukemia, cell survival, and DNA repair. The protein can thus be considered as a hub protein and an interesting target to treat multiple diseases. Knowledge on the interaction between LEDGF/p75 and IN has resulted in a new class of inhibitors (LEDGINS) blocking HIV replication that are in advanced preclinical and early clinical studies. LEDGINS are now being used as research tools to unravel HIV integration. They provide a good example of translating basic research findings into novel therapeutics.

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Role of Regulatory T Cells During HIV Infection

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Definition

Human immunodeficiency virus (HIV) causes a gradual loss of immune competence, leading to acquired immunodeficiency syndrome (AIDS). HIV-associated defects in cell-mediated immunity (CMI) are of particular importance, as these impairments lead to poor control of HIV replication and of other pathogens whose clearance depends on CMI. Importantly, a number of immune deficits caused by HIV infection can be

partially restored *in vitro*, suggesting the existence of active regulatory mechanisms. Several mechanisms are involved in the regulation of the immune system; among these is the pivotal role of regulatory T cells (Treg), a subset of CD4⁺ T cells, which has been recognized for several years. In addition to their role in controlling peripheral tolerance, Treg promote the establishment of persistent infections (viral, bacterial, parasitic, and fungal). Treg may thus contribute to inefficient CMI during chronic HIV infection. However, Treg may also play a beneficial role, because their control of immune activation may limit the availability of cellular targets for HIV infection, as well as decrease the pathology associated with immune activation. The mechanism(s) underlying the accumulation and role of Treg during HIV/SIV infection remain poorly understood. The aim of this entry is to discuss the recent findings on Treg origin and function in the setting of HIV infection.

Regulatory T Cells

Treg are a subpopulation of CD4⁺ T cells that originate from either the thymus (tTreg) or the peripheral conversion of conventional CD4⁺ T cells (Tcon) (pTreg). tTreg are positively selected in the thymus through MHC class II-dependent T-cell receptor (TCR) interactions resulting from a high-avidity selection. Multiple pathways of peripheral conversion have been described. The most studied involves TCR stimulation in the presence of the immunosuppressive cytokine, transforming growth factor beta (TGF- β). In addition, dendritic cell (DC)-mediated signals such as indoleamine 2,3-dioxygenase 1 (IDO) or retinoic acid (RA) induce or stabilize Treg conversion. Treg, regardless of their origin, are characterized by a high and constitutive expression of the IL-2 receptor alpha chain (CD25), the low expression of IL-7 receptor (CD127), and expression of the transcription factor, FOXP3. Unfortunately, so far, no single marker allows for a reliable phenotypic comparison between tTregs and pTregs.

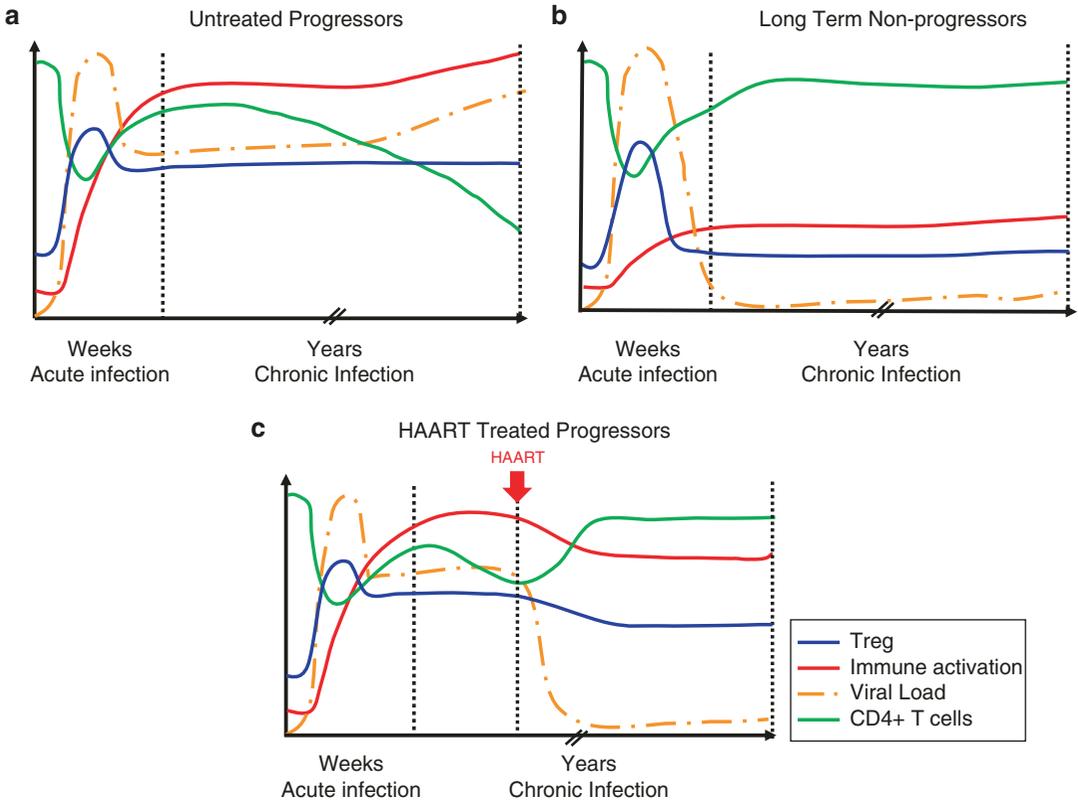
Immune Control by Regulatory T Cells

Treg are key regulators of the immune system. During viral infections, Treg are essential to prevent exacerbated inflammatory processes. However, in excess, Treg can impair the development of effector immune responses, thus promoting the establishment of chronic infections. Treg have been shown to suppress both innate and adaptive immune responses, based on their ability to produce immunomodulatory cytokines such as TGF- β and IL-10, as well as their capacity to mediate suppression through cell contact-dependent mechanisms. Among these, the interaction of lymphocyte antigen 4 (CTLA-4) with CD80/CD86 in DCs promotes the production of IDO.

Treg also contain high levels of cyclic adenosine monophosphate (cAMP), which inhibits T-cell differentiation and proliferation and which can be transferred to target cells through the formation of gap junctions (GJ) between Treg and their targets. Additionally, Treg express the ectonucleotidases CD39 and CD73, enzymes that hydrolyze extracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP) into adenosine monophosphate (AMP) and then into adenosine, respectively. Adenosine consequently activates adrenergic receptors which increases intracellular cAMP.

Regulatory T Cells During Acute HIV Infection

Little is known about Treg dynamics during the acute phase of HIV infection. In this setting, Treg expansion was reported and the percentage of circulating Treg inversely correlated with CD8⁺ T-cell activation (Ndhlovu 2008). During the non-pathogenic SIV infection in the African green monkey, Kornfeld et al. observed a very early (24 h post infection) and strong induction of FOXP3 mRNA expression and an increased percentage of circulating CD4⁺CD25⁺ T cells in the blood (Kornfeld 2005). In the context of pathogenic SIV infection in rhesus macaques, relative Treg accumulation occurred 2 weeks



Role of Regulatory T Cells During HIV Infection, Fig. 1 Treg kinetics during HIV infection. Change in peripheral Treg frequency over the course of HIV infection and its relationship with the levels of immune hyperactivation, HIV viral load, and CD4+ T-cell counts. **(a)** Untreated typical progressor: during the acute phase of HIV infection, the viral load (dotted yellow line) rapidly rises, the CD4+ T-cell count (green line) rapidly falls, while markers of immune activation (red line) and Treg frequency (blue line) start to rise. During the late acute and early chronic phase, the viral load is controlled, CD4+ T-cell levels increase, immune hyperactivation persists, and Treg frequency declines but remains higher than normal levels. During the late chronic phase, the viral loads as

well as immune hyperactivation gradually increase; Treg frequency also increases despite a progressive loss of CD4+ T cell. **(b)** Long-term nonprogressors: during the late chronic phase, viral loads decrease to very low levels and immune activation is low. CD4+ T-cell counts and frequency increase and can reach normal levels. Treg frequency remains comparable to that observed in healthy individuals. **(c)** HAART-treated HIV progressor: viral loads quickly fall to undetectable levels, and CD4+ T-cell counts and frequency are rapidly reestablished but not to levels comparable to uninfected individuals. HAART ameliorates Treg frequency and immune activation; however, Treg frequency never normalizes completely

postinfection in both mucosal tissues and peripheral lymph nodes (Allers 2010; Ji 2009). Importantly, macaques vaccinated by an attenuated vaccine showed significantly higher levels of Treg after SIV challenge than control animals (Genesca 2012). However, important differences exist depending on the model employed, as rapid loss of Treg occurred in the highly pathogenic model of SIV infection of pigtailed macaques, suggesting that the effect of acute HIV/SIV on

Treg frequency is dependent on multiple factors (Fig. 1a).

Regulatory T Cells During Chronic HIV Infection

High frequency of circulating Treg has consistently been found during progressive HIV infection (Presicce 2011; Rueda 2013). Increased Treg

percentages were also detected in lymph nodes, tonsil, gastrointestinal mucosa, and thymus (Nilsson 2006; Rueda 2013). In addition, chronically SIV-infected rhesus macaques with high viral loads had higher FOXP3 expression in the spleen, lymph nodes, and gut than macaques with a lower viral load (Boasso 2007). Despite this relative accumulation of Treg, their absolute numbers decrease over the course of infection (Presicce 2011; Rueda 2013; Fig. 1a).

In contrast, the majority of studies in long-term nonprogressors (LTNP), particularly elite controllers, reported decreased Treg frequency in peripheral blood and rectal mucosa compared to HIV progressors. Taken together, these data suggest that Treg imbalance is associated with progressive infection and uncontrolled viral replication (Fig. 1b).

Regulatory T Cells in HAART-Treated Patients

Discrepant results have been reported concerning the effect of HAART treatment on circulating Treg. HAART treatment was shown to either decrease (Presicce 2011) or not to modify (Lim 2006) the frequency of circulating Treg, although Treg frequency is not fully normalized by HAART (Presicce 2011; Fig. 1c). Differences between studies could come from the heterogeneity of HAART-treated patients. Indeed, recent studies comparing patients with immunological and non-immunological response to HAART suggest that Treg frequency was reduced only in patients with immune reconstitution (Rueda 2013). Treg absolute counts also increase post HAART, although they remain lower than in healthy donors (Presicce 2011; Rueda 2013). This alteration was associated with the persistent mucosal barrier dysfunction and activation (Rueda 2013).

Treg Susceptibility to HIV Infection

Although all studies agree that Treg can be infected by HIV, their relative susceptibility to

infection compared to Tcon remains controversial. Some studies have shown that activated Treg were more susceptible to infection by an HIV R5 strain than memory T cells, but Treg replicated the virus to a similar extent. Conversely, Treg have been shown to be less susceptible to infection with HIV R5 strains than Tcon but were more susceptible to X4 viruses (Moreno-Fernandez 2009). Data on Treg infection by HIV in vivo are scarce. In chronically HIV-infected patients, Treg were not preferentially infected compared to non-Treg (Chase 2008). During SIV infection of rhesus macaques, productive infection was detected in some FOXP3⁺ cells present in the gut-associated lymphoid organs and in the mucosal surface, but relatively less than in non-Treg. This suggested that Treg may be selectively spared from SIV/HIV-mediated cell death (Allers 2010). Additional work is needed to address the role of different viral factors in the dynamics of Treg infection by HIV.

Another unanswered question is whether Treg constitute a reservoir for HIV infection. In chronically infected HIV patients on suppressive HAART, the frequency of HIV DNA harboring cells is higher in resting Treg than in resting non-Treg. Similar results were found in mucosal Treg from SIV-infected macaques (Allers 2010), thus suggesting that Treg could contribute to viral reservoir.

Mechanisms of Treg Accumulation During HIV Infection

Preferential survival. As mentioned above, Treg are susceptible to HIV infection both in vitro and in vivo. However, their low susceptibility to productive infection compared to that of non-Treg could lead to their preferential survival. In the same line, Treg exposure to HIV selectively promoted their survival via a CD4-gp120-dependent pathway (Nilsson 2006).

Increased proliferation. Increased expression of Ki67, a marker of cell cycle, was found in mucosal and blood Treg from SIV-infected macaques and in circulating Treg from HIV-infected patients, suggesting that local

expansion of Treg could contribute to increased Treg frequency in lymphoid tissues and blood (Allers 2010; Presicce 2011). Of note, HIV may directly induce Treg expansion, as in vitro interaction of HIV with Treg induced their expansion and increased the expression of FOXP3, CD25, and CTLA-4 (Amarnath 2007).

Tissue redistribution. Some authors hypothesized that redistribution of Treg from blood to lymphoid tissues could explain the increased tissue frequency. Ji et al. showed that HIV-1 binding upregulated expression of the homing receptor CD62L and integrin $\alpha 4\beta 7$ by CD4⁺CD25⁺ Treg, which in turn could result in more rapid Treg migration to peripheral and mucosal lymphoid tissues where HIV replication occurs (Ji 2009). In agreement with this hypothesis, Treg prevalence correlated better with tissue viral load than with plasma viremia (Boasso 2007). However, using flow cytometry characterization of Treg, increased Treg frequency have been found in the peripheral blood of infected compared to uninfected subjects (Chase 2008; Presicce 2011), which does not support increased Treg homing as a mechanism for their accumulation in tissue.

Increased peripheral conversion. Several results suggest a contribution of peripheral conversion to the increased Treg pool. First, HIV-exposed plasmacytoid dendritic cells (pDC) convert allogeneic non-Treg into CD4⁺CD25⁺FOXP3⁺ Treg, while unexposed pDC do not (Manches 2008). Second, lymph node DC from untreated HIV-infected subjects induced a Treg phenotype in normal allogeneic T cells, an ability that was lost after ART. Third, tissue myeloid dendritic cells (mDC) from chronic SIV-infected rhesus macaques were more efficient at inducing the expression of CD25 and FOXP3 in autologous non-Treg than mDC from uninfected macaques (Presicce 2012). However, the contribution of this mechanism to increased Treg frequency may depend on the stage of infection and/or the body compartment, as blood mDC infected in vitro with HIV were not able to convert non-Treg into Treg (Presicce 2012).

Mechanisms that could be involved in increased DC-mediated peripheral conversion during HIV are not well characterized.

HIV-exposed pDC induced Treg through IDO (Manches 2008), and IDO levels are high in many organs of SIV-infected macaques (Boasso 2007). Another molecule involved in Treg conversion is TGF- β . During HIV infection, TGF- β levels are high (Estes 2007). Interestingly, although CD103⁺ mDC are considered critical for Treg conversion in GALT, the enhanced capacity of mDC from SIV-infected macaques to induce FOXP3 was not directly associated with the presence of CD103⁺ DC, suggesting that other molecules such as PD-L1 may be involved in this conversion (Presicce 2012).

Phenotype and Functional Activity of Treg During HIV Infection

During chronic HIV infection, Treg express high levels of activation markers, cell cycle, and regulatory molecules, such as CD69, PD-1, GITR, CTLA-4, and CD39, compared to healthy controls (Presicce 2011; Rueda 2013). Expression of most of these molecules is normalized in individuals with an immunological response to HAART but not in those without a response (Chase 2008; Presicce 2011). In vitro, Treg exposed to HIV or gp120 maintained their suppressive activity (Moreno-Fernandez 2009). Treg from HIV-infected individuals can suppress proliferation, cytotoxicity, and cytokine production in response to HIV proteins, but no marked difference in suppression is observed between acute and chronic HIV infection (HAART-treated or untreated HIV patients) or between progressors and elite controllers (Chase 2008; Kared 2008; Ndhlovu 2008; Rueda 2013). HIV-driven Treg expansion also appears to hamper immune control of other persistent pathogens, as Treg from HIV-infected individuals suppress immune responses to recall antigens such as CMV and PPD, suggesting that Treg may contribute to the clinical reactivation of CMV in untreated AIDS patients as well as attenuate the immune response to vaccines.

In the past years, new observations have suggested that Treg act as a double-edged sword during HIV-1 infection and that Treg may also play

a beneficial role during HIV infection by controlling immune activation. During SIV acute infection, CTLA-4 blockade decreases the level of IDO1 and paradoxically increased the viral replication in mucosal tissue due to intense immune activation, suggesting that the suppressive action of Treg is necessary to control the pathogenic process induced by SIV (Hryniewicz 2006). Likewise, SHIV-vaccinated rhesus macaques were protected from uncontrolled viral replication after SIV challenge, and this protection was associated with increased Treg frequency and decreased immune activation (Genesca 2012). Similarly, because the frequency of CD4⁺CD25^{high} Treg positively correlated with CD4 T-cell count and inversely correlated with CD4 T-cell activation during primary infection, Treg were postulated to play a largely beneficial role during the acute phase of HIV-1 infection (Kared 2008). In utero, activation of Treg may contribute to a lack of vertical transmission by decreasing T-cell activation (Legrand 2006). Moreover, individuals with strong HIV-specific Treg function in vitro had significantly lower levels of plasma viremia and higher CD4:CD8 ratios than individuals in whom Treg activity could not be detected (Kinter 2004).

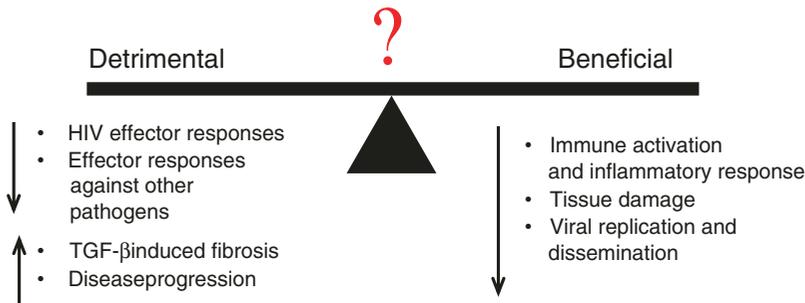
Consistent with these in vivo findings, Treg can control the frequency of HIV-infected Tcon cells in an in vitro model of acute infection by a process independent of their effect on Tcon cell proliferation. Cyclic AMP was critical for this antiviral effect, acting either by direct transfer of cAMP through gap junctions or by the extracellular pathway, which involves CD39 activity (Moreno-Fernandez 2011). Recently, Treg were shown to decrease HIV infection in dendritic cell-Tcon clusters using both CTLA-4 and cAMP mechanism, suggesting that Treg could reduce HIV dissemination, which would be beneficial to the host in the early stages of infection (Moreno-Fernandez 2014). In addition, Treg have been shown to decrease infection of murine macrophages by a pseudotyped HIV virus and, consequently, to attenuate HIV-associated neurodegeneration (Liu 2009).

On the other hand, Treg also play a detrimental role during HIV infection. Treg have been shown

to be a major source of TGF- β . Treg contributed heavily to the development of fibrosis in SIV-infected rhesus macaques as TGF- β exacerbates collagen deposition within T-cell zones that destroys the lymphatic tissue architecture and hampers CD4 reconstitution in the lymphoid tissues of infected animals (Estes 2007). In addition, as described above, Treg suppress HIV-specific immune responses. Depletion of Treg from mononuclear cells enhanced the T-cell response to HIV/SIV antigens, and their activity contributed to the diminution of HIV-specific immune responses in the early stages of HIV infection (Hryniewicz 2006; Kinter 2004). Rhesus macaques acutely infected with SIV have increased Treg frequency that correlated with inefficient SIV-specific CD8⁺ responses. Furthermore, a high-perforin/FOXP3 ratio was associated with nonprogressive disease, suggesting that the immune control of virus replication represents a balance between CMI and Treg-mediated counter regulation of such responses (Nilsson 2006). Confirming this hypothesis, an important study recently showed that HIV-specific CD8⁺ T cells bearing protective HLA alleles (HLA B*27 and B*57) are able to evade Treg suppression (Elahi 2011). This suggests that alternative mechanisms to evade Treg suppressive activity have emerged to counteract their deleterious effect on HIV-specific immune responses.

Treg could decrease HIV-specific responses via several mechanisms. The role of CTLA-4 is suggested by the fact that in vivo CTLA-4 blockade increased CD4⁺ and CD8⁺ T-cell effector function during the chronic phase, when given in combination with ART (Hryniewicz 2006). Blockade of CD39 partially decreased the suppressive effect of Treg on effector T cells (Nikolova 2011). Of note, a CD39 gene polymorphism that leads to low CD39 expression is associated with the relative protection against development of AIDS (Nikolova 2011). Together these data demonstrate that Treg activity may contribute to the inability of the host to clear HIV infection both in vivo and in vitro.

Potential role of Tregs during HIV infection



Role of Regulatory T Cells During HIV Infection, Fig. 2 Role of Treg during HIV infection. Treg have both a detrimental and beneficial role during the acute and chronic phases of HIV infection. Increased Treg frequency during the early acute phase of HIV infection (*right side*) may have an overall beneficial effect, by controlling T-cell activation, inflammation, and tissue damage and by decreasing the availability of target cells for HIV

replication. During the late acute and chronic phase (*left section*), the increased frequency of Treg is likely deleterious as they dampen specific immune responses against HIV and other pathogens. Treg also contribute to lymphoid tissue fibrosis by secreting large amounts of TGF-β. At this stage, increased Treg frequency likely favors disease progression. However, protection against tissue damage may persist

In vivo studies directly evaluating the role of Treg during HIV/SIV infection are scarce. One study in chronically SIV-infected African green monkeys treated with Ontak (a fusion protein that combines a recombinant IL-2 and a cytotoxic diphtheria toxin moiety that depletes CD25⁺ cells) showed that treated animals had significantly higher levels of immune activation and a significant increase in viral replication compared to control animals suggesting that Treg can control virus infection in vivo. However, it is important to note that the data from this study are difficult to interpret, as the frequency of FOXP3⁺ cells was not significantly decreased in the Ontak-treated animals, suggesting that Ontak may have acted through another mechanism. Notably, the higher levels of immune activation induced by Ontak treatment may have increased the numbers of available cellular targets for HIV infection.

Conclusion

Excessive activation and regulatory processes coexist through the entire course of HIV infection. As HIV infection progresses, a relative expansion of Treg cells occurs in the early stages when viral replication is high and before severe damage of

the GALT occurs. At this stage, Treg might control Tcon activation, reducing the proliferation and the number of target cells for the virus (Fig. 2, right section). This effect of Treg is thought to result in decreased HIV replication and limited disease progression. At the post-acute stage when the viral load is reduced and HIV-specific immune responses are established, the balance between regulatory and effector cells is lost and the increased frequency of Treg may become deleterious due to their suppression of HIV-specific immune effector mechanisms (Fig. 2, left section). In addition, Treg inability to suppress systemic hyperactivation and their promotion of tissue fibrosis may potentiate their role in HIV pathogenesis (Fig. 2, left section). Gaining a better understanding of the mechanisms regulating Treg dynamics and their role during HIV infection remains an important question, as this knowledge will help in determining whether Treg function or number can be successfully manipulated to the advantage of the infected host.

Cross-References

- ▶ [T-Cell Homeostasis](#)
- ▶ [Th17 Cells](#)

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Role of Transportin-SR2 (Transportin-3, TRN-SR2, TNPO3) in HIV Replication

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Definition

TRN-SR2 (TNPO3, transportin-3) is a karyopherin of the importin- β superfamily. As such it imports protein cargos from the cytoplasm

into the nucleus. Alike for all members of this family, the import of cargos is driven by the GTP/GDP gradient between the cytoplasm and the nucleus of the cell and therefore regulated by RanGTP-induced release of the cargo in the nucleus. TRN-SR2 is known to import SR-rich proteins in to the nucleus and has been proposed to be responsible for PIC nuclear translocation essential for provirus formation and a bottleneck in HIV replication.

Nuclear Import, a Critical Step in HIV Replication

Lentiviruses, such as the human immunodeficiency virus type 1 (HIV-1), are unique among the retroviruses because of their capacity to infect nondividing cells. The lentiviral DNA, packed in the nucleoprotein pre-integration complex (PIC), is recognized by the cellular nuclear transport machinery and traverses the nuclear envelope in an active- and energy-dependent manner to integrate into human chromatin. Gammaretroviruses, however, such as murine leukemia virus (MLV) require nuclear envelope breakdown during mitosis to gain access to the cellular chromatin and efficiently integrate. Although this process has been studied over decades, implementing different viral components and cellular nuclear import factors in the process, only recently the importin- β like karyopherin TRN-SR2 (transportin-3, TNPO3 (Lai et al. 2000, 2001, 2003; Yun et al. 2003)) has been proposed to play an important role in HIV nuclear import. In 2008 three independent reports using either siRNA screening (Brass et al. 2008; Konig et al. 2008) or yeast two-hybrid (Christ et al. 2008) identified TRN-SR2 as an important cellular factor for HIV replication. In the yeast two-hybrid approach, HIV integrase was identified to interact with TRN-SR2 (Christ et al. 2008), indicating a role of TRN-SR2 in the nuclear import of the viral PIC.

TRN-SR2 (Transportin-3, TNPO3) an Importin of the Importin- β Family

TRN-SR2 (Lai et al. 2000) shuttles essential splicing factors, the serine-/arginine-rich proteins

(SR proteins), between the cytoplasm and the nucleus of cells and therefore is involved in the regulation of mRNA splicing. The recognition of the SR proteins by TRN-SR2 relies on the conserved RS domain and requires phosphorylation (Lai et al. 2000, 2001). Yun et al. showed that TRN-SR2 like its splicing variant TRN-SR1 is encoded by *TNPO3* and is expressed via alternative splicing (Yun et al. 2003). In most tissues and in all cell lines analyzed so far, only TRN-SR2 is expressed, whereas the TRN-SR1 isoform is undetectable (Yun et al. 2003). By yeast two-hybrid screening, multiple non-SR proteins have been identified as binding partners of TRN-SR2. The most prominent binding partner is the RNA-binding motif protein 4 (RBM4), a zinc finger protein involved in splicing regulation (Lai et al. 2003). TRN-SR2 is a member of the importin- β family of nuclear importins and therefore depends on the RanGTP/RanGDP gradient between cytoplasm and nucleus for efficient nuclear import of its cargos. Despite the lack of knowledge regarding the structure of TRN-SR2, its close relation and homology with importin- β proteins suggests that its three-dimensional structure consists of stacked HEAT domain repeats containing two antiparallel α -helices linked by a flexible loop. No clear independent domains can be assigned, but overlapping functional domains can be defined: (1) the N-terminal RanGTP-binding domain, (2) the central nuclear pore-binding domain, and (3) the C-terminal cargo-binding domain. Indeed the C-terminal domain of TRN-SR2 was shown to be involved in cargo binding (Lai et al. 2000).

TRN-SR2 and HIV Replication

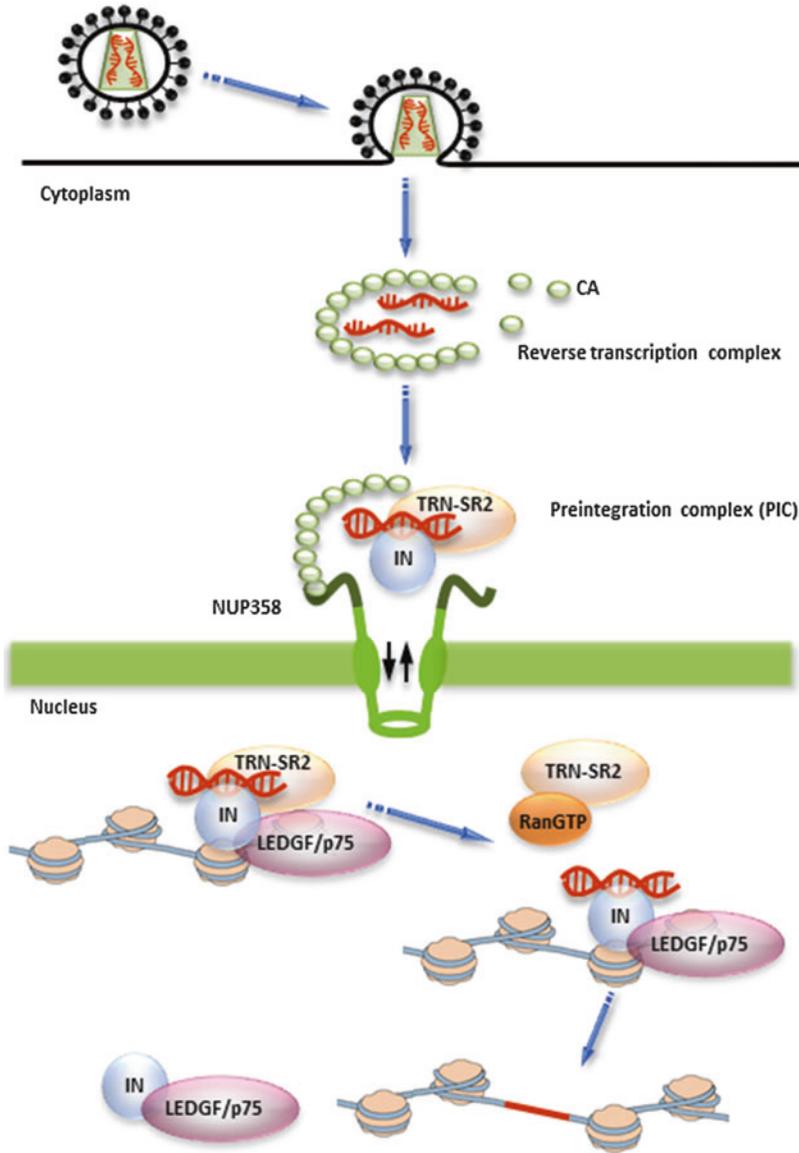
Between the first reports of the involvement of TRN-SR2 in HIV replication (Brass et al. 2008; Konig et al. 2008; Christ et al. 2008) in 2008 and today, multiple groups have been elaborating on its function in HIV replication (Christ et al. 2008; Cribier et al. 2011; De Iaco and Luban 2011; Krishnan et al. 2010; Lee et al. 2010; Logue et al. 2011; Ocwieja et al. 2011; Schaller et al. 2011; Thys et al. 2011; Valle-Casuso

et al. 2012; Zhou et al. 2011). Although consensus is reached that TRN-SR2 indeed is an important cofactor of early HIV replication acting prior to provirus establishment in the host chromatin, the detailed underlying mechanism is still controversial. TRN-SR2 was identified as an interaction partner of the viral protein integrase in yeast two-hybrid screening (Christ et al. 2008). A reverse screen confirmed the exclusive interaction between both proteins excluding interactions with other viral proteins under these experimental settings. The high-affinity interaction was independently confirmed by others (Krishnan et al. 2010). Two groups though demonstrated that the *in vitro* interaction with TRN-SR2 is not exclusive for lentiviral integrases (Krishnan et al. 2010; Thys et al. 2011) but is also observed with other retroviral integrase such as MLV and RSV that do not depend on TRN-SR2 for viral replication (Krishnan et al. 2010; Logue et al. 2011; Thys et al. 2011). Thus the lentiviral specificity of the TRN-SR2 phenotype does not depend on the integrase but must be determined by at least one second viral determinant. The early report that a mutant, N74D, of the viral capsid protein apparently rendered HIV independent of TRN-SR2 knockdown brought the capsid protein into the picture (Lee et al. 2010). The N74D mutant had been selected to be resistant for the overexpression of a deletion mutant (385) of the cleavage and polyadenylation specific factor 6 (CPSF6) which is in turn a SR-rich protein and therefore a likely cargo of TRN-SR2. Although to date no direct evidence has been provided that CPSF6 is indeed a cargo of TRN-SR2, the CPSF6_385 deletion mutant, lacking the SR-rich domain, does not translocate to the nucleus and stays in the cytoplasm where it most likely binds to the viral capsid and slows down the cytoplasmic uncoating of the viral particle (Lee et al. 2010). Even though the detailed mechanism underlying the link between both processes is not understood, the data suggest coupling between nuclear import and uncoating of the viral particle. In further support of this notion, the TRN-SR2 requirement for viral replication was shown to be associated with the viral entry pathway into the cell (Thys et al. 2011). VSV-G-pseudotyped HIV

vectors enter the cell through endocytosis, whereas HIV enters through membrane fusion. These different entry pathways likely affect the uncoating process. This underlines the care to be taken when analyzing the early steps of HIV replication (prior to ► [integration](#)) with pseudotyped vectors instead of HIV-enveloped replication competent viruses. In fact the initial finding that the N74D capsid mutant replicates independently from TRN-SR2 (Lee et al. 2010) needs to be revised after analyzing the replication of the N74D molecular clone. The replication competent virus with N74D capsid is still dependent on TRN-SR2 (Thys et al. 2011).

Different groups have studied the physical interaction of TRN-SR2 with capsid but no consensus on the functional implications has been reached (Valle-Casuso et al. 2012; Zhou et al. 2011). *In vitro* interaction studies of capsid protein with potential binding partners might be biased by the multimeric state of capsid and therefore should be interpreted with care unless a specific high-affinity interaction with reconstructed core particles can be demonstrated. Independent confirmation in literature will be needed to further support the interaction between capsid and TRN-SR2 and to clarify the underlying mechanism.

During HIV replication not all reverse transcribed cDNAs are integrated in the cellular chromatin, but nonproductive byproducts, the 2-LTR circles, are formed. The general belief is that such circles are formed in the nucleus and therefore they are taken as a measure of nuclear import. Interpretation of the absolute number of 2-LTR circles has to take into account that additional blocks of ► [integration](#) will lead to an increase of circles that can mask any reduction caused by a nuclear import defect. Several groups have studied how knockdown of TRN-SR2 affects the number of 2-LTR circles. In 2008 a 2- to 3-fold decrease in 2-LTR circles after siRNA-mediated knockdown of TRN-SR2 was reported (Christ et al. 2008). Although several studies confirmed this initial finding (Logue et al. 2011; Schaller et al. 2011), other groups could not detect the decrease (De Iaco and Luban 2011; Valle-Casuso et al. 2012; Zhou et al. 2011). Although final word



Role of Transportin-SR2 (Transportin-3, TRN-SR2, TNPO3) in HIV Replication, Fig. 1 Schematically drawing of the early steps of HIV replication. After fusion to the cell membrane, the viral core is released into the cytoplasm. While the RNA is reverse transcribed into a cDNA copy and the reverse transcription complex and later the PIC travels through the cytoplasm in direction to the nucleus, uncoating progresses. Eventually uncoating has reached a state where the inner structure of the PIC is accessible to other cellular cofactors, such as TRN-SR2 (Christ et al. 2008). CA then docks the PIC to the nuclear

pore basket via its interaction with Nup358 (Schaller et al. 2011) and enables the nuclear import factor to interact with the nuclear basket leading to the passaging of the PIC into the nucleus where TRN-SR2 draws the PIC in the proximity of the preferred integration sites of HIV (Ocwieja et al. 2011), RanGTP displaces the PIC from TRN-SR2, and, via the interaction of IN with LEDGF/p75, the PIC is tethered to the host chromatin leading to provirus formation. More experimental evidence is needed to support and test the proposed hypothesis of HIV-PIC nuclear import

needs to be spoken regarding the formation of 2-LTR circles, all reports confirm that knockdown of TRN-SR2 leads to a decreased provirus formation confirming the importance of TRN-SR2 for early steps of the replication cycle (Christ et al. 2008; Cribier et al. 2011; De Iaco and Luban 2011; Logue et al. 2011; Ocwieja et al. 2011; Schaller et al. 2011; Valle-Casuso et al. 2012; Zhou et al. 2011). Analyzing the cellular localization of fluorescently labeled PICs in cell culture supports the notion that TRN-SR2 is directly involved in nuclear import. Knockdown of TRN-SR2 with siRNA or shRNA excludes the fluorescently labeled PICs almost completely from the nucleus (Christ et al. 2008). The Bushman group demonstrated that depletion of TRN-SR2 alters the ► [integration](#) site distribution of HIV but not MLV, pointing toward a direct or indirect role of TRN-SR2 in ► [integration](#) site selection (Ocwieja et al. 2011). Although several questions remain unanswered, it is clear today that TRN-SR2 is an important cofactor of HIV replication (Christ et al. 2008; Cribier et al. 2011; De Iaco and Luban 2011; Logue et al. 2011; Ocwieja et al. 2011; Schaller et al. 2011; Valle-Casuso et al. 2012; Zhou et al. 2011). As the single steps which lead to provirus formation (uncoating of the incoming viral particle, reverse transcription, nuclear import, and integration) are interdependent, the detailed understanding of the TRN-SR2 mechanism is a challenging task. In any case it has become clear that nuclear import is very tightly linked to uncoating and even that uncoating might only be completed through nuclear import since CA promotes the docking of the PIC to the nuclear pore protein, NUP358 (Schaller et al. 2011). Future work will help to elucidate the interplay of TRN-SR2 with integrase and/or capsid and will shed light on the function of TRN-SR2 in lentiviral replication.

Conclusions

TRN-SR2 (TNPO3, transportin-3) is a classical importin- β like nuclear import factor present in all cell types. Multiple lines of evidence suggest an important role of TRN-SR2 in HIV

replication, although the detailed underlying mechanism still needs to be understood. The interaction of TRN-SR2 with HIV-IN seems at least partially responsible for the nuclear import of the PIC. Other tightly associated viral processes such as uncoating are rate limiting for the nuclear import and are therefore tightly linked to the nuclear import of the HIV-PIC (Fig. 1).

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Salmonellosis and Other Bacterial Enteric Infections and HIV

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Definition

Enteritis by definition is inflammation of the intestinal wall; this inflammation could be small intestine or large intestine or both. Inflammation may be infectious or noninfectious in origin. Among infectious etiologies, bacterial organisms are most commonly associated with acute infectious enteritis. In this entry enteric bacterial infections in persons living with HIV are discussed as they have been an important cause of morbidity and mortality in this vulnerable population. The rates of gram-negative bacterial infections are at least tenfold higher in persons living with HIV than in the general population. The risk of symptomatic disease varies according to the immune status (as measured by CD4 count) of the host. The greatest risk is in individuals with CD4 count less than 200 cells/mm³. In this entry we aim to provide a concise review of the enteric bacterial pathogens that play a very important role in

causing enteritis in the HIV patient population. The first, historical perspective about the enteric bacterial infections during the late 1980s and 1990s when immune compromised HIV patients were the norm will be presented. This will be followed by data on enteritis from the current era of widespread use of antiretroviral therapy which may alter the presentation and frequency of these pathogens. While emerging bacterial pathogens will be discussed, pathogens such as mycobacteria, parasites, or viruses will not be discussed.

Overview

The most commonly encountered enteric bacterial pathogens are salmonella, shigella, and campylobacter and the diarrheagenic *Escherichia coli*. There has been a recent emergence of *Clostridium difficile* and enteroaggregative *Escherichia coli* infections among persons living with HIV. Infection with any of the above pathogens can have differing clinical presentations depending on the region of the gut infected and the patient's immune status. Infections of the small bowel present as watery, often dehydrating, diarrheal illnesses, and infections of the colon are more likely to be associated with smaller volume often bloody or inflammatory stool. Colitis in persons living with HIV may be complicated by bacteremia with extra-intestinal manifestations (AIDS info 2016).

Salmonella

Early in the AIDS pandemic, salmonella was recognized as an important opportunistic pathogen. Patients with AIDS had a 20–100-fold increased incidence of salmonellosis (Angulo and Swerdlow 1995). Salmonellosis heralded the development of other opportunistic infections and is an AIDS-defining condition (Celum 1987). The incidence of salmonella bacteremia is high in persons living with HIV, and recurrence is common due to the intra-macrophagic persistence of salmonella organisms (Casado 1999). With the institution of antiretroviral therapy in the 1990s, mortality from nontyphoidal salmonella bacteremia decreased significantly (Arthur 2001).

Microbiology

Salmonella is a motile gram-negative bacilli that is oxidase and lactose negative and produces hydrogen sulfide. Salmonella grows well in both aerobic and anaerobic conditions. Salmonella grows on XLD (xylose lysine deoxycholate) agar. All of the clinically relevant salmonella fall under *Salmonella enterica* subsp. *enterica*. This is further subdivided into multiple serotypes each causing a different clinical disease (Tindall 2005).

Serotype (serogroup)	Clinical syndrome
<i>S. paratyphi A</i> (A)	Enteric fever
<i>S. paratyphi B</i> (B)	Gastroenteritis or enteric fever
<i>S. typhimurium</i> (B)	Gastroenteritis
<i>S. paratyphi C</i>	Enteric fever
<i>S. choleraesuis</i> (C)	Bacteremia
<i>S. newport</i> (C)	Gastroenteritis
<i>S. typhi</i> (D)	Enteric fever
<i>S. enteritidis</i> (D)	Gastroenteritis
<i>S. dublin</i> (D)	Bacteremia

Epidemiology and Transmission

Typhoidal Salmonella Typhoid and paratyphoid are major public health problems in the resource-limited setting. In 2010 there was an estimated 13.5 million cases of typhoid fever globally. Incidence (episodes per 100,000 person years) of typhoid fever was highest in the sub-Saharan Africa (724.6), followed by South Asia (394.2), and East and South East Asia (29.2). With

regard to paratyphoid fever, the incidence was highest in sub-Saharan Africa (77.4) and South Asia (394.2) followed by East and South East Asia (29.2). North America had an incidence of 0.1 for typhoid fever (Buckle 2012).

Typhoid is endemic in regions of the world which are overcrowded with poor sanitation (Crump 2004). Humans are the only reservoir of *S. enterica serotype typhi*. So contact with a known typhoid case or carrier is needed to cause transmission. History of travel to typhoid endemic areas with poor sanitation is also one of the major causes of typhoid in United States (Lynch 2009).

Nontyphoidal Salmonella The global burden of nontyphoidal salmonellosis was estimated to be 93 million in 2006 with an incidence of 1140 per 100,000 person years (Majowicz 2010). Outbreaks of nontyphoidal salmonellosis were most commonly associated with poultry and eggs. Reptiles and amphibians especially when they were pets can also carry and transmit salmonellosis (Bennett 2015).

Clinical Syndrome

Clinically, *Salmonella* can present in one of the following five ways (Bennett 2015; Cohen 1987):

- Asymptomatic chronic carrier state
- Gastroenteritis
- Enteric fever
- Bacteremia and endovascular infections
- Extraintestinal focal infections

Typhoidal *Salmonella* (*S. typhi*, *S. paratyphi A*, *S. paratyphi B*, and *S. paratyphi C*) causes enteric fever. Typical enteric fever is characterized by onset of symptoms, 5–21 days after the ingestion of the causative microorganism. During the first week, a patient may experience rigors from bacteremia often with a relative bradycardia or a pulse temperature dissociation. During the second week, the patient may develop “rose spots” which are faint salmon-colored macules on the trunk and abdominal pain. During the third week, if left untreated patients can develop intestinal bleeding or intestinal perforation due to the

ileocecal lymphatic hyperplasia of the Peyer patches (Stuart and Pullen 1946). HIV infection in itself does not change the course of enteric fever. But in AIDS patients, enteric fever may have an atypical presentation, have an increased rate of relapse, and develop into a chronic carrier state (Gutuzzo 1991).

Nontyphoidal *Salmonella* (NTS) cause gastroenteritis with or without bacteremia. Presence of NTS bacteremia in the absence of any other immunodeficiency can be an early sign of HIV infection. Also HIV confers a significant risk for bacteremia (primary and secondary) and focal extraintestinal infections. The extraintestinal sites of infections are the urinary tract, lung, pleura, joints, bones, heart, and central nervous system (Rodriguez 2006; Cohen 1987). NTS bacteremia has a predilection to seed the vasculature especially the large vessels with atherosclerotic plaques and may cause mycotic aneurysms of the aorta (Benenson 2001).

Shigella

Microbiology

Shigella species are a major cause of bacterial dysentery worldwide. *Shigella* are facultative anaerobes and are gram-negative rods. There are four species of *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. *Shigella* are nonflagellated, indole positive, and urea and oxidase negative, ferment glucose, and don't produce hydrogen sulfide. They grow well in MacConkey agar.

Epidemiology and Transmission

The global burden of shigellosis was estimated to be 165 million each year (Kotloff 1999). In the resource-limited setting, most cases are transmitted by the fecal–oral route from people with symptomatic infection. In the resource-sufficient world, outbreaks of shigella infection have been reported among MSM. In the person living with HIV, raw vegetables traced to a common supplier, cold salads, and day care centers and areas with crowded living condition are all risk factors for shigellosis (Niyogi 2005; Aragon et al. 2007). Men who have sex with men (MSM) have an increased risk of shigellosis due to direct oral–anal contact (Aragon et al. 2007).

Clinical Syndrome

Shigellosis, also known as acute bacillary dysentery, is an infectious inflammation of the colon. The disease is characterized by a prodrome of fatigue, malaise, and anorexia followed by diarrhea. Initially, diarrhea can be watery (*S. sonnei*), and then it can progress to bloody diarrhea with tenesmus (*Shigella dysenteriae* 1 or *Shigella flexneri*) (Khan 2013). In a normal host, the course of the disease is generally self-limited and will resolve within 7 days if left untreated. HIV infection in itself is not clearly associated with increased risk of *Shigella* gastroenteritis (Angulo and Swerdlow 1995). Intestinal complications include intestinal perforation, toxic megacolon, and rectal prolapse (Bennish 1991). *Shigella* bacteremia is a rare occurrence in the general population. But it has been more prevalent in the HIV patient population, occurring both in the early and later course of HIV infection with increased mortality (Keddy 2012). Recurrent shigella bacteremia was also found among AIDS patients (Angulo and Swerdlow 1995). Other systemic complications include encephalopathy manifesting as seizures, electrolyte abnormalities from dehydration, microangiopathic hemolytic anemia, and post-infectious inflammatory arthritis (Niyogi 2005).

Campylobacter

Microbiology

Campylobacters are motile gram-negative bacteria which can be of spiral, rod, or curved shape. They are microaerophilic and nonspore forming (Mandell 2015). *Campylobacter jejuni* and *Campylobacter coli* are the most important species to cause human infections. *Campylobacter* spp. multiply slowly, and isolation of these organisms requires growth in a blood-based, antibiotic-containing media (Bennett 2015). *C. upsaliensis* and *C. concisus* are emerging pathogens in patients with gastroenteritis (Man 2011).

Epidemiology and Transmission

The incidence of campylobacter cases has been increasing globally over the past decade. Incidence in the United States in 2012 is 14.3 per 100,000 population. In Europe, incidence ranges

from 0.92, in Poland from 2011 to 2012 to a maximum of 81.4 per 100,000 population in Germany from 2005 to 2011 among reported studies. The prevalence of campylobacter gastroenteritis in China and India ranges from 5% to 16% in the last decade (Kaakoush 2015). Risk factors for campylobacteriosis are international travel, exposure to animals, and contaminated food and water (Kaakoush 2015). With regard to international travel, the greatest risk for acquiring infection is travel to Asia, Africa, Latin America, and the Caribbean (Mughini-Gras et al. 2014). Among foods, consumption of poultry products especially broiler chicken and unpasteurized milk is associated with infection. Domesticated animals like cattle, sheep, pigs, dogs, and cats are known reservoirs of campylobacter and have been implicated in human infections (Kaakoush 2015).

Clinical Syndrome

Campylobacter enteritis is characterized by a prodrome of fever, malaise, and abdominal pain which lasts for about 12–24 h. This is followed by diarrhea, which can range from loose watery stools to bloody stools. Diarrhea can last for up to 7 days, but the organism can be shed in the stool for several weeks (Bennett 2015). In the HIV patient population, the disease tends to be severe and persistent. HIV patients are also at risk of developing recurrent episodes of enteritis, extra-intestinal campylobacter infections, and long-term carriage. The incidence of campylobacter bacteremia has not consistently been increased in patients with AIDS (Angulo and Swerdlow 1995; Molina 1995).

Escherichia coli (*E. coli*)

E. coli infection which has acquired diarrheagenic virulence factors is a common cause of diarrheal illness worldwide. *E. coli* are a part of the normal flora of the human gut. But the acquisition of genes for virulence factors such as adhesins or toxins by *E. coli* allows *E. coli* to cause different kinds of diarrheal illness in humans. The currently recognized pathotypes are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli*

(EAEC). Among these enteroaggregative *E. coli* is associated with an increased risk in patients with HIV. In HIV patients especially those who are immunocompromised, infection with EAEC causes chronic diarrhea and malnutrition (Mayer and Wanke 1995). There has been an association of EAEC in the stool of chronic diarrheal patients with low CD4 counts and weight loss. Thus it may be easier for an immunocompromised host to develop EAEC infection (Wanke 1998).

Clostridium difficile Infection in HIV

Clostridium difficile infection (CDI) was found to be the most common cause of bacterial diarrhea in a study among persons living with HIV from 1992 to 2002 (Sanchez 2005). In another study from 2003 to 2011 among the persons living with HIV, clinical manifestations among persons living with HIV did not differ significantly from the disease in non-HIV patients. A CD4 count of less than or equal to 50 cells/mm³ was identified as an independent risk factor for acquisition of CDI. Other risk factors for acquisition of CDI were the use of gastric acid suppressants, the use of antibiotics, a recent hospital stay, and the use of immunosuppressive medications (Haines 2013).

Proctitis

The most common bacterial causes of infectious proctitis are *Neisseria gonorrhoea*, *Chlamydia trachomatis*, and *Treponema pallidum* (Hoentjen and Rubin 2012). The most common cause of proctitis is anal-receptive intercourse from an individual infected with gonorrhoea or chlamydia; the most common etiology is gonorrhoea followed by chlamydia. More than one infectious agent may occur concurrently in proctitis at the same time (Klausner 2004). Rectal inflammation in proctitis is associated with an increased risk of acquiring HIV infection (Craib 1995).

Evaluation

The evaluation of diarrhea in a person living with HIV does not need to be fundamentally different than the evaluation of diarrheal illness in the general population. Thorough history taking is a

crucial part of the initial evaluation; questions related to characterization of diarrhea should be gathered in detail and should include duration of diarrhea, associated symptoms, quality of diarrhea (is blood present), frequency of diarrhea (stooling), and presence of tenesmus. Weight loss, if present, should be noted. Then, specific emphasis should be focused on exposures: animals, pets, recent travel, and sick contacts. Dietary history including consumption of raw foods, salads, and green leafy vegetables, method of food preparation, dining at restaurants, and travel history is important. A detailed review of medications the patient is taking currently or in the recent past is also important. It is important to keep in mind that antiretroviral therapy in itself can cause diarrhea as a side effect.

Laboratory evaluation should begin with complete blood count and renal function tests to assess hydration status in the person living with HIV; CD4 count should be determined to assess which pathogens may occur. In the person living with HIV, blood cultures should also be drawn as incidence of salmonella, and campylobacter bacteremia is high in this patient population. Stool microscopy, ova and parasite screen, and stool cultures should be part of the standard evaluation for diagnosis. Specification of which pathogens are being sought will allow the microbiology lab to perform the appropriate stains or tests. Stool testing for *Clostridium difficile* toxin by EIA or PCR should be added to patients who are at risk of *Clostridium difficile* infection. The Widal test which detects *Salmonella* antibodies has been used for the diagnosis of salmonellosis, but it has limited clinical utility due to insufficient sensitivity and specificity. Additional workup to look for nonbacterial causes of diarrhea is also important. Other possible differential diagnoses in this patient population would be *Cytomegalovirus*, *Mycobacterium avium complex* infections, and malignancies like Kaposi's sarcoma and lymphoma. Endoscopic evaluation would be beneficial in cases in which the standard evaluation is uninformative. Based on the symptomatology, upper gastrointestinal endoscopy or colonoscopy or both are done to evaluate the intestinal mucosa (Wilcox and Saag 2008; AIDS info 2016).

Prevention and Prophylaxis

Regular handwashing with soap and water or alcohol-based cleaners is the best way to prevent enteric bacterial infections. The MSM patient population should be advised to avoid unprotected sexual practices such as anal sex and oral–anal contact as there is a risk of oral exposure to feces. People recovering from typhoid fever or those who have become chronic carriers of *Salmonella* should be excluded from food preparation activities. Killed or live vaccine is available for typhoid prevention.

Routine antimicrobial prophylaxis is not recommended to prevent enteric bacterial infections in HIV patients. Fluoroquinolones, azithromycin, or rifaximin can be considered as antimicrobial prophylaxis for travelers with severe immune compromise. Patients who are already taking trimethoprim–sulfamethoxazole benefit from limited protection for travelers' diarrhea, although many pathogens have become resistant to trimethoprim–sulfamethoxazole.

Treatment

The cornerstone of treatment of infectious diarrhea is adequate rehydration. The use of sufficient fluid and electrolytes, whether given by the oral or intravenous route, is crucial. Most patients can take fluid in the form of oral rehydration fluid. Calculating the loss of total body water and matching that with the rate of water and fluid repletion can have a beneficial impact on outcomes. Below are the individual antibiotic regimens to pathogens in HIV patients.

Salmonella

Typhoid fever: All persons infected with HIV who are diagnosed with typhoid fever should be treated. This is due to the increased risk of salmonella bacteremia in this patient population. The choice of the empiric antibiotic regimen depends on the clinical setting, susceptibility testing of the bacteria, severity of illness, and ability of the patient to tolerate oral antibiotics. For the patient who is not severely ill, the preferred regimen is

ciprofloxacin 500–750 mg oral every 12 h for duration of 5–7 days. For patients in areas of increased incidence of resistance to nalidixic acid or documented resistance to quinolones, azithromycin or cefixime is the preferred choice. Alternative regimens used in case of allergy or contraindications are co-trimoxazole, amoxicillin, or chloramphenicol.

In patients who are severely ill or not able to tolerate oral antibiotics, admission to the hospital and initiation of intravenous antibiotics are crucial (Bhan 2005). Given the increasing resistance of salmonella to quinolones, intravenous treatment with a cephalosporin such as ceftriaxone 2 g daily is often started until resistance testing is available.

The duration of therapy depends on the immune status of the patient, presence or absence of bacteremia, and presence of metastatic foci of infection. When the CD4 count is <200 cells/mm, a 2–6-week course is recommended. When CD4 count is >200 cells/mm, a 7–14-day course is recommended. A longer duration is recommended when bacteremia persists or if there is a metastatic foci of infection. Recurrent salmonella bacteremia in itself constitutes an AIDS-defining illness. Management of HIV with antiretroviral therapy and suppression of the viral load lead to decreased risk of recurrence. Secondary prophylaxis is warranted for patients who have a CD4 count of <200 cells/mm with severe diarrhea and patients with recurrent gastroenteritis with or without bacteremia. Secondary prophylaxis can be discontinued when the CD4 count is >200/mm and sustained viral suppression is achieved (AIDS info 2016).

Management of chronic carrier state is based on the presence of abnormal biliary anatomy like the presence of gallstones. Preferred antibiotic regimen for the eradication of chronic carrier state is the use of ciprofloxacin 750 mg oral twice daily for 28 days or norfloxacin 400 mg orally twice daily for 28 days. Cholecystectomy is required for patients with gallstones in addition to antibiotics (Bhan 2005).

Nontyphoidal Salmonella (NTS)

Gastroenteritis caused by NTS is usually self-limiting and needs supportive care. But in HIV

patients, a short-course treatment of 3–7 days with quinolones is helpful to diminish symptoms and prevent long-term carriage. Quinolones are the preferred first-line antibiotics. Alternatives are ceftriaxone, cefotaxime, trimethoprim–sulfamethoxazole, and ampicillin. Carbapenems are the drug of choice when multidrug resistance is encountered. HIV patients are at an increased risk of NTS bacteremia and metastatic foci of infections. NTS can seed large vessels leading to mycotic aneurysm and rupture. Seeding of an aneurysm can be a source of recurrent bacteremia (Benenson 2001). Management of endovascular infections involves a combination of prolonged antibiotic therapy and surgical procedures including endograft placements. Duration of antibiotic therapy will depend on the immune status of the patient (CD4 count), presence of metastatic foci of infection, adequacy of source control, and retained foreign material. Duration can range anywhere from 4 weeks to 12 weeks, and sometimes the patient will need oral antibiotic suppression after completion of the treatment course.

Shigella

Treatment of shigellosis in HIV patients is recommended to shorten the duration of illness and prevent the spread of infection to others. The preferred regimen is fluoroquinolone like ciprofloxacin 500–750 mg PO Q12 hourly. Alternative regimens especially in areas of quinolone resistance are trimethoprim–sulfamethoxazole and azithromycin. Typical duration of treatment for gastroenteritis in immunocompromised patients is 7–10 days. When oral azithromycin is used, duration can be shortened to 5 days. But azithromycin is avoided when there is concomitant bacteremia. For shigella bacteremia treatment duration is extended to 2 weeks (Niyogi 2005; AIDS info 2016).

Campylobacter

Campylobacter gastroenteritis is a self-limiting disease, and no intervention is required in mild cases with intact immunity (Ternhag 2007). If there is persistent diarrhea, if the disease is moderate to severe, or if the patient is

immunocompromised, treatment is recommended. Ciprofloxacin 500–750 mg PO twice daily (7–10-day course) or azithromycin 500 mg PO daily (5-day course) is the preferred regimen. There is an increasing rate of fluoroquinolone resistance worldwide (Gupta 2004). Antibiotic therapy should be guided based on the susceptibility report. In patients with bacteremia, empiric ciprofloxacin and an aminoglycoside are used until the sensitivities are back. Duration of antimicrobial therapy in bacteremia is 14 days and recurrent bacteremia is treated for 2–6 weeks (AIDS info 2016).

Others

HIV patients infected with enteroaggregative *E. coli* gastroenteritis have been treated with a course of ciprofloxacin 500 mg oral twice daily for 7 days. This regimen leads to eradication of EAEC from the stool culture, decrease in frequency of diarrhea, and improved general sense of well-being (Wanke 1998). Treatment of gonococci or chlamydia proctitis is standard as in non-HIV patients. It is treated with a regimen consisting of ceftriaxone 500 mg IM one dose with azithromycin 1 g oral once. Treatment of *Clostridium difficile* infection in HIV patients is similar in the general population.

Conclusion

Salmonella and other enteric pathogens are an important cause of morbidity and mortality in the HIV patient population. The risk of acquiring these infections is significantly associated with the immune status of the patient. Thus initiating early antiretroviral therapy is an important part of management of these infections. As a patient develops symptoms, it is important to obtain the necessary cultures and sensitivities. Due to the emergence of multiple resistances to commonly used antibiotics, it is important to reevaluate if the patient is not responding to the empiric regimen. In bacteremic and metastatic infections, prolonging the course of antibiotics and adequate source control is needed to prevent relapses.

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Sex Work and HIV Prevention

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Definition

The phrase “sex work and HIV prevention” refers to any strategy undertaken to prevent the acquisition or transmission of HIV/AIDS in the context of commercial sex transactions (e.g., where there is exchange of sex for money between consenting adults). This may include HIV prevention among sex workers or their clients (sex buyers) or both, though the vast majority of programs and research to date have focused on sex workers alone. While HIV prevention within sex work typically focused on prevention of sexual acquisition or transmission of HIV among sex workers and their clients, it may also include drug-related transmission routes where there is overlap between sex workers and clients who use drugs. This entry will focus on HIV prevention through sexual contact but will

also discuss HIV prevention within sex work where there is overlap with drug use.

Global Burden of HIV Among Sex Workers

Worldwide, sex workers remain highly vulnerable to HIV in most settings, due to a complex interplay of structural and environmental factors (Shannon et al. 2015). Sex work remains hidden and marginalized in many countries, due to extreme gender-based discrimination and social stigmatization of sex work (UNAIDS 2009). Laws surrounding sex work further drive sex work underground, making it difficult for HIV prevention to reach sex workers and clients (UNDP 2012). While there are some examples of environments of sex work that have been conducive to implementing HIV prevention, treatment, and care, many other environments compromise sex workers' health and safety due to vulnerability to violence, coercive and exploitative management, debt bondage, economic insecurity, low pay, poor working and housing conditions, and insufficient access to health and social resources and services (e.g., male and female condoms and water-based lubricants, post-exposure prophylaxis following unprotected sex and rape, management of sexually transmitted infections (STIs), drug treatment) (UNAIDS 2009). Even if services are available to sex workers, use of services may be limited by stigmatization or coercive practices (UNAIDS 2009). The lack of availability of safe, supportive channels for sex workers to report rights abuses further exacerbates harm to sex workers (UNAIDS 2009). The vulnerability of sex workers to HIV is also affected by the local epidemic structures, including level of antiretroviral (ARV) coverage, STI epidemics, overlap with HIV epidemics among key populations (e.g., injection drug users, men who have sex work men), and the type of epidemic (e.g., "generalized" among general population or "concentrated" epidemics among most-at-risk populations (MARPS)).

A recent systematic review of the global HIV prevalence among sex workers in low- and

middle-income countries found that overall HIV prevalence was 11.8% (95% confidence intervals (CI): 11.6–12.0%) (Baral et al. 2012). Overall, sex workers had 13.5-fold higher odds of having HIV compared with general populations of women, irrespective of whether the HIV epidemic in that setting was a generalized or concentrated epidemic. Of note, two thirds of countries in this review had no HIV surveillance data, largely attributed to the same structural constraints (e.g., criminalization, stigmatization) that enhance HIV vulnerability in sex work (Baral et al. 2012).

HIV Prevention Approaches and Sex Work

Main Types of HIV Prevention Approaches

Different terms have been used to describe HIV prevention approaches among sex workers. A brief survey of some of the main types of approaches used is described here. "Large scale" and "small scale" are terms used to describe the size and coverage of the prevention program or intervention, usually with respect to the numbers or proportions of individuals served. "Core group" approaches refer to programs and interventions that are specifically designed for groups of individuals among whom the largest concentration of HIV infections is observed, most often including sex workers (and sometimes their clients) and men who have sex with men, as well as drug users and truck drivers, depending on the context.

Traditionally, HIV prevention in sex work has focused on biomedical and behavioral HIV prevention developed for sex workers, rather than by or along with sex workers. While sex workers have been wrongly portrayed as "vectors of disease" and "drivers" of HIV epidemics and sex work itself framed as a risk pathway, biological and behavioral factors (e.g., the use of condoms and type of sex acts within commercial transactions, high numbers of sex partners, presence or absence of STIs) more accurately shape HIV risk structures in sex work, as in sexual exchanges outside of sex work. "Biomedical" approaches typically focus on addressing HIV risk through biological factors that affect the transmission and acquisition of HIV (e.g., reducing

transmission probability by treatment of STIs or use of ARVs). “Behavioral” approaches have been influenced by a number of theoretical principles of behavior change and include an individual-level focus on changing behavior to reduce HIV risk (e.g., education to increase condom use).

There has been increasing recognition of the importance of structural and environmental factors (e.g., discrimination, stigmatization, gender inequality) in shaping increased risk for HIV within commercial transactions and along with this the development of HIV prevention interventions and programs that aim to reduce vulnerability to HIV within commercial sex transactions on structural levels (Shannon et al. 2015). Accordingly, the important roles of other individuals who are a part of commercial sex transactions besides sex workers, including clients and managers (e.g., pimps, madams, brothel owners), are beginning to be acknowledged as key in HIV prevention within sex work. In brief, “structural” approaches address upstream or root causes of HIV risk to sex workers, structural factors that shape or constrain the behavior of individuals. “Community-level” HIV prevention can describe HIV prevention implemented on a community or structural level and/or refer to an approach where HIV risk is considered an issue for communities rather than individuals to address. The integral role that sex workers should play in decisions regarding their own health, safety and well-being, and in particular to HIV prevention, has been cemented in recent years (UNAIDS 2009). “Sex worker-led” or “community-led” approaches are structural interventions that are initiated, developed, and/or implemented by relevant affected community of sex workers (e.g., sex worker organizations and/or collectives, including sex workers themselves). If a program or intervention is “peer-based,” sex workers themselves have been included as peers. “Comprehensive” approaches to HIV prevention typically refer to approaches that include multiple and different types of components. Comprehensive HIV prevention that is tailored for local contexts is increasingly identified as key in reducing sex workers’ HIV vulnerability (UNAIDS 2009). It is important to note that multiple terms are often used to describe the same type of approach and

many prevention programs can be best described as using multiple approaches (e.g., “community structural intervention” or “community-/sex worker-led structural interventions”).

- **Biomedical HIV prevention strategies**

Having an STI, including both ulcerative and nonulcerative, is thought to increase the probability that HIV will be transmitted upon sexual contact by causing lesions or inflammation that facilitates transmission of the virus (Wasserheit 1992). This relationship may exist in the other direction, with HIV amplifying the probability that STIs will be transmitted (Wasserheit 1992). Several main STI control strategies exist and have been integrated into comprehensive HIV prevention, particularly in resource-poor settings (Shahmanesh et al. 2008). These strategies include diagnosis and treatment based on observed STI symptoms (i.e., syndromic management) (Steen and Dallabetta 2003), regular screening of sex workers, and presumptive treatment (Steen and Dallabetta 2003). Rapid, point-of-care tests also exist now for many STIs (Peeling 2011). There has been little acknowledgement or research on how these approaches can constitute violation of sex workers’ rights, nor how these approaches can further marginalize and stigmatize sex workers (UNAIDS 2009; Shannon and Csete 2010; UNDP 2012). There have been increasing calls by international organizations (United Nations, World Health Organization) to not endorse the inclusion of regular screening or presumptive treatment of sex workers for these reasons. Moreover, results regarding the effectiveness of regular screening for STIs and periodic presumptive treatment of STIs on HIV among sex workers have been mixed, with strong evidence lacking regarding the effectiveness of STI screening and presumptive treatment alone (Shahmanesh et al. 2008).

Antiretroviral therapy (ART) has increasingly been accepted as part of comprehensive HIV prevention globally, particularly with the established role of treatment as prevention (TasP) (Montaner 2011). Suppressed viral

load drastically reduces the likelihood of onward transmission and can also substantially reduce HIV/AIDS-related morbidity and mortality among HIV-positive individuals (Montaner et al. 2006). While the role of TasP at a population level in onward transmission of HIV is now widely acknowledged, with strong global commitments to TasP at a policy level by international bodies, there remain limited data available on implementation across various settings (in part due to publication lag). Implementation research needs to evaluate and monitor role out of TasP on human rights of individuals and communities and ensure noncoercive practices (e.g., confidential, voluntary HIV testing) that are respected in all cases.

- **Behavioral HIV prevention strategies**

Inconsistent or non-condom use within the context of commercial sex transactions has historically been the primary means through which sex workers contract HIV through sexual transmission. Research suggests that lower condom use with intimate or other nonpaying male partners could increasingly be a key means by which sex workers contract HIV, especially in settings where interventions have shown success in increasing condom use in commercial sex transactions (Deering et al. 2011). Male condom use is thought to be the primary means with which HIV can be prevented through reducing the probability of transmission during sex contact, with condom use decreasing the per-contact probability of male-to-female transmission of HIV to about 5% when used correctly. Increased numbers of sex contacts also plays a role in increasing the likelihood that individuals will come into sexual contact with person living with HIV.

Sex workers have typically been expected to shoulder the responsibility for correct and consistent use of condoms and having reduced numbers of sex contacts to prevent the spread of HIV. In terms of HIV prevention, condom use and numbers of sex contacts have historically been conceptualized in terms of “behavior,” on an individual level, with behavioral HIV prevention influenced by a number of

theoretical principles of behavior change, including social cognitive theory and the theory of reasoned action (Fishbein and Middlestadt 1989). Aspects of social cognitive theory include knowledge, self-efficacy, and outcome expectancy, with behavior change thought to occur through activities such as observation, role modeling, performance, positive feedback, and social support. Under the theory of reasoned action, individuals’ intentions to act are thought to play the largest role in determining behavior. Individuals’ attitudes, perceptions of social norms, and personal beliefs about the consequences of behavior are thought to influence intentions. Increased education, risk reduction counseling, and condom promotion, including that provided by peers (e.g., condom demonstrations, social support), are key components of behavioral HIV prevention and have been effective at reducing HIV risk through increased condom use (Patterson et al. 2008).

Behavioral HIV prevention is mostly directed toward sex workers instead of clients, with little acknowledgement of the role that clients play in determining the use of condoms. However, HIV prevention programs and interventions have increasingly been including clients. For example, the *Avahan Indian AIDS Initiative* (“Avahan”) included a behavior change communication program that aimed to increase consistent condom use (i.e., 100%) among male clients of sex workers in four southern Indian states through the use of outdoor static promotional materials, interpersonal communication, and mid-media activities, with evidence suggesting that this program increased condom use over two years (Lipovsek et al. 2010).

- **Decriminalization of sex work as HIV prevention**

There are several main legislative approaches to sex work globally, with decriminalization of sex work increasingly cited as being a critical issue to address in HIV prevention for sex workers. In most settings most aspects of sex work either criminalized or quasi-criminalized (UNDP 2012). In brief, sex work can be

criminalized, where the buying and selling of sex itself are illegal (e.g., China, the United States, many African countries), although it is still practiced in some if not all cases (UNDP 2012). Sex work can be quasi-criminalized (e.g., Canada, India, United Kingdom) where some but not all aspects of sex work are criminalized, effectively making the practice of sex work nearly impossible without breaking laws (UNDP 2012). Another variation of quasi-criminalized approach to sex work adopted in Sweden and Iceland is the “Nordic model” where the selling of sex is legal (e.g., being a sex worker) but the purchasing of sex is criminalized (e.g., being a client/sex buyer) (UNDP 2012). In relatively few countries, sex work is legalized (e.g., sex work is legal and regulated), often in specific contexts such as Victoria, Australia, and Nevada, USA, where sex work is legal in brothels. Sex work can also be decriminalized (e.g., criminal laws prohibiting sex work are removed – e.g., New Zealand, Senegal, and parts of Australia (UNDP 2012)).

Criminalization of sex work is highly tied to stigmatization and increased HIV risk to sex workers (Shannon et al. 2015). Under criminalized and quasi-criminalized legislative frameworks, sex workers may experience police harassment as well as physical and sexual violence as part of police crackdowns, from which they have no legal protection or means to report (UNDP 2012). Police may also commit acts of sexual violence against sex workers in exchange for not arresting them, including forced and coercive sex (UNAIDS 2009; UNDP 2012). These tactics force sex work underground, displacing sex workers to unsafe areas as a means of avoiding police harassment, where they are at higher risk for client physical and sexual violence, further away from safer sex resources, HIV/STI testing, treatment, and care services (UNDP 2012). Sex workers may be inhibited from carrying condoms due to fear of police confiscation and harassment or as evidence of sex work (UNDP 2012). Sex workers may avoid HIV-related prevention, treatment, and care in criminalized

settings because of fear of disclosure of sex work. In a legalized environment, sex workers and business owners face substantial regulations and licensing not applied to other businesses (e.g., mandatory HIV/STI testing, requiring sex workers to carry permits). Mandatory HIV/STI testing of sex workers is a human rights violation and results in avoidance of care and increased HIV risk.

In a decriminalized sex work environment, sex work is regulated by the same laws that regulate other businesses (e.g., tax, employment laws, occupational health, and safety standards) (UNDP 2012). A number of international bodies, including the Global Commission on HIV and the Law, United Nations Development Programme, United Nations Programme on HIV/AIDS, and the World Health Organization, have called for the removal of criminal sanctions targeting sex workers and clients as a critical step in reducing HIV risk to sex workers, and decriminalization is therefore an important component of comprehensive HIV prevention for sex workers on a structural level (Shannon and Csete 2010; UNDP 2012). Instead, international bodies promote a public health and human rights approach, including decriminalization, addressing police harassment and violence, prohibiting mandatory HIV testing of sex workers, and providing safe spaces where sex workers can work without fear of police harassment and arrest (UNDP 2012). Emerging research from New Zealand suggests that decriminalization has had positive impacts on sex work, including reduced violence and stigmatization.

- **Violence prevention in HIV programming**
Sex workers experience high rates of occupational violence (e.g., within the context of sex work) from a variety of perpetrators, including clients, police and managers, and exploitative business owners, as well as violence by intimate or other nonpaying partners (Deering et al. 2014), which has increasingly been linked to increased HIV risk (Shannon et al. 2012). Sexual violence can increase risk for HIV/STIs through the potential damage to

vaginal tracts, facilitating transmission of infection, and the reduced likelihood of condoms being used during acts of sexual violence. Men who commit violent acts against women may themselves be more likely to have higher-risk behavior and be living with HIV/STIs (Dunkle et al. 2006). Fear or the threat of violence by clients has been linked to being pressured into not using condoms or reduced ability to insist on condom use with clients (Shannon et al. 2012). Coercive sex, rough sex, and a history of experiencing violence and sexual assault have been associated with reduced condom use and condom breakages (Shannon et al. 2012).

Despite sex workers' high risk for violence and the link between violence and HIV, there are only a few examples of HIV prevention interventions that have incorporated anti-violence strategies (Shannon et al. 2012). One of the largest-scale HIV prevention interventions that has included antiviolence strategies is Avahan, which has addressed occupational violence against sex workers from police and clients using a comprehensive approach, including state-wide training to police officers, legal literacy training for sex workers, increased mobilization and collectivization of sex workers, crisis response teams, and legal representation for sex workers (Beattie et al. 2010). Sex workers identified the inclusion of antiviolence programming in HIV prevention with Avahan as a critical priority. Evidence suggests that Avahan has been associated with a reduction in the proportion of sex workers reporting sexual violence in follow-up surveys compared to at baseline (Beattie et al. 2010).

- **Safe sex work environments and HIV prevention**

Sex work environments influence sex workers' vulnerability to HIV, and this relationship is context specific. In criminalized settings, soliciting for clients in street or public places often isolates sex workers from other workers, forcing them to work alone and to take clients to hidden outdoor places to provide services, including clients' cars, streets, alleys, and parks, in order to avoid police (Shannon and

Csete 2010). This practice can place sex workers at higher risk for violence by clients and result in sex workers having reduced agency in terms of clients' condom use and negotiations relating to transactions (e.g., amount charged) (Shannon and Csete 2010). Working in more visible settings results in sex workers or their clients being more likely to be harassed by police, displaced to more remote or isolated areas, and less able to access safer sex resources, as well as HIV prevention. In contrast, working indoors (e.g., in homes, motels, brothels) can afford some protection from violence and increased access to HIV prevention. In some settings, however, exploitative brothel owners and operators (e.g., pimps) can increase sex workers' HIV vulnerability by making decisions about condom use with clients, allowing violence by clients and controlling HIV prevention (Shannon and Csete 2010). In settings where sex work is legalized or regulated, sex workers may be able to work indoors but can be subject to human right abuses such as mandatory HIV/STI testing and forced registration with governments, practices which act as barriers to HIV prevention (Shannon and Csete 2010). This can concentrate HIV vulnerability, forcing some sex workers to work outside the legally regulated areas and making the practice of sex work even more dangerous.

Addressing the lack of safe spaces for sex workers to solicit for clients and provide services is an integral component of comprehensive HIV prevention for sex workers. Decriminalization of sex work can have a dramatic impact on improving the safety of sex work environments and reducing sex workers' vulnerability to HIV (Shannon and Csete 2010). In decriminalized environments where sex workers are allowed to organize and collectivize, sex workers have more control over their work environments, which can be structured to meet the health and safety needs of sex workers. Even in settings where sex work is criminalized, safe sex work spaces have been developed collaboratively between sex workers and local organizations and have

highlighted the crucial importance of such spaces in reducing HIV vulnerability. For example, in Canada, unsanctioned indoor sex work environments in low-barrier, supportive housing options for women promoted enhanced control within transactions with clients, including enabling condom use and reducing violent encounters (Krusi et al. 2012). In the Dominican Republic and Nevada, USA, brothels with supportive management policies and physical security measures were shown to promote condom negotiation with clients (Kerrigan et al. 2006). Safer-environment programs (e.g., mobile outreach) can modify sex work environments to improve safety of sex workers and increase access to condoms and connections with health services.

- **Sex work collectivization and destigmatization**

In most settings, criminalization of sex work can obstruct sex workers from supporting one another through formal or informal collectivization processes, isolating sex workers from one another and limiting their involvement in HIV prevention. Perhaps the best-known HIV prevention model that focused on sex work collectivization is the *Sonagachi Project* (“Sonagachi”), a community- and sex worker-led intervention implemented in India in 1992. Sonagachi, highlighted as a United Nations Program on HIV/AIDS/World Health Organization “best practice,” placed emphasis on reconceptualizing sex work risks as occupational hazards, focusing on community empowerment, collectivization, and engagement of sex workers to reduce HIV risk, and evidence similarly suggests that this intervention has been successful in reducing HIV risk to sex workers (Jana et al. 2004).

Sonagachi (and other programs that followed) also placed importance on including sex workers as key stakeholders in all aspects of project design, as well as addressing stigma and discrimination and its negative impacts on HIV risk as part of its community development and health promotion approach (Jana et al. 2004). Stigmatizing and discriminatory

attitudes toward sex work, along with laws criminalizing sex work and punitive sanctions against sex work, increase sex workers’ vulnerability to HIV through reduced use and access of HIV prevention (UNAIDS 2009). Male sex workers and sex workers who do not identify as a traditionally recognized gender or sexual identity can face intensified stigma and discrimination from police and community members (UNAIDS 2009). Sex workers living with HIV experience even more intensified stigma, inhibiting them from accessing HIV treatment (UNAIDS 2009). Reducing stigmatization and discrimination by health service providers, law enforcement officers, and community members is increasingly shown to be an integral part of HIV prevention among sex workers. Sex worker-led structural HIV preventions that focus on individual and collective empowerment developed for/by sex workers (e.g., education, literacy, training, self-esteem support, activism, organizing to protest rights abuses) as well as changing community attitudes toward sex work, including those surrounding safer sex practices within sex work, have contributed toward reducing gender inequalities and decreased HIV risk and HIV/STI prevalence among sex workers. Avahan has included elements to increase public awareness of sex work, as well as police sensitization training and educating sex workers on their legal rights, which are suggested to be important in reducing HIV risk to sex workers (Beattie et al. 2010). Structural interventions that are not implemented with substantial sex worker involvement (e.g., government sponsored 100% condom use campaign as in Thailand and Brazil) have been shown to have mixed effects by reducing HIV vulnerability among some populations while pushing the most marginalized sex workers underground and away from HIV prevention, treatment, and care supports.

- **Economic empowerment**

Most sex workers sell sex to meet economic needs; however, this can range from sex workers in poverty to higher-end sex workers

who engage in sex work for increased social stature, mobility, and economic opportunities. In some settings where credit or financial support outside marriage, employment, and education opportunities for women are limited, sex work may be one of the few economic options for sex workers to support themselves (UNAIDS 2009). The financial insecurity associated with sex work, particularly within criminalized settings, can place sex workers at higher risk for HIV (UNAIDS 2009). Sex workers often have to make difficult decisions regarding their health and safety in the context of economic insecurity. For example, sex workers may feel pressured to agree to sex without a condom or exchange higher-risk sex services (e.g., anal sex) in exchange for a higher fee. Such situations may be exacerbated for sex workers who use drugs or among sex workers who may be forced to charge less for sexual services (e.g., older sex workers in some settings due to competition with younger workers). Sex workers are furthermore prevented from accessing benefits and financial services available to the general population, such as bank accounts, savings schemes, loans and legal forms of credit, insurance, pensions, and other employment benefits, inhibiting sex workers from managing their own finances and planning for the future (UNAIDS 2009). Sex workers living with HIV experience heightened economic insecurity (UNAIDS 2009).

A number of examples exist of successful microcredit and microfinance programs that provide economic opportunities for women and girls and people living with HIV. Within these programs, economic empowerment can reduce stigma, enhance social inclusion, and provide alternate economic options. However, few programs have been specifically designed for sex workers. Many of these types of programs that do exist for sex workers have been criticized for placing heavy emphasis on exiting sex work without providing viable economic alternatives for sex workers (UNAIDS 2009). These “rehabilitation” programs often assume that sex workers *want* to leave sex

work, which is not always the case, with such programs more often reflecting stigmatizing and discriminatory attitudes and policies with hidden agendas to rid society of sex work. Unsuccessful programs can further stigmatize sex workers and leave them in debt and in worse financial situations than prior to the program (UNAIDS 2009). Nevertheless, economic empowerment programs can play an important role in HIV prevention for sex workers, and such programs have shown promise for reducing sex workers’ vulnerability to HIV (e.g., Brazil, where sex work is decriminalized; India and Kenya, where sex work is criminalized) (UNAIDS 2009). These programs address gender-based inequalities that limit women’s economic options and promote gender-based equality within homes and work environments, as well as in social, legal, and political environments. Recognizing sex work as an occupation within decriminalized environments would allow for sex workers to access the same occupational and financial rights as other workers and reduce HIV risk.

- **Substance use programs**

Sex workers who use illegal substances may experience increased HIV-related vulnerability within overlapping sex work and drug use HIV risk environments (Rhodes et al. 2005). In the context of drug and alcohol use, sex workers may experience barriers to HIV prevention including dopesickness, withdrawal, and the immediate need to use drugs, factors that can prevent sex workers from assessing and screening dangerous clients and negotiating safety within potentially high-risk situations (Shannon et al. 2008). Drug or needle sharing with clients or other partners, which has been associated with gender-based power imbalances favoring the male partner, may facilitate increased risk of violence, HIV, or other blood-borne infection transmission (Strathdee et al. 2011). Dual stigmatization of sex work and drug use inhibits sex workers from accessing drug-related HIV prevention, including harm-reduction resources. Drug use is criminalized in most settings and increases harms through enforcement-based policing

approaches toward drug use that increase violence on a community level (Werb et al. 2011), as well as microlevel displacement of drug users to unsafe drug use spaces (e.g., shooting galleries, drug dealing, scoring and distributing drugs).

Addressing substance use can be an important component of comprehensive HIV prevention for sex workers who use drugs. As with HIV prevention that aims to prevent the transmission of HIV through sexual routes, substance use programs have included biomedical, behavioral, and structural components, with growing research supporting the need for comprehensive programs that include all components. Harm-reduction programs including methadone maintenance therapy and needle and syringe programs have been integrated in many settings. Safer-environment interventions (e.g., mobile outreach) along with inclusion of peers in HIV prevention to drug-using sex workers can be highly effective. Drug treatment modalities, including opiate substitution therapies, have had success in increasing economic empowerment within other populations and should be tailored to meet the needs of sex workers.

Future Research and Evaluation of HIV Prevention in Sex Work

Future research on HIV prevention in sex work should include a focus on better understanding of structural factors and their relationship to HIV risk and prevention among sex workers and their clients, as well as the complexities of HIV risk and prevention among sex workers. To help support a strong evidence base to inform HIV programmers and policy makers, improving the evaluation of HIV prevention in sex work should be a priority. The evaluation of HIV prevention programs and interventions remains challenging. Conventional methods (e.g., randomized control trials of communities) are often unethical and/or impractical to implement, particularly in vulnerable populations including sex workers (Boily et al. 2007; Piot 2010). In order to better understand the impact

of HIV prevention, a formal multipronged evaluation framework is necessary, as has been developed for large-scale HIV prevention programs (e.g., *Avahan*) (Boily et al. 2007). Support for community-based organizations to implement and evaluate, in partnership with research institutions, existing interventions remains critical. New evaluation strategies should be developed to understand the impact of modifying complex and interrelated factors that exist on multiple levels of influence and on HIV risk and prevention, including those on a macrostructural (e.g., policy reforms, collectivization of sex workers) and microlevel (e.g., gender-based power dynamics that influence condom use by men).

Conclusion

While there have been some positive examples of comprehensive HIV prevention acknowledging the importance of structural change in reducing sex workers' vulnerability to HIV alongside behavioral and biomedical components of HIV programming and interventions, much more work needs to be done in terms of addressing upstream factors that shape sex workers' risk environments for HIV. Furthermore, while behavioral or biomedical interventions may be deemed successful in some contexts, rigorous evaluation of the potential negative effects is needed (e.g., potential further stigmatization and criminalization, institutional barriers accessing to care for sex workers). To achieve effective community engagement and empowerment and successfully engage sex workers in HIV prevention, it is necessary for sex workers to be involved in the design, research, implementation, monitoring, and evaluation of policies and programs that affect them. New strategies should be developed to reach hidden populations of clients, with increased research scaled up among clients. Accordingly, clients should be involved in the design of HIV prevention *for* clients. Governments and policy makers should make use of the growing evidence base of peer-reviewed research, as well as community knowledge and expertise, to guide decisions surrounding sex work policy as

HIV prevention. Alongside global calls by sex workers, sex worker allies and advocates and researchers to decriminalize sex work to improve sex workers' health and safety (UNAIDS 2009; UNDP 2012), decriminalization of sex work on a global scale, should be considered critical to facilitate effective HIV prevention among sex workers.

Violence against sex workers, which is highly exacerbated within criminalized settings, should be recognized as a key determinant of increased vulnerability for HIV among sex workers, with antiviolence programming incorporated as part of HIV prevention. To reduce vulnerability, structural HIV prevention should include components addressing economic empowerment, destigmatization, empowerment, and collectivization. Supportive environments for sex workers need to be developed, including safe work spaces for sex workers. Partnerships with sex workers and organizations that work with sex workers need to be strengthened, with support available for sex workers who both wish to exit and remain in sex work. Emphasis must be placed on ensuring that all sex workers have universal access to safe, confidential, and voluntary access to HIV prevention, treatment, care, and support.

A growing movement advocates for shifting the focus of HIV prevention away from sex workers and, rather, acknowledging how sex work environments can shape or constrain risk for sex workers and clients (Shannon et al. 2015). HIV prevention would therefore focus on supporting sex work to be a safer occupation instead of placing responsibility on sex workers themselves. Comprehensive HIV prevention must address multiple key factors in combination, with both short- and long-term commitments, and urgently be scaled up to reduce further harms to sex workers.

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SIV Infection in Mandrills

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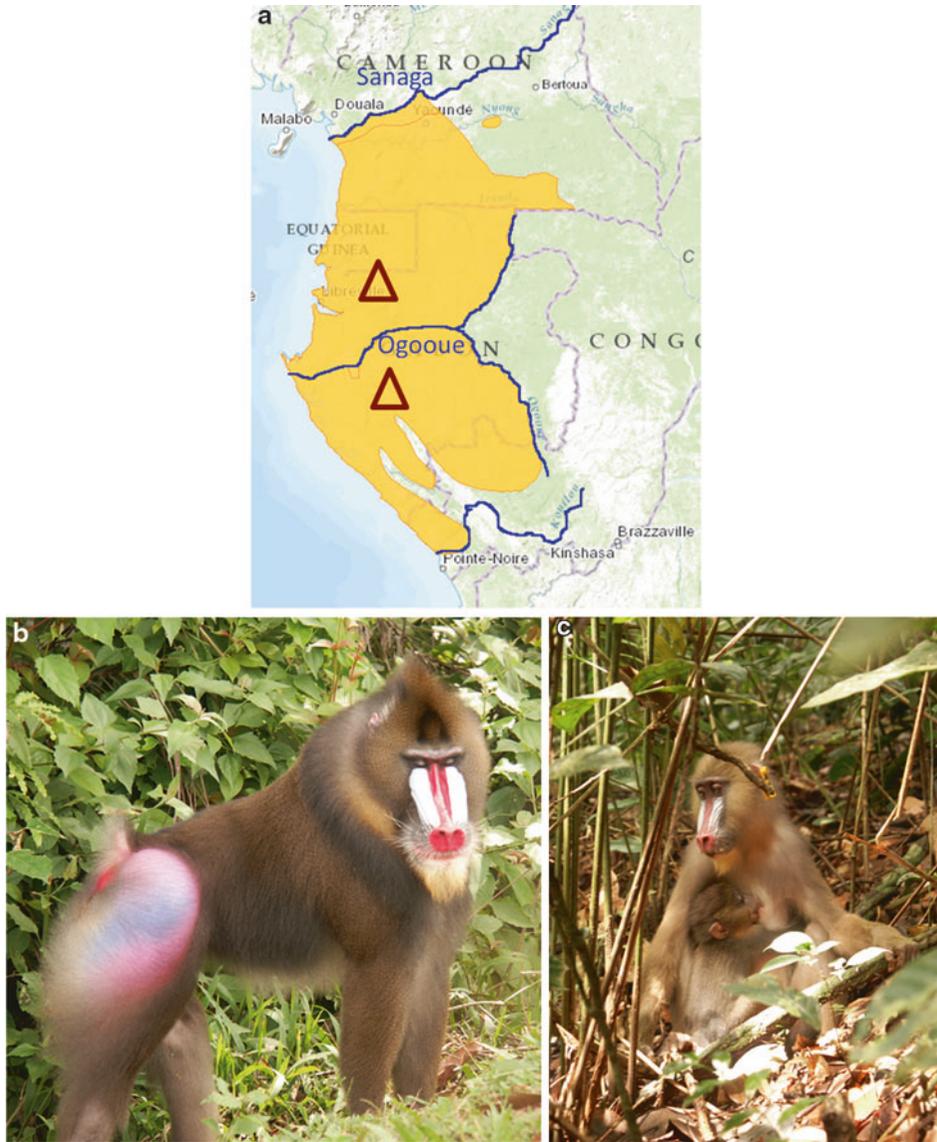
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Retrovirology Department, CIRMF, Franceville, Gabon Year 2001-2004

Definition

Thousands of years ago, the first simian immunodeficiency virus (SIV) infections occurred in wild African papionine species, specifically among the African mandrills (*Mandrillus sphinx*, Linnaeus 1758). In contrast to human immunodeficiency virus (HIV) infections, the SIV infection among mandrill populations (SIVmnd) is considered to be nonpathogenic and occurs without clinical symptoms of immune deficiency. Similar to HIV, SIVmnd targets activated CD4 T lymphocytes and leads to high viral loads in the peripheral blood, but the infection is not associated with inflammation and is characterized by low antiviral immune responses. Mandrill SIV infection is thus used as a nonpathogenic model to understand the pathogenesis of AIDS and host protection mechanisms.

Introduction

African mandrills (*Mandrillus sphinx*) have distinct physical characteristics. Their large size and colorful face (Fig. 1) make them recognizable



SIV Infection in Mandrills, Fig. 1 (a) Map of the area inhabited by mandrills in West Central Africa, with the main River limits in blue. Highlands are depicted by *open triangles*: north of Ogooué River, Mont de Cristal, south of

Ogooué River, Massif du Chaillu. (b) Male mandrill. (c) Female and her offspring within the semi-free enclosure in CIRMF (©P. Roques 2004)

among all the African monkey populations. While the mandrills are considered iconic for all African monkeys, they only inhabit a small area in Western Central Africa. Their habitat spans from the Kouilou River in the Republic of Congo and the south of Gabon to the Sanaga River in Cameroon. Mandrills occupy a coastal forest habitat with their eastern limit defined by the Ivindo and

Ogooué Rivers in Gabon (map Fig. 1a); however some literature suggests their range may extend further east. Mandrills are prevalent in their endemic area and are hunted for meat by locals. As such, their populations have decreased in size over the past three decades. Since 1986, mandrills have been deemed “vulnerable” by the International Union for Conservation of Nature (IUCN

2008), in addition to their classification within Annex 1 of the Convention on International Trade in Endangered Species (CITES).

Since the early 1980s, the Centre International de Recherche Medicale de Franceville (CIRMF), Gabon, has housed a unique semi-free colony of mandrills. The first isolated strain of SIVmnd (designated GB1 in 1988) was obtained from a wild-born mandrill female, identified as F17, kept in the semi-free enclosure at CIRMF since 1983 (Tsujimoto et al. 1988). SIVmnd GB1 was identified and sequenced (Tsujimoto et al. 1989) after the first identification of SIVs from African green monkeys in 1986 (Kanki et al. 1985), at a time when SIV was also isolated from sooty mangabeys living in captivity in the USA (see “► SIV Infection of African Green monkeys” and “► Nonpathogenic SIV Infection of Sooty Mangabeys”). Within the CIRMF enclosure, the infected mandrills seemed perfectly healthy. No further exploration was done in the following 10 years. At this time, the colony had grown from 15 wild born animals to more than 200 mandrills, and these animals represent the best source of information about the natural history of SIV infection in this genus. Indeed, many scientific collaborations have chosen these animals to examine the course of SIV infection and have compared their data to natural SIV infections in other species and in accidental cross-species HIV/SIV infections involving humans and macaques. Herein, we review virology, immunology, and physiopathology data on SIV infection in mandrills obtained during the last 20 years.

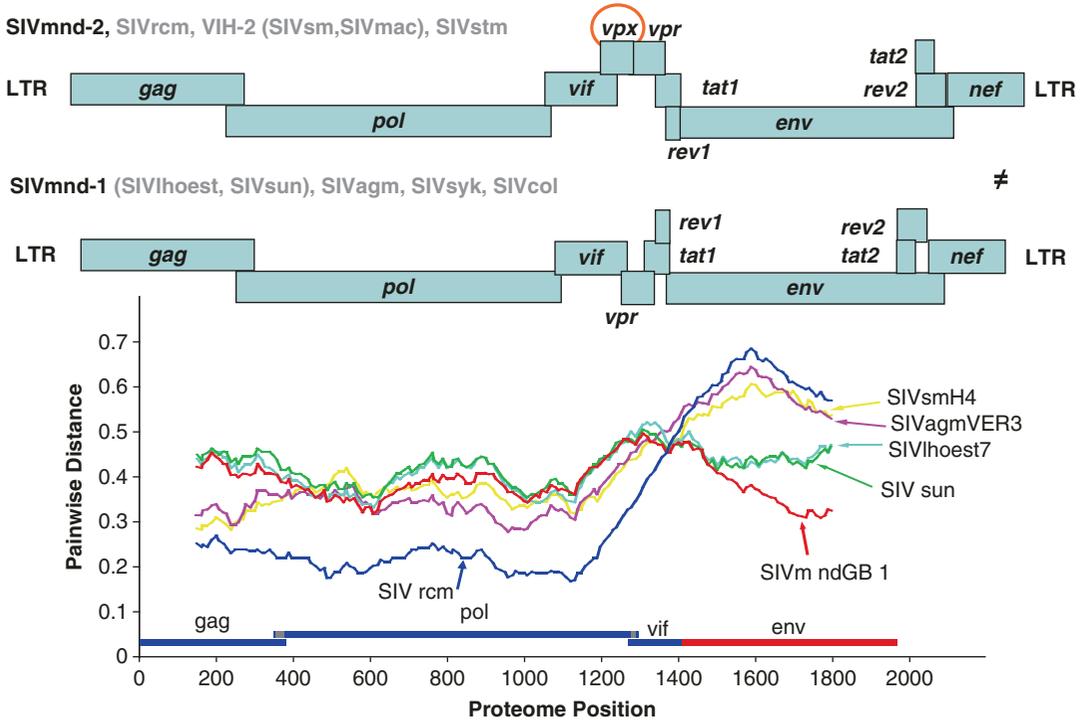
Discovery, Natural History, and Physiopathology of SIVmnd

The GB1 SIVmnd strain was the only representative of the SIVmnd lineage until two *Cercopithecus* viruses, one from L’Hoest’s monkey (*Cercopithecus l’hoesti l’hoesti*, Sclater 1899) and another from the recently discovered sun-tailed monkeys (*Cercopithecus l’hoesti solatus*, Harrison 1988), were shown to cluster in the same lineage (Beer et al. 1999; Hirsch et al. 1999). Sun-tailed monkeys are considered

to be a descendent of the l’hoesti monkey superfamily, which today is restricted to a forest area in Gabon (Forêt des Abeilles). The SIVmnd GB1 genome has the same organization as that of the *Cercopithecus* SIVs but does not match that of the papionine SIVs. Because the habitats of mandrills and sun-tailed monkeys overlap in Gabon, it has been suggested that the SIVmnd GB1 jumped across species barriers, starting in sun-tailed monkeys and moving to mandrills. The lineage was subsequently renamed the L’Hoest lineage (Hirsch et al. 1999). SIVmnd GB1-related viruses were present in up to 60% of tested wild adult mandrills, indicating that the virus is capable of spreading efficiently between animals of this species.

Within the CIRMF colony, western-blot and PCR detection results clearly defined two separate groups of monkeys. In one group containing five male mandrills, viruses were detected using anti-SIV p27 monoclonal antibodies and anti-HIV-1 polyclonal assays. In a second group containing F17 and four of her offspring, the Gag antigen of SIVmnd is detectable only with the HIV-1 polyclonal assay. Pol gene sequences from these animals were strikingly different. These results, together with experiments demonstrating that both viruses are present in wild animals and can spread within the CIRMF’s colony without causing disease, led to defining two distinct viruses: SIVmnd-1 and SIVmnd-2 (Souquiere et al. 2001).

The *env* region of SIVmnd-2 is phylogenetically similar to that of SIVmnd-1; however, the *gag pol* gene resembles that of SIVrcm, which is the SIV derived from the red-capped mangabey (*Cercocebus torquatus torquatus*, Kerr 1792) (Fig. 2). Various groups, including CIRMF researchers, screened drills (*Mandrillus leucophaeus*, Cuvier 1802), the closest living relative of mandrills, and found that wild drills were infected with a species-specific virus SIVdrl closely related to SIVmnd-2. Furthermore, some *Mandrillus sphinx* individuals living in captivity were found to be infected with “SIVdrl” variants. These observations led to the new hypothesis that SIVdrl, SIVmnd-2, and SIVrcm are different strains which may have evolved from an ancestral SIVrcm-like virus.



SIV Infection in Mandrills, Fig. 2 (a) Genomic structure of SIV mandrill type 1 and type 2 (SIVmnd-1; SIVmnd-2). Diversity plot comparing SIVmndM14 (the SIVmnd-2

representative) to SIVmndGB1 (the SIVmnd-1 representative), SIVrcm, SIVsun, SIVlhoest, SIVsm, and SIVagm. Derived from Souquiere et al. (2001)

Thus, under the current classification, the SIVmnd-1 of the SIVlhoest lineage is deemed a “Cercopithecidae-like” SIV. Despite its recombinant structure, SIVmnd-2 is considered more closely related to Papionini viruses, SIVdrl, and SIVrcm.

Screening of human populations also identified a case of probable infection with an SIVmnd (Souquiere et al. 2001). However, this remains a very rare event as no case has been reported since 2003. Human SIVmnd infection is probably an epidemiological dead end, similar to HIV-2 groups D and E (see “► Transmission HIV-2: Origin, Epidemiology, and Phenotype”), although these viruses were previously observed to replicate easily in human peripheral blood mononuclear cells (PBMCs) in vitro (Hu et al. 2003).

SIVmnds Are Ancient Viruses Coevolving with Their Host

Studies of the mandrill cytochrome b gene suggest mandrill populations cluster in two mitochondrial

haplotypes (Telfer et al. 2003), which appear to be associated with a distinct geographical origin. The Ogooué River in Gabon, which divides the mandrill’s range, separates mandrill populations in Cameroon and northern Gabon from those in southern Gabon (Fig. 1). This geographic clustering is concordant with that of known SIVmnd species as well as with the distribution of other viruses, such as the mandrill-associated simian T lymphoma virus (STLVs) and the simian foamy virus (SFV) of which distinct strains circulate north and south of the Ogooué River. Therefore, mandrill phylogenetic groups and viruses may have followed similar evolutionary trajectories.

Both genetic and climatic data suggested a separation of two distinct mandrill populations during a dry period, eight hundred thousand years ago, at a time when forest cover retreated to the highlands of the Mont de Cristal (north of Ogooué River) and of the Massif du Chaillu

SIV Infection in

Mandrills, Fig. 3

Map depicting the superposition of natural habitats of mandrills (orange), drills (dashed dark gray), red-capped mangabeys (*Torquatus* west of brown line), and sun-tailed monkeys (*Sol. bright red*)



(south of Ogooué River). These two highlands are found south of the Sanaga River. This river is the frontier which separates the mandrill and drill, which began evolving into distinct populations for an even longer period of time. A drill subspecies (*Mandrillus leucophaeus poensis*, Zukowsky 1922) inhabits Bioko Island, which separated from the African mainland more than 10,000 years ago. Despite this geographical separation, both drill subspecies still have SIVmnd-2/SIVdrl virus. Thus SIVdrl has evolved within its current host for more than 10,000 years (Worobey et al. 2010). Finally, drill and mandrill species share this virus, which is also closely related to the SIV found in the commensal papionine, the

red-capped mangabey. The red-capped mangabey lives in coastal wet forests and shares similar feeding habits as the mandrills and drills. This suggests these locations and behaviors play an important role in spreading infection between the two species (Map Fig. 3). To support this hypothesis, it was recently shown that groups of drills and red-capped mangabeys may compete when feeding (Astaras et al. 2011), suggesting this close proximity could spread the virus. However, the hypothesis that the ancestral virus SIVmnd-2/drl existed before the speciation of drill and mandrill cannot be ruled out.

In parallel, SIVmnd-1 is found only in mandrills and has a mosaic structure that may be the

result of recombination with multiple SIVs from the *l'hoesti solatus* group found south of the Sanaga River. The rearrangement of SIVmnd-1 likely occurred in more recent ancient history than the introduction of SIVmnd-2 in mandrills, and species jump of SIVsun like viruses may have occurred sometime after the separation of the two mandrill haplotypes.

SIVmnd Infections Are Not Associated with an Immunodeficiency in Mandrills

No pathology has been associated with SIVmnd infection (type 1 or type 2) either in the wild or in the semi-free colony in CIRMF, with one exception. The most recent biological data on the founder female F17, collected after 20 years of SIVmnd carrier status, showed a very low level of CD4 T lymphocytes, a high viral load, and increased immune inflammation when corrected for her age (Pandrea et al. 2001). Because of the difficulty in following wild mandrills, it is very challenging to determine whether this is a common feature among mandrill populations. Nevertheless, there is a very low probability of SIVmnd-associated disease among wild and semi-free colonies. Despite the numerous zoo mandrills which are natural carriers of SIVmnds, only very few reports cite animals with diseases suggestive of AIDS in elderly animals [two in (Pandrea et al. 2001) and one in Zoller et al. (2012)].

Mother to Infant Transmission or Horizontal Transmission?

SIV prevalence was assessed to be as high as 60% in wild mandrill groups via serology and PCR testing. Detailed studies of prevalence compared to age, as has been done with African green monkeys (see “► [SIV Infection of African Green Monkeys](#)”), are lacking; however a cumulatively increasing SIV seroprevalence is expected as transmission occurs horizontally through sexual contact and physical contact during fights over dominance among males (but not in females). This may affect a large number of animals, as mandrill social groups, such as those in Lope National Park, Gabon, may consist of 30–600 or more animals depending on the season (Kate Abernethy, CIRMF annual report).

One of the more surprising observations in the semi-free colony was the finding of SIVmnd-1 infection in young offspring of the F17 female (Souquiere et al. 2001). Vertical transmission (from mother to infant) was suggested as the cause, but routine annual sampling could not determine the time of infection. Experimental infection of a group of females after delivery and during lactation resulted in very low transmission to the offspring, despite continuously detectable viral load in the female breast milk. Offspring infection was detected only after 6 months of age when the experimentally SIV-infected mothers and infants were housed together in a larger enclosure. None of the animals showed clinical symptoms of infection, whether they were housed in pairs or in social groups. The young mandrills gained body mass and built relationships with their peers that were comparable to the animals in the semi-free ranging groups (Pandrea et al. 2008). In another trial, pregnant mandrills were infected with SIVmnd-1 during their last trimester and vertical transmission (as shown by a high viral blood load at birth) occurred in one out of four infants. Thus, even if rare, vertical transmission of the SIVmnd-1 may occur. There is no data for mother-to-infant transmission of SIVmnd-2 as only males were positive at the beginning of the CIRMF colony history.

The possibility that susceptibility to SIVmnd-1 infection may vary with genetic background was also proposed by studies of the CIRMF colony, but the potential mechanisms involved have yet to be investigated (Fouchet et al. 2012). The diversity of the mandrill population in the colony and their mating behaviors must be accounted for when trying to elicit such a relationship (Charpentier et al. 2005; Setchell et al. 2013).

Molecular Description of the Virus: In Vivo and In Vitro Variation

Molecular analysis of the SIVmnd-1 and SIVmnd-2 viruses highlights the diversity among different SIVs (Fig. 2). There are several differences between these two SIVmnds. The main three are (i) their phylogeny (SIVmnd-1

and SIVmnd-2 belong to different SIV phylogenetic lineages inferred from various genomic regions); (ii) their genomic structures (SIVmnd-1 lacks the *vpx* gene, whereas it is present in SIVmnd-2) (Fig. 2); and (iii) their antigenic properties (SIVmnd-1 and SIVmnd-2 behave differently in commercial EIA and p24 antigen assays).

Thus, two different genera of SIVs can infect mandrills. This is similar to HIV infection encountered in humans, who are susceptible to infection by two lentiviral types, HIV-1 and HIV-2, which have different origins, different genomic structures, and different antigenic properties. The two types of SIVmnd that naturally infect *M. sphinx* can be considered as a model for studying the complexity of HIV evolution and their interaction within humans.

The viral protein Vif of a species-specific SIV is involved in breaking the innate immune response against SIV infection via its interaction with the host factor APOBEC3G. Vif protein is able to interact with exogenous APOBEC and thus is involved in the species barrier break. SIVmnd-1 Vif and SIVrcm Vif were tested by trans-complementation of HIV lacking *vif*. It was expected that only Vif from SIV/HIV viruses found in humans (SIVsm, SIVcpz in addition to HIV-1/2) may complement HIV in vitro to aid in human cell infection. SIVrcm Vif (which is closely related to SIVmnd-2), but not SIVmnd-1 Vif, was found to occasionally complement HIV. Surprisingly, SIVmnd-1 is still capable of replication in HUT78 that expresses both APOBEC3G and APOBEC3F (Gaddis et al. 2004). Thus, the species barrier is not limited to cellular restriction, and species-specific modulation of Vif function is quite complex.

Interestingly, phylogenetic analysis of the *vpx* and *vpr* genes of SIVmnd-2 from similar strains isolated from drills showed that they form a monophyletic cluster that groups with *vpr* from SIVagm (Hu et al. 2003). Unfortunately, there are no studies of the role of Vpr within these viruses versus its role in HIV. However, Vpx was recently found to antagonize SAMHD1, another species-specific lentiviral restriction factor. One possible explanation could be that the deletion of *vpx* from SIVmnd-2 would contribute to the evolutionary

gain of a new function of Vif in SIVmnd-1 and hence provide new trans-species infection capacity. SIVcpz evolved in a similar manner, mounting infection in a human that ultimately led to today's HIV (Etienne et al. 2013).

In terms of viral entry receptor and co-receptor usage, prior studies showed that SIVs primarily use the CD4 receptor on lymphocytes for initiating infection. Initially, the SIVmnd-1 strain GB1 was described as using just the chemokine receptor CXCR4 as a viral co-receptor. Subsequent reports demonstrated that SIVmnd-1 isolated from the same animal (F17) (Pandrea et al. 2001) also used CCR5, as did all the genotype 1 and 2 viruses isolated from other mandrills. In addition SIVmnd-1 and SIVmnd-2 can use other chemokine receptors, such as CXCR6 and GPR15 (also known as Bonzo and Bob, respectively). Similar to HIV, in vitro experimental infection of monocytes shows the use of various co-receptors is driven by SIVmnd envelope protein motifs (Sakai et al. 1992).

Pathophysiology of Experimental and Natural Infection

Pathophysiology of SIVmnd-1 and SIVmnd-2 Infections in Their Natural Host

A pattern consistent with high levels of active viral replication was observed both for SIVmnd-1 and SIVmnd-2 in experimental infection studies (Table 1). A peak in viremia occurs around days 9–11 postinfection (p.i.), and the peak viral load (VL) values range between 10^6 and 10^9 SIV RNA copies/ml of plasma (RNA cp/ml). Immediately following the peak in viremia, the viral load declines rapidly, dropping between 3 and 4 logs. This decrease is observed between days 14 and 28 p.i. This occurs concomitantly with the development of immune responses to the infection, as suggested by the seroconversion of all animals between days 21 and 28 p.i. Starting from day 42 p.i., the set-point values of VL were around 10^5 RNA cp/ml and were remarkably stable up to 2 years later (Table 1). Interestingly there was no variation in the evolution within the animals, regardless of the quantity of virus injected.

SIV Infection in Mandrills, Table 1 Summary of the available immuno-virological data for SIVmnd infection in mandrills and rhesus macaques. Data for SIVagm infection in African green monkeys (AGM) are shown as a reference for nonpathogenic infection.

	Viral load				Immune markers	
	Mandrills	AGM	Macaque + SIVmnd1, 2	Mandrills	AGM	Macaque + SIVmnd-1, 2
	VL peak between d7 and d10 postinfection with 10^7 to 10^9 vRNA/ml plasma					
Acute phase (or primary infection)	10^7-10^8 DNA cp/ 10^6 PBMC	10^4-10^5 DNA cp/ 10^6 PBMC	10^5-10^6 DNA cp/ 10^6 LN cells	Depletion of lymphocyte CD4+ subset according to time of VL peak, inflammation		
	High viral load in LN + PBMC	Low viral load in LN + PBMC	High viral load in LN + PBMC	CD4, CD8 activation, sharp but transient depletion of lymphocytes (4 + 8) in blood (nt in gut)		
	$5 \times 10^5-5 \times 10^6$ DNA cp/ 10^6 LN cells	10^3-10^5 DNA cp/ 10^6 LN cells	$5 \times 10^3-10^5$ DNA cp/ 10^6 LN cells	CD4, CD8 activation, strong but transient depletion of CD4 in blood and small depletion in gut		
Early chronic phase (<1 year)	Plasma vRNA: 10^2-10^7 cp/ml	Around one log below than	Plasma vRNA: 10^2-10^4 cp/ml	Return to normal value in all tissues		
	10^5 DNA cp/ 10^6 PBMC	mandrills – no difference with chronic long term below	Lower DNA SIV-mnd-1 versus-mnd-2 (10^3 cp/PBMC) (10^5 cp/PBMC)	Low (20%) but persistent depletion in macaque infected either by SIVmnd-1 or SIVmnd-2		
	Medium VL (10^4-10^5) in plasma and LN		10^3-10^6 DNA cp/ 10^6 LN cells	Decrease of CD4 but associated with loss of CD4 expression with age; double CD4-/CD8- lymphocytes compensates this defect		
Chronic phase (>2-3 years)	Plasma vRNA: $5 \times 10^4-10^7$ cp/ml	Plasma vRNA: 10^4-10^6 cp/ml	Plasma vRNA: 10^4-10^6 cp/ml	Slight decrease of CD4 + memory after >5 years of infection		
	Trend -2 PVL >-1 PVL			CD4 decrease with age		
	Medium viral load (10^4-10^5) in PBMC and lymph nodes	10^2-10^3 DNA cp/ 10^6 PBMC	Rebound of peripheral viral load	No increase of sCD14 (normal gut barrier)		
		10^2-10^3 DNA cp/ 10^6 LN cells	10^3-10^4 DNA cp/ 10^6 LN cells			

Despite this very active viral replication, there is an absence of primary infection syndrome with all variations of SIV_{mnd} in experimentally infected mandrills. No fever was observed during the first month. After a one-year follow-up, none of the animals had lost weight, nor was there an increase in the size of their lymph nodes (LNs). No opportunistic infections (Onanga et al. 2002, 2006) could be detected, even after 3 years.

Within the semi-free colony at CIRMF, the follow-up of SIV in the “naturally” infected mandrills was performed once or twice a year (sometimes over a larger timespan) in accordance with Animal Health control protocols. In this group the viral load in the animals varied from 10^5 to 5×10^6 regardless of the estimated time of infection (between 1 and 15 years, mean 5 years) (Pandrea et al. 2003; Greenwood et al. 2014).

When animals were inoculated with a high viral dose, T cell populations after the peak of viral replication (day 14 p.i.) were on average 35% lower than those at baseline before infection. CD4+ T cell numbers were then rapidly restored and returned to baseline values by day 60 p.i. All CD4+ T cell subsets were somewhat depleted during acute infection; however, a significant depletion (defined as a cell count more than 80% below baseline, $p = 0.0005$) occurred only in CD4+ effector memory T cells (CD28–) at the peak of viral replication. Restoration of peripheral effector memory CD4 T cells occurred rapidly within 2 months, and normal counts were reached 3 months postinfection. No significant changes were observed for CD8+ T lymphocytes and for B lymphocytes (CD20+ cells). During the acute phase, immune activation of both CD4 and CD8 lymphocytes was observed as a transient increase in Ki67+ and high HLA-DR+ cell counts. However, similar to lymphocyte depletion, the activation level returned to normal values less than 1 month after the highest recorded viral load (Onanga et al. 2002, 2006). Various mechanisms, which are shared with AGM/SIV_{agm} and Mangabey/SIV_{sm} infection, are suspected to be involved in the observed moderate acute infection. These include low counts of CD4+ CCR5+ cells (Pandrea et al. 2008) and Nef inhibition of CD3+ cell activation.

To conclude the experimental infection studies performed in STLV_{neg} animals, close monitoring of infected mandrills over a 3-year period did not reveal any significant modification to lymphocyte subsets nor to peripheral viral load. Additionally, immunodeficiency was never observed in the animals.

Due to the semi-free enclosure in CIRMF, chronically infected animals can be explored while considering the complexity of the “natural life.” This means that external factors have to be evaluated, such as the large age dispersion, the gender distribution (ratio of males to females), the possibility of multiple infections within an individual mandrill, and lastly the amount of stress associated with social relationships or breeding periods. Two studies carried out with animals from the semi-free enclosure showed the numbers of naïve lymphocytes (either CD4+ T, CD8+ T or B cells) are inversely correlated with age and lymphocyte numbers decline faster in male mandrills than in females (Sumpter et al. 2005; Greenwood et al. 2014). Greenwood et al. (2014) used a large number of animals to show that the absolute number of CD4+ T cells was inversely correlated with the first positive time point for SIV infection using a linear model that included age as a covariate. They also showed that viral load in females (with STLV and age as covariates) was significantly higher than in male mandrills and suggested that the breeding season may be associated with high peripheral VL in females. After more than 5 years of SIV infection, the number of CD4+ memory cells was lower than baseline, whereas these cells were not affected in non-infected animals despite a large distribution of their values (Greenwood et al. 2014). Nonetheless, as observed in the colony for many years, there was no difference in the prevalence of other diseases between infected animals and non-infected animals. Although the gut barrier appeared to be intact (as assessed by soluble CD14 concentrations), lymphocyte activation was high (as assessed by Ki-67 and MHC-II levels) in CD8+ cells and MHC-II levels were low in CD4+ cells. During the 3 years of continued observation, the experimentally infected mandrills did not present these same levels of

activation (Onanga et al. 2002, 2006; Souquiere et al. 2009).

Thus, evidence from the long-term monitoring of infected mandrills suggests that while the virus is not fully controlled, it is unable to induce immunodeficiency within the normal lifespan of a mandrill (approximately 15 and 20 years for males and females, respectively).

SIVmnd Superinfection Within Mandrills and Cross-Species Transmission to Nonhuman Primates

Further investigation of the natural course of SIV infection is necessary to elucidate the mechanisms of control and the potential differences between SIVmnd-1 and SIVmnd-2. Mandrills are the only primates infected *in natura* with two very different retroviruses that harbor different supplemental genes, like HIV-1 and HIV-2 among humans (see “► [Update on HIV-1 and HIV-2 Dual Infection](#)”). To date, only two major questions have been addressed: (1) Could interference between the two viruses explain why only one strain is present in a given animal? This can be tested using simultaneous infection, or superinfection, with both viruses. (2) If there is no difference between the viruses in their natural hosts, could experimental infection of non-African macaques reveal any differences?

The apparent mutually exclusive nature of viral infection was tested using coinfection with both viruses. Superinfection of SIVmnd-1- or SIVmnd-2-positive animals (stabilized at 10^5 RNA copies/ml) with the missing viral counterpart showed that infection with both viruses is possible. The initial peak in peripheral viral load of the new injected virus was not observed; however, the two viruses (at least during the first year) reached a steady state of replication identical to that of single infection (i.e., around 10^5 RNA copies/ml) without any significant modification to the immune status. There was no evidence of recombination between the two strains. After 3 years, the SIVmnd-1 VL decreased, whereas SIVmnd-2 VL remained high. Despite this demonstration of superinfection in an experimental setting, it has never been observed in the wild. In the CIRMF colony, superinfection was reported once, despite the co-circulation of the two viruses

(as assessed by an annual follow-up) (Souquiere et al. 2012). These observations are useful for the understanding of the properties of SIV infection in a natural environment and deserve further investigation. In particular, it would be interesting to investigate whether recombination occurs between SIVmnd-1 and SIVmnd-2. In humans, recombination between HIV-1 and HIV-2 has not been demonstrated in coinfecting patients, leading to much discussion about this possible scenario.

Cross-species transmission of SIVmnd-1 or SIVmnd-2 to the rhesus macaque (*Macaca mulatta*) results in two distinct manifestations of infection: SIVmnd-2-infected rhesus macaques maintained normal numbers of CD4+ T cells during a 2 year follow-up period (Table 1). However, these cells were significantly depleted in SIVmnd-1-infected rhesus macaques in the presence of high amounts of T cell activation, suggesting that SIVmnd-1 is more pathogenic than SIVmnd-2 (Souquiere et al. 2009). Both the number of activated T cells and the amount of antibodies in SIVmnd-infected rhesus macaques were higher than in SIVmnd-infected mandrills. In addition, SIVmnd-infected rhesus macaques had higher concentrations of proinflammatory cytokines, with the most significant differences observed for IFN- γ .

Overall, studies of cross-species transmission suggest that *Cercopithecus*-specific SIVs are associated with low pathogenicity in rhesus macaques. Viral competition experiments involving superinfection show that SIVmnd-2 is better than SIVmnd-1 at mounting persistently higher levels of viral replication in mandrills. SIVmnd-1 adaptation to its relatively “new” mandrill host is probably the best explanation for the relatively high pathogenicity of this virus in rhesus macaques, as illustrated by persistent infection and CD4+ T cell depletion.

Levels of T cell activation and plasma IFN- γ secretion were identical for both types of SIVmnd in rhesus macaques. This is surprising given the different pathogenic outcomes of the two infections. However, SIVmnd-2 may progress toward AIDS in infected rhesus macaques after a longer incubation period, ultimately rendering both viruses highly pathogenic. Alternatively, the pathogenicity of these two viruses may differ because of different mechanisms of CD4+ T cell depletion

during SIVmnd-1 and SIVmnd-2 infections. Particular clones of the SIVmnd-1 strain GB1 (from which the virus strains used in experimental infection in CIRMF were derived) are CXCR4-tropic SIVs and probably promote CD4⁺ T cell depletion at extra-intestinal mucosal sites. In contrast, pure CCR5-tropic SIVs, such as SIVmnd-2, may promote intestinal depletion of effector memory CD4⁺ T cells, as described previously for SIVsmm strains (see “► [Nonpathogenic SIV Infection of Sooty Mangabeys](#)”).

The difference in pathogenic potential for rhesus macaques between SIVmnd-2, which induced persistent infection (Takehisa et al. 2001; Souquiere et al. 2009), and SIVrcm, which is controlled in these animals, may also appear somewhat surprising in the context of the close phylogenetic relationship between these two viruses. However, this difference could also be associated with the fact that these viruses use different co-receptors, specifically CCR2b in the case of SIVrcm (Beer et al. 2001) and CCR5 in the case of SIVmnd-2 (Hu et al. 2003). This difference may have given the ancestor of SIVmnd-2 an evolutionary advantage when making the species jump.

Conclusion

The relatively high propensity for successful cross-species transmission of the SIVmnd viruses suggests that host-species barriers can be overcome relatively easily through recombination and adaptation in cross-species-transmitted SIVs. Mandrills and SIVmnd infections should be considered as a system to explore the various mechanisms involved in trans-species transmission and as a model to predict the risk associated with human-primate contact.

For example, some strains of other retroviruses, such as STLVs/HTLVs or foamy viruses, have been found in both humans and mandrills but with a geographical separation between the north and south of the Ogooué River. Host restriction factors are poorly understood, but examination of viral mutations may provide some clues in clarifying the connections between cross-species transmission and immune response adaptation.

The semi-free enclosure in CIRMF allows for close observation. Taking into account the protected status of the mandrills as well as their natural behavior, these enclosures provide a unique opportunity to study the natural history of the SIVmnd/nonpathogenic infection of SIVmnd/mandrill. Current findings also suggest the importance of following SIV-positive animals not only in nature but also in captive animals living on continents where mandrills have been imported and breeding for years.

Experimental infection of AGM or sooty mangabey has been more extensively investigated than in mandrills. Nonetheless, the overall results of the SIV/mandrill studies in connection with other nonpathogenic SIV infection reveal tolerance to the viral infection. Decreased cell-related and antibody-related immune responses are cumulatively a sign of viral tolerance. However, the mechanisms of this tolerance may stem from the virus, the host, or a combination of both.

This review is dedicated to Prof. François Simon who gave me the opportunity to work within CIRMF in Gabon, Prof. Françoise Barré-Sinoussi who encouraged me to go back to Africa, and the late Prof. Dominique Dormont, my mentor in the HIV/AIDS field. Thanks to Michaela Müller for her sharp mind, friendly comments, and ideas and to Jo Setshell and Beatrice Jacquelin for their corrections and questions.

Cross-References

- [Nonpathogenic SIV Infection of Sooty Mangabeys](#)
- [SIV Infection of African Green Monkeys](#)
- [Transmission HIV-2: Origin, Epidemiology, and Phenotype](#)
- [Update on HIV-1 and HIV-2 Dual Infection](#)

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SIV Infection of African Green Monkeys

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Definition

Human and simian immunodeficiency virus (HIV and SIV) are both *Lentiviridae*, a subgroup of the *Retroviridae*, which are characterized by the presence of regulatory genes in addition to the *gag*, *pol*,

and *env* genes. At least 12 independent cases of SIV transmission from chimpanzees, gorillas, or sooty mangabeys (SMs) to humans have been documented. HIV-1M, the epidemic strain of HIV, was likely introduced into the human population via an SIV-infected chimpanzee around the beginning of the twentieth century. Sooty mangabey SIV (SIVsmm) gave rise to both HIV-2, which is largely restricted to West Africa, and SIVmac (Munch and Kirchhoff 2012). In contrast to natural hosts, Asian macaques are not infected with SIVmac in the wild.

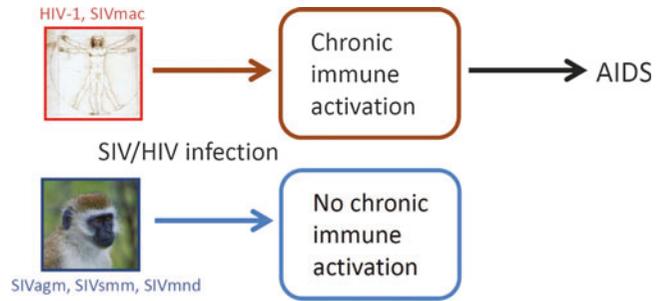
Over 40 African nonhuman primate species are infected with species-specific SIVs. Infection has been extensively studied in only four of these species: African green monkeys (AGMs), sooty mangabeys, mandrills, and chimpanzees. The first three have a nonpathogenic outcome of infection and the last shows an attenuated disease profile. Other species, such as L'Hoest's and Sykes' monkeys, have not been sufficiently studied to state outcome of infection. In this chapter, the term natural hosts will be used to describe AGMs, SMs, and mandrills as in these species a lack of SIV pathogenesis has been demonstrated (► [SIV Infection of Mandrills](#) and ► [SIV Infection of Sooty Mangabeys](#)). AGMs represent the largest reservoir of SIV in the wild, given their seroprevalence rates (mean of approximately 45%) and their wide distribution throughout sub-Saharan Africa (Fig. 1). Comparison of SIV infection in AGMs to HIV infection in humans and SIVmac infection in macaques can give insights into AIDS pathogenesis (Fig. 2) (Diop et al. 2002; Liovat et al. 2009).

AGM Classification

AGMs live throughout sub-Saharan Africa, with the exception of tropical forests and deserts. Four AGM subspecies have been described: vervet (*Chlorocebus pygerythrus*), sabaues (*Chlorocebus sabaues*), grivet (*Chlorocebus aethiops*), and tantalus (*Chlorocebus tantalus*). Vervets inhabit East and South Africa, sabaues West Africa, grivets East Africa, and tantalus Central Africa (Fig. 1). More AGM subspecies are currently being identified by next-generation

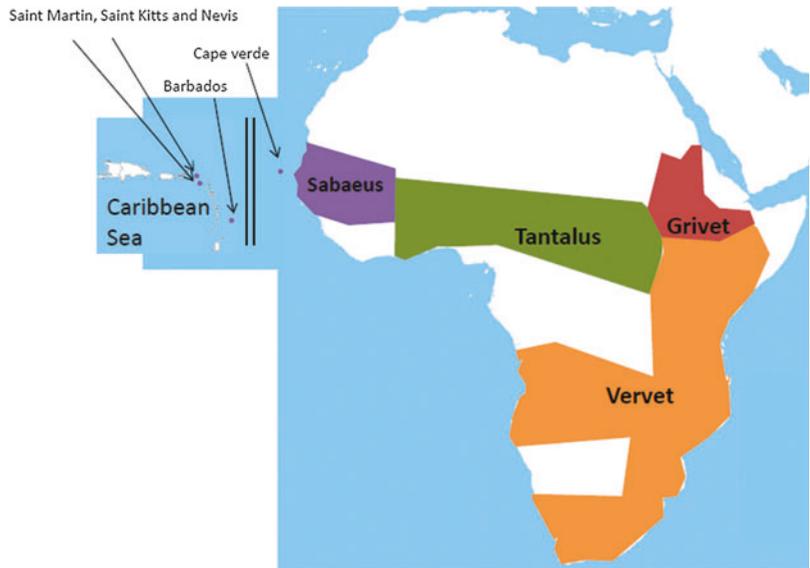
SIV Infection of African Green Monkeys,

Fig. 1 Geographical distribution of AGM subspecies. The figure shows the regions that represent the habitat of the four AGM subspecies in Africa and the Caribbean



SIV Infection of African Green Monkeys,

Fig. 2 Pathogenic and nonpathogenic infection of humans and nonhuman primates. During acute infection, African green monkeys rapidly resolve inflammation and immune activation. The absence of chronic immune activation leads to an asymptomatic chronic infection



sequencing studies. AGMs found in the Caribbean belong mostly to the sabaeus subspecies and are descendants of animals introduced during the slave trade in the seventeenth and eighteenth centuries. In contrast to AGMs in Africa, AGMs that live in the Caribbean are SIV negative. A likely explanation for this is that the transferred animals were pets, which are usually captured as infants. Such young AGMs are generally negative for SIV (Jacquelin et al. 2012).

Seroprevalence, Modes of SIVagm Transmission and Cross-Species Infection

The mean level of SIV infection in wild AGMs is approximately 40–50%. In adults,

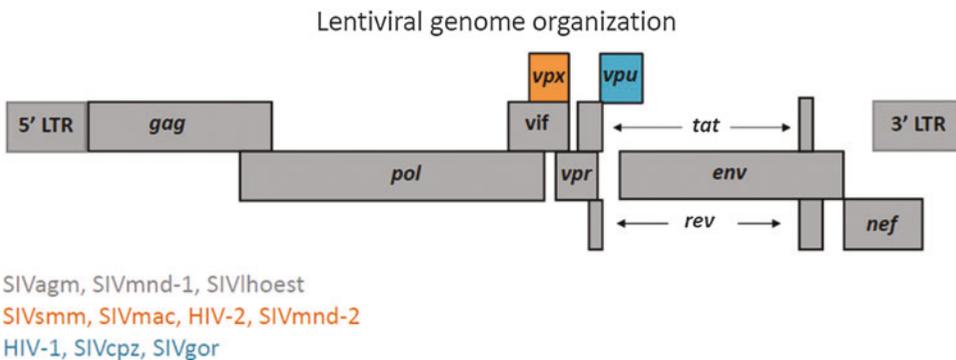
seroprevalence rates of up to 60–90% have been found, with females infected at a higher rate than males. In contrast to HIV infection in humans, mother-to-child transmission is rare in AGMs. Correspondingly, neonate animals are rarely infected, and juvenile animals are infected at an estimated rate of 10–20%. After sexual maturation, seroprevalence increases quickly, indicating that the main mode of SIVagm transmission could be sexual. However, SIV can also be transmitted through biting. SIV infection does not affect AGM lifespan. *In natura*, cases have been described of patas monkeys and baboons infected by SIVagm. Such cross-species infection in the wild may occur through either sexual contacts between sympatric species, biting, or hunting of smaller animals by larger species (Jacquelin et al. 2012).

SIVagm Phylogeny and Gene Function

The four recognized AGM subspecies all carry their own species-specific SIVs: SIVagm.gri in grivet, SIVagm.sab in sabaeus, SIVagm.tan in tanzania, and SIVagm.ver in vervet. The four SIVagm subtypes genotypically cluster by host rather than by geographical distribution. For instance, SIVagm.tan viruses from Uganda and the Central African Republic cluster together, while SIVagm.ver and SIVagm.gri strains that were both isolated in Ethiopia do not. This might be explained by the circulation of SIVagm in the AGM population since before the AGM subspecies diverged. The four SIVagm subtypes display up to 30% differences in the *pol* gene, a similar diversity as observed between distinct HIV-1 groups. SIVagm.sab is a mosaic virus, clustering for most of its genome with the other SIVagms, except for a region in the long terminal repeat (LTR), as well as the 3' *gag* and 5' *pol* region, in which it clusters with the SIVsmm lineage. This is the first example of a recombination event between two distinct types of SIV. It led to a recombinant virus, which outcompeted the native SIVagm.sab strain (Munch and Kirchhoff 2012).

The lentiviral genome is approximately ten kilobase pairs long and contains eight or nine genes encoding fifteen different proteins. All SIVs/HIVs have the virulence genes *vif*, *rev*, *tat*, *vpr*, and *nef* in addition to *gag*, *pol*, and *env*. Three

classes of SIVs are further categorized based on genomic organization: (1) SIVs/HIV-2 that have the additional gene *vpx* (e.g., SIVsmm and SIVmac), (2) SIVs/HIV-1 with the additional gene *vpu* (e.g., SIVcpz), and (3) SIVs without either of these genes (e.g., SIVagm) (Fig. 3) (Munch and Kirchhoff 2012). The absence of the *vpx* and *vpu* genes in SIVagm viruses is at least partially compensated for by additional functions of the *vpr* gene in this lineage. The *vpr* gene is closely related to the *vpx* gene and might share some of its functions, such as the degradation of SAM domain and HD domain-containing protein 1 (SAMHD1), a restriction factor found in both humans and nonhuman primates. Also, the SIVagm *vpr* gene has been shown to counteract the restriction factor tetherin, a function performed by *vpu* in HIV-1 (Munch and Kirchhoff 2012). SIVagm *vpr* can cause cell cycle arrest and apoptosis and is required for import of the pre-integration complex into the nucleus. SIVagm *vif* can increase infection of AGMs peripheral blood mononuclear cells (PBMCs) but not human PBMCs. SIVagm *vif* counteracts the restriction factor apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) in vivo, similar to HIV-1 *vif*. Nef is another virulence factor in SIVagm, with delta *nef* viruses replicating with lower viral loads than wild-type viruses (Muller and Barre-Sinoussi 2003). The *nef* gene in SIVagm, but not in HIV-1 or other SIVs of



SIV Infection of African Green Monkeys, Fig. 3 Three classes of SIV based on genome organization. The first class of lentiviruses comprises among other SIVsmm, SIVmac, and HIV-2 and is defined by the

presence of the viral protein x (*vpx*). The second class, containing HIV-1, is characterized by the viral gene u (*vpu*). The third class, to which the SIVagms belong, has neither of these genes

the *vpu*-containing group, can downregulate CD3 and CD28 on infected cells. This downregulation has been suggested to account for the lower levels of immune activation seen in SIV-infected natural hosts (Munch and Kirchhoff 2012). The *tat* and *vpr* proteins of SIVagm can transactivate the viral long terminal repeat (LTR), albeit to a lower level than HIV-1. In absence of *tat*, the SIVagm LTR shows a greater basal promoter ability than HIV-1 or SIVmac, possibly due to the absence of negative regulators in the SIVagm LTR (Muller and Barre-Sinoussi 2003).

Pathogenic Potential of SIVagm Infection

Rare examples of AIDS exist in natural hosts. In 1992, a case was described of a 12-year-old AGM that was coinfecting with SIV and STLV-1 that developed a severe enlargement of peripheral lymph nodes, diarrhea, and loss of appetite leading to cachexia (Pandrea et al. 2009).

Furthermore, experimental infection of pig-tailed macaques with SIVagm strains SIVagm.sab92018 and SIVagm.ver90, but not SIVagm.ver155, leads to AIDS in the infected animals. In contrast, SIVagm infection of patas monkeys and baboons is nonpathogenic (Hirsch et al. 1995; Jacquelin et al. 2012). Rhesus macaques that are infected with SIVagm control viral replication. These observations demonstrate that a virus-host interplay dictates the outcome of SIV infection, rather than an absence of pathogenic potential of SIVagm (Jacquelin et al. 2012).

Natural Course of SIVagm Infection

Most information on SIVagm infection has been obtained by studying both experimentally and naturally infected AGMs. The majority of studies have been carried out using the vervet and sabaues subspecies infected with SIVagm.ver and SIVagm.sab, respectively. Both intravenous and intrarectal infections lead to a similar infection course, recapitulating all parameters seen in the chronic phase of naturally infected AGMs (Liovat et al. 2009).

AGM peripheral blood CD4⁺ T cells are transiently depleted during acute SIV infection, with higher baseline CD4⁺ T cell levels correlating with a stronger depletion. The CD4⁺ T cell level in the blood of AGMs is dependent on the age of the animal. Levels in young adult AGMs vary between 800 and 2,900 cells/ μ L (with a median of 1,400 cells/ μ L) and decline by approximately 50 cells/ μ L per year in both infected and uninfected animals. In elder, uninfected animals, the levels of CD4⁺ T cells can fall below 200 cells/ μ L of blood, in the absence of any symptoms of AIDS (Jacquelin et al. 2012). During SIVagm infection, the depletion of AGM CD4⁺ T in the gut is as rapid and dramatic as in SIVmac infection. However, these cells partially rebound in AGMs in the chronic phase of infection (Table 1) (Liovat et al. 2009).

In acute SIVagm infection in AGMs, viremia reaches peak levels with RNA copy numbers between 2×10^4 and 10^9 RNA copies/mL. This is in the same order of magnitude as found for SIVmac infection in macaques and HIV-1 in humans. After the acute phase, viremia decreases several logs to a steady state. While the set-point viremia level between individual AGMs varies, the viral load remains stable over time. This is in contrast to HIV-1 and SIVmac infection where the viral load increases in late-stage infection (Diop et al. 2000). Most strikingly, the viremia in the chronic phase remains high, as high as in macaques progressing toward AIDS. Natural hosts resemble in that aspect a rare group of HIV-infected individuals who display elevated viremia but do not, or only slowly, progress to AIDS, called viremic non-progressors (► [Viremic Non Progressors](#)).

Similarities Between SIVagm and HIV-1/SIVmac Infections

High Mutation Rate

A hallmark of HIV is the high mutation rate throughout infection. Mutations are introduced by both an error-prone reverse transcriptase and recombination events in superinfected cells. This ability to mutate, coupled with a high tolerance for mutations by the virus, is conserved between

SIV Infection of African Green Monkeys, Table 1 Similarities and differences between SIVagm infection and pathogenic HIV-1/SIVmac infections. The table indicates the presence (+) or absence (–) in

pathogenic infection (middle column) and SIVagm infection (right column) of the characteristics described in the left column

Characteristic of SIV/HIV infections	Pathogenic infections (macaque or human)	AGM infection
Virus characteristics		
Cytopathicity of the virus	+	+
CD4 primary receptor + coreceptors	+	+
DC-SIGN-mediated capture of virions by DCs	+	+
Downregulation of CD3 by nef	–	+
CD4+ T cells		
Loss of CD4+ T cells in blood and gut during acute infection	+	+
High levels of CCR5 expression on CD4+ memory T cells	+	–
CD4 downregulation on activated CD4+ T cells	–	+
Maintenance of CD4+ T cells in blood in chronic infection	–	+
Partial restoration of CD4+ T cells in gut in chronic infection	–	+
CD4+ T cell bystander apoptosis	+	–
Viral replication		
Replication in activated, short-lived cells	+	+
High viral turnover in blood and gut	+	+
Rapid accumulation of viral mutations in vivo	+	+
Adaptive immune response partially controls viraemia	+	+
Preservation of memory T cells	–	+
Replication in germinal center of lymph node	+	–
Viral trapping by follicular dendritic cells in lymph node	+	–
Immune activation		
Acute inflammation	+	+
Acute T cell activation	+	+
Chronic inflammation	+	–
Chronic T cell activation	+	–
Effects of infection		
Altered T helper subset balance	+	–
Loss of intestinal integrity	+	–
Microbial translocation	+	–
Disruption of lymph node architecture	+	–
Depletion of peripheral CD4+ T cells	+	–
Infection leads to AIDS	+	–

SIVagm and HIV. This shows that the lack of SIVagm-associated pathogenicity is not due to an inability to escape the immune system (Muller and Barre-Sinoussi 2003).

Inefficient Antiviral Adaptive Immune Responses

Antiviral antibodies are elicited with complete seroconversion occurring about a month after SIVagm infection, as for SIVmac infection in

macaques. For unknown reasons, AGMs and other natural hosts rarely have antibodies against p27. AGMs have low levels of IgG broadly neutralizing antibodies, similar to what is observed in humans. Furthermore, SIV-infected AGMs generate functional SIVagm-specific T cells, at a similar or lower frequency than SIVmac-infected macaques (Jacquelin et al. 2012). Simultaneous depletion of CD8+ and CD20+ cells in chronically infected AGMs increases viral loads,

without leading to depletion of CD4⁺ T cells (Schmitz et al. 2009). This shows that (1) the adaptive immune response partially controls viral load, as in humans, and (2) the absence of AIDS is not due to low levels of viral replication.

Cell Tropism

Like HIV and SIVmac, SIVagm infects CD4⁺ T cells and is cytopathic for infected cells (Broussard et al. 2001). Infection of these cells corresponds with the usage of CD4 as the primary receptor. The coreceptor usage of SIVagm is slightly different from HIV-1 but similar to SIVmac. All SIVs can use CCR5 to infect, they generally do not use CXCR4, and use additional coreceptors as good as, or even better than, CCR5 (Liovat et al. 2009). SIVagm preferably uses Bonzo as alternative coreceptor, while SIVmac and SIVsmm preferentially use Bob. For *in vitro* infection, activation of CD4⁺ T cells is required. *In vivo*, the bulk of SIVagm replication is sustained by short-lived, activated CD4⁺ T cells (Liovat et al. 2009). However, macrophage-tropic SIVagm strains have been isolated from both monocyte-derived macrophages and cerebrospinal fluid macrophages (Broussard et al. 2001). *In situ* hybridization has been used to detect SIVagm-infected alveolar macrophages. In contrast, immunohistochemistry of the gut did not reveal any infected macrophages. Similarly, human intestinal macrophages are poorly infected *in vivo*, as they downregulate CD4 and CCR5 upon migration to the gut. Whether other cell types, such as dendritic cells, are infected in SIVagm infection is currently unclear (Apetrei et al. 2007; Jacquelin et al. 2012).

Tissue Tropism

In acute SIVagm infection, high viral loads are found in the lung, lymph nodes, gut, and in many other lymphoid tissues. Approximately 70% of all CD4⁺ T cells in the body are located in the gut, often displaying an activated phenotype (Jacquelin et al. 2012). They are thus preferential target cells for HIV/SIV, and between 30% and 60% of memory CD4⁺ T cells are infected during the peak of SIVmac infection. Infected cells in the gut can be found in both Peyer's patches and in the

lamina propria in all primate lentiviral infections (Muller and Barre-Sinoussi 2003; Liovat et al. 2009). In the chronic phase, the level of SIVagm RNA and DNA decreases in tissues such as the lung, thymus, and brain. The majority of virus is located in the gut during chronic SIVagm infection.

Viral Capture by Dendritic Cells

Dendritic cells of humans, macaques, and AGMs all have the ability to capture HIV/SIV via cell surface molecules, among them lectin receptors such as DC-SIGN. After HIV/SIV capture, dendritic cells can transfer virions to CD4⁺ T cells, leading to efficient replication in activated CD4⁺ T cells, a process called trans-infection. This process has been suggested to be involved in the acquisition of SIVmac during mucosal transmission. Moreover, viral capture might contribute to dissemination of the virus to lymph nodes (Sodora et al. 2009; Jacquelin et al. 2012). Thus, SIVagm has the same abilities for rapid viral dissemination as SIVmac.

Recapitulating, SIVagm infection in AGMs shows many similarities to HIV infection in humans and SIVmac in macaques including (a) rapid viral mutation; (b) high viremia; (c) partial control of viral replication after acute infection; (d) preferential replication in activated CD4⁺ T cells and the bulk of viral replication is sustained by activated, short-lived cells; (e) cytopathicity of the virus; (f) DC-SIGN-mediated dendritic cell capture and transfer of virus; (g) broad dissemination to all lymphoid tissues in acute infection with persistently high replication in the gut; and (i) dramatic loss of intestinal CD4⁺ T cells in the acute phase (Table 1) (Chahroudi et al. 2012; Jacquelin et al. 2012).

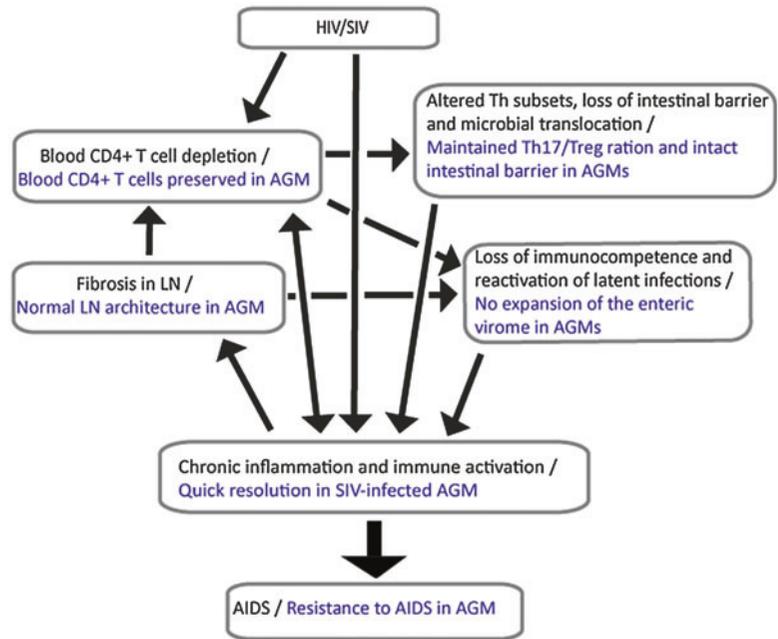
Differences Between SIVagm and HIV-1/SIVmac Infections

Absence of Chronic Immune Activation

The major differences between SIV infections in natural hosts, such as AGMs, and pathogenic SIV/HIV infections are the absence of chronic

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Fig. 4 Events suggested to contribute to chronic immune activation in HIV-infected individuals and the potential mechanisms by which AGMs avoid this. Upper lines of text, depicted in *black*, display the mechanisms contributing to chronic inflammation in pathogenic SIV/HIV infection. Observations made for AGMs are depicted in *blue*. LN lymph nodes, *Th* Thelper, *Treg* Regulatory T cell



immune activation (IA) and the preservation of peripheral CD4⁺ T cells in natural hosts. During the acute phase of infection in AGMs, proliferation of CD8⁺ T and CD4⁺ T cells increases in the blood, yet levels return to baseline during chronic infection. Lymph nodes display only a moderate peak of CD8⁺ T cell proliferation, and no symptoms of acute viral infection, such as fever or lymphadenopathy, are observed. This is in contrast to humans infected with HIV or macaques infected with SIV that have a sustained increase in CD8⁺ T and CD4⁺ T cell proliferation. The difference in IA between asymptomatic and pathogenic HIV/SIV infections reinforced the notion that IA is the driving force behind the depletion of CD4⁺ T cells. Indeed, the level of CD8⁺ T cell activation before seroconversion is a strong, independent predictive marker of time to progression to AIDS in HIV infection. Even in patients with undetectable viral loads (patients under treatment or viral controllers), the percentage of activated CD8⁺ T cells is still higher than in HIV-negative individuals. This increased IA associates with the loss of CD4⁺ T cells more strongly than viremia (Hunt 2012).

There are multiple proposed contributors to chronic IA in HIV and SIVmac infection:

(1) viral proteins, (2) disruption of the lymph node architecture and function, (3) homeostatic proliferation, (4) intestinal barrier disruption and subsequent microbial translocation, (5) (subclinical) viral reactivation, and (6) chronic inflammation. Figure 4 lists suggested differences from pathogenic infections that could allow SIVagm-infected AGMs to resolve IA and sustain an asymptomatic chronic SIV infection (Jacquelin et al. 2012).

Resolution of Inflammation

AGMs do not show chronic inflammation during SIVagm infection, indicated by the absence of high expression levels of inflammatory markers (e.g., TNF α), monocyte activation markers (e.g., soluble CD14), coagulation markers (e.g., D-dimer), and interferon-stimulated genes (e.g., IP10) (Jacquelin et al. 2012). The reasons for the absence of chronic inflammation in SIV-infected AGMs are still unknown. AGM plasmacytoid dendritic cells (pDCs) readily sense SIV (Diop et al. 2008). Indeed, in acute SIVagm infection, cytokines such as IFN α , IFN γ , and IP10 are strongly upregulated, indicating a robust innate immune response. This corresponds to the recruitment of activated plasmacytoid dendritic cells

(pDCs) and myeloid dendritic cells (mDCs) to the lymph nodes. However, cytokines such as TNF α and IL-18 are not upregulated, or upregulated to a lesser extent, in AGMs than in macaques during acute SIV infection. Before the end of acute SIV_{agm} infection, all inflammatory markers return to baseline levels in AGMs. This shows the remarkable capacity of AGMs to resolve inflammation despite ongoing viral replication (Kornfeld et al. 2005; Jacquelin et al. 2012).

Upon HIV-1 or SIV_{mac} infection, an inflammatory response is rapidly elicited in humans and macaques, respectively. This response is characterized by high levels of IFN α , IFN γ , and IP10 as for AGMs. However, markers as TNF α and IL18 are more strongly upregulated in pathogenic infection than in SIV_{agm} infection (► [Inflammatory Cytokines](#) and ► [Immunopathogenesis of SIV_{mac} Infection in Macaques](#)). Furthermore, chronic HIV-1 and SIV_{mac} infection are characterized by elevated levels of markers for inflammation, monocyte activation, and coagulation. While IFN α and IFN γ are not detectable in the asymptomatic part of chronic infection, many interferon-stimulated genes are still elevated compared to preinfection (Jacquelin et al. 2012). The relevance of chronic inflammation has been established recently when chronic inflammation was shown to be stronger associated with mortality than T cell activation in HIV infection (Hunt 2012).

Low Viral Load in Lymph Nodes in the Chronic Phase of SIV_{agm} Infection

While high viral levels are found in lymph nodes of acutely infected AGMs, the amount of SIV-infected cells there declines during chronic infection (Diop et al. 2000; Chahroudi et al. 2012). A similar infection profile has been observed in the lymph nodes of SMs. In natural hosts, SIV is confined to the T cell zones and does not infiltrate the germinal centers. There is also no trapping of SIV_{agm} by follicular dendritic cells. In contrast, in pathogenic SIV_{mac} infection, viral replication takes place in the T cell zone during acute infection but then predominantly shifts to the germinal centers. The majority of SIV_{mac} in lymph nodes can be found in follicular T helper

cells (T_{fh}) or trapped by follicular dendritic cells in germinal centers. Concomitantly, there is an expansion of T_{fh} cells, increase of germinal center size, and infiltration of CD8⁺ T cells into the germinal centers. These alterations could have an impact on the B cell maturation process, leading to incorrectly matured B cells in pathogenic HIV-1/SIV_{mac} infections.

In SMs, it has been demonstrated that T_{fh} cells are not infected, which is likely also true for AGMs, as there is no viral replication in the germinal centers of AGMs. Low levels of T cell infection in lymph nodes correlate with maintenance of the lymph node architecture in AGMs. SIV_{agm}-infected AGMs also do not have infiltration of CD8⁺ T cells into the germinal centers of their lymph nodes (Diop et al. 2000). In contrast to AGMs, the lymph nodes of humans and macaques become fibrotic during HIV-1/SIV_{mac} infection. This fibrosis has been linked to the production of TGF β in macaques. TGF β is not produced by AGMs chronically infected with SIV, further protecting the lymph node integrity of these animals. The disruption of the lymph node architecture in humans and macaques could impact B and T cell education, leading to an impaired immune capacity after infection. This would hinder adaptive immune responses to pathogens, lead to the reactivation of persistent infections, and drive chronic IA (Chahroudi et al. 2012; Jacquelin et al. 2012).

Low Levels of SIV Infection in Central Memory T Cells in AGMs

In natural hosts, central memory CD4⁺ T cells are infected at lower levels than in macaques. This correlates with a lower level of CCR5 expression on these cells. Furthermore, in AGMs, upon activation of CD4⁺ T cells, the expression of the *CD4* gene is downregulated. This leads to a population of double-negative memory T cells, which do not express CD4 or CD8, but preserve a helper function similar to CD4⁺ T cells (Jacquelin et al. 2012). A relative resistance to infection by central memory cells might contribute to the lower lymph node viral load in AGMs and SMs as a large proportion of these long-lived cells are found in the lymph nodes.

Central memory CD4⁺ T cells are pivotal for immunity as they provide immunological memory, so a preservation of these long-lived cells might contribute to the immunocompetent state maintained in SIV-infected natural hosts in contrast to HIV and SIVmac infections (Chahroudi et al. 2012; Paiardini and Muller-Trutwin 2013). A similar lower contribution of central memory CD4⁺ T cells to the viral reservoir has been observed in some HIV controllers and in posttreatment controllers (► [Posttreatment Controllers](#)).

A loss of central memory CD4⁺ T such as observed in HIV infection can contribute to the reactivation of normally latent viruses, such as *Cytomegalovirus*. These reactivated viruses can further contribute to the IA observed in HIV-1/SIVmac infections. A study on the enteric virome in AGMs and macaques has shown that there is no such viral expansion in AGMs (Handley et al. 2012). Avoiding the (subclinical) reactivation of latent viruses could help the prevention of chronic IA in these animals.

Low Proviral Loads in AGMs

While AGMs and macaques have similar blood SIV RNA viral loads, they differ in proviral load. The proviral load of AGM PBMC in the chronic phase ranges from 1 to 10³ SIV DNA copies per 10⁶ PBMCs, approximately tenfold lower than what is observed in HIV-infected individuals. Furthermore, AGMs display lower infectious titer of PBMC (Muller and Barre-Sinoussi 2003). The reasons for this relatively high viral RNA/DNA ratio and its implications for pathogenesis are still unclear.

Absence of Bystander Apoptosis of CD4⁺ T Cells

Most of the CD4⁺ T cells that undergo apoptosis during pathogenic SIV/HIV infections are uninfected, a process called bystander apoptosis. In AGMs there is no bystander apoptosis of CD4⁺ T cells during SIV infection, while the level of CD8⁺ T cells undergoing bystander apoptosis is slightly increased compared to preinfection. This bystander apoptosis has been linked to chronic IA

and inflammation via several hypotheses: (1) interferon alpha can induce expression of proapoptotic molecules on the surface of CD4⁺ and CD8⁺ T cells. One such molecule, TRAIL, was found to be upregulated in the CD4⁺ cells of lymph nodes of macaques, but not AGMs. (2) IL-18, which can induce caspase-1-mediated apoptosis, is downregulated in AGMs compared to macaque (Jacquelin et al. 2012). (3) NKp44L is upregulated on uninfected, activated CD4⁺ T cells in macaques, which could lead to NK-mediated killing of these cells. Homeostatic proliferation in response to loss of CD4⁺ T cells by bystander apoptosis has been proposed in turn to contribute to the chronic IA in pathogenic SIV/HIV infection. This proliferation can lead to immune exhaustion, diminishing the efficiency of antiviral immune responses (► [HIV-Associated Immune Exhaustion](#)). As AGMs lack bystander apoptosis, homeostatic proliferation is only required to replace the cells killed by SIV. This is a minor percentage compared to macaque where also the apoptotic, uninfected cells need to be replaced (Jacquelin et al. 2012).

Microbial Translocation

AGMs show a preservation of the Th17/regulatory T cell (Treg) ratio during infection, while Th17 cells are preferentially depleted in pathogenic SIV infections. Th17 cells are known to secrete interleukin 22 (IL-22), a cytokine required for the homeostasis of epithelial cells. The preferential Th17 cell loss in macaque is associated with the loss of the epithelial barrier integrity and a circulation of products derived from gut microbiota in the blood (► [Microbial Translocation](#)). Bacterial products, such as lipopolysaccharides (LPS), can activate monocytes and macrophages and thus contribute to the chronic IA observed in pathogenic infection (► [Macrophages in HIV Immunopathogenesis](#)) (Hunt 2012). In contrast, in SIV-infected AGMs, the intestinal barrier remains intact and there is no microbial translocation. This has been proposed to contribute to the absence of inflammation and IA in chronically SIV-infected AGMs. Indeed, administration of LPS to AGMs leads to elevated CD8⁺ T cell

activation levels, demonstrating the importance of microbial translocation (Chahroudi et al. 2012; Jacquelin et al. 2012).

Conclusions

SIVagm infection of its natural host, AGMs, has served as a useful model to gain insight into the causes of AIDS in HIV infection. In particular, studying nonpathogenic SIV infection has contributed to acknowledging the importance of immune activation in the progression to AIDS. SIVagm infection in its natural host is asymptomatic despite rapid viral mutation and high levels of viral RNA in the blood and intestine. Remarkably, AGM lymph nodes are infected with lower levels of virus in the chronic phase of infection, and the lymph node architecture is maintained. Less infection of central memory CD4+ T cells and follicular helper T cells as well as a lack of viral trapping in the germinal centers is thought to underlie this. AGMs are capable to resolve acute inflammation and chronic immune activation is absent. Studying this model may yield further insights into HIV pathogenesis, contributing to identify the precise mechanisms of HIV-1/SIVmac-induced immune activation.

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SIVmac Infection of Macaques, Immunopathogenesis of

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Definition

The simian immunodeficiency virus of macaques (SIVmac) induces an AIDS-like disease in rhesus macaque monkeys with similar symptoms and immunological consequences seen in HIV-infected humans. SIVmac was discovered in rhesus macaques that were accidentally infected in captivity with SIV of sooty mangabey monkeys (SIVsmm). Unlike rhesus macaques that are not naturally exposed to SIV, sooty mangabeys show a high prevalence of SIVsmm infection in the wilderness without developing overt signs of disease. The SIVmac virus was named for rhesus macaques within which it was discovered rather than for its original source. SIVmac lifelong persistently replicates within the macaques' immune system that continuously tries to counterattack this virus infection and thereby induces a chronic immune activation. Thus, macaques develop SIV-related immunopathological symptoms while the inadequate immune responses fail to eradicate the virus. The SIVmac-infected rhesus macaque is an outstanding nonhuman primate (NHP) animal model to investigate virus-host interactions, intricate viral pathogenesis, and progressive immunopathological host responses. This animal model is the premier model for preclinical exploration of HIV vaccine candidates and treatment interventions aimed to prevent infection and/or control of viremia.

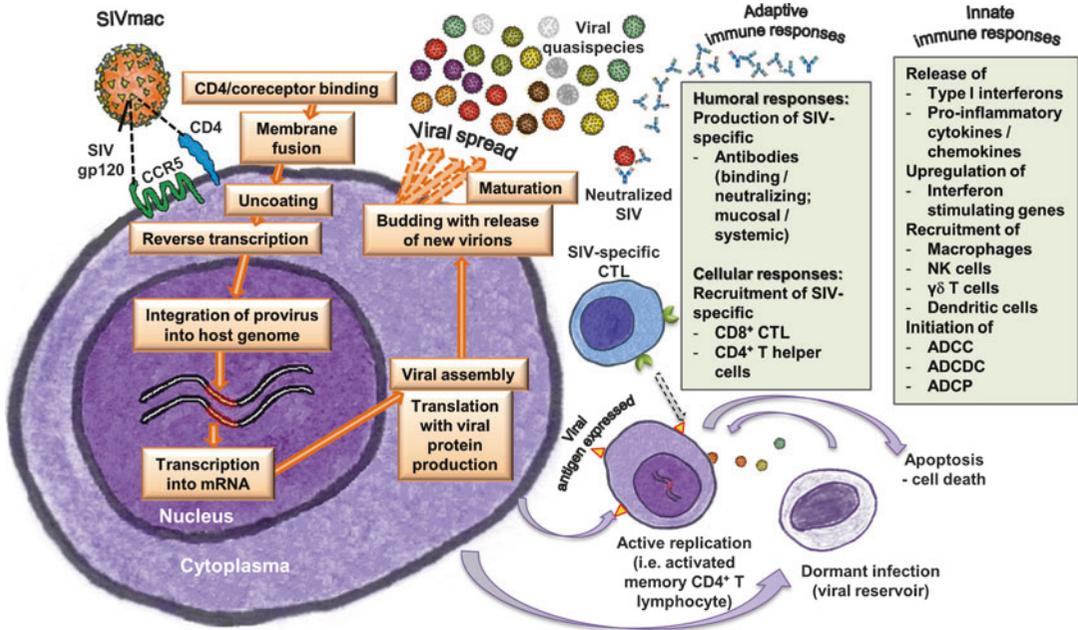
Overview

Rhesus macaques were first exposed to SIV when they were cohoused in primate center corrals in the United States with sooty mangabeys that were,

unsuspectingly, naturally infected with SIVsmm. Traces of the first detected SIVmac strain go back to a colony housed at the Caribbean Regional Primate Research Center (CRPRC) in Puerto Rico in the 1960s–1970s (Gardner 2003; Pancino et al. 2011). Apparently healthy rhesus macaques carrying SIVmac without symptoms were then imported in the 1970s to the New England Primate Research Center (NEPRC) that did not house any sooty mangabeys. These SIVmac-infected rhesus macaques built the seeds for further spread among cohoused rhesus macaques (horizontal transmission) and their offspring (vertical transmission) (Gardner 2003; Pancino et al. 2011). An increased incidence of opportunistic infections and lymphomas in rhesus macaques consistent with simian acquired immunosuppressive disease syndrome (simian AIDS) prompted medical investigations in search for a potential infectious causative agent that ultimately led to the discovery of SIVmac at the NEPRC in 1985. At that time, HIV was used to be called human T-lymphotropic virus III (HTLV-III). Therefore, the newly discovered virus was originally named simian T-lymphotropic virus III (STLV-III) of macaques and later renamed SIV (Daniel et al. 1985; Kannagi et al. 1985; Gardner 2003).

As SIVmac infection in rhesus macaques derived from SIVsmm in sooty mangabeys, human HIV-2 infection groups A through G similarly originated from cross-species transmission of SIVsmm by proximate human contact to sooty mangabeys in West Africa. In the HIV-2 endemic areas of West Africa, an exposure risk to SIVsmm exists through blood contact with infected bushmeat or pet sooty mangabeys. A comparable crossing of a species barrier happened by the spread of SIV in chimpanzees (SIVcpz) to humans that is considered the origin of HIV-1. In contrast to endemically SIV-infected African NHP species (e.g., sooty mangabeys, African green monkeys), Asian NHP (e.g., rhesus macaques) are not naturally infected with SIV (Gardner 2003; Pancino et al. 2011).

SIVmac is phylogenetically closely related to HIV-1 but even more closely to HIV-2. Large parts of the genomic structure and sequence are shared between SIVmac, SIVsmm, and HIV-2.



SIVmac Infection of Macaques, Immunopathogenesis of, Fig. 1 Virus-host interactions between SIV and macaque. Binding of SIV Env gp120 to CD4 and cytokine coreceptor on the surface of the host cell lets the envelope of the SIVmac virion fuse with the cell membrane of the macaque CD4(+) cell, enabling a single virus to enter the cell. Upon uncoating of viral material into the host cytoplasm, the SIV RNA template is reverse-transcribed in an error-prone process into cDNA, allowing mutations to occur. After entering the host cell nucleus, viral DNA is integrated into the host genome as provirus enabling viral replication. Newly assembled SIV virions bud off the host cell, mature, and spread SIV infection. Innate immune

responses immediately attempt to counteract infection, before adaptive cellular and humoral immune mechanisms respond specifically, but mostly inefficiently, as viral quasi-species or escape mutants recurrently evade immune responses that lag behind in adapting to the viral evolution. As SIV-specific CTL, MHC class dependent, recognize viral antigen-expressing activated CD4(+) T cells and kill infected cells, they play a pivotal role in the control of SIV replication, especially during acute infection. Selective pressure causes the virus to develop CTL escape mutants. Likewise, SIV-specific antibodies that are rarely broadly neutralizing fail to eliminate the virus. The ongoing chronic viral replication results in a continuous T-cell loss (Not to scale)

The viral structural genes *gag*, *pol*, and *env*, the essential regulatory genes *rev* and *tat*, and additional accessory regulatory genes (including *nef*, *vpr*, and *vif*) that do not occur in other retroviruses are characteristic for primate lentiviruses (Coffin et al. 1997; Pancino et al. 2011; King et al. 2012). SIVmac and HIV-2 differ from HIV-1 by the presence of the nonstructural *vpx* instead of a *vpu* gene and by more than 50% sequence deviation (King et al. 2012).

Viral Determinants of SIVmac Infection

To enter a host cell, the SIVmac virion surface glycoproteins, especially areas of the Env protein,

have to interact with specific cell surface receptors of the CD4(+) T cells. To facilitate entry of an SIVmac virion, the susceptible recipient cell needs to express both a CD4 protein and a chemokine receptor on its cell membrane (Coffin et al. 1997; King et al. 2012) (Fig. 1). C-C chemokine receptor type 5 (CCR5) and alternatively C-X-C chemokine receptor type 4 (CXCR4) are predominantly used as coreceptors or second/fusion receptors by SIVmac strains. Low expression of the coreceptor or its blockage decreases viral entry (Pancino et al. 2011; King et al. 2012).

After the SIVmac envelope trimeric glycoprotein gp120 binds to CD4 and the coreceptor, the envelope of the SIVmac virus fuses with the cell plasma membrane allowing it to enter the cell.

Subsequently, the virus is uncoated and releases its genetic material including ribonucleic acid (RNA) and the enzyme reverse transcriptase into the CD4(+) host cell. With the help of this enzyme, SIVmac RNA is transcribed in the host cell cytoplasm into complementary deoxyribonucleic acid (cDNA) (Coffin et al. 1997; King et al. 2012). As typical for retroviruses, the genetic information is transferred “backwards” (or in Latin *retro*) from RNA to DNA (King et al. 2012).

After the integration of the retroviral DNA into the host chromosomal DNA as provirus, the host cell is enabled to produce new copies of SIVmac with tropism for CD4(+) cells (Coffin et al. 1997). During the reverse transcription, a high rate of genetic recombination and variation takes place. Consequently, an abundance of genetically diverse viral copies or virions develop so that viral quasi-species are formed differing from each other in their functional properties such as virulence and protein expression (Coffin et al. 1997). The newly assembled SIVmac virions are released from the host cell by a process called budding that uses the host plasma membrane for the capsid assembly of the virion (Coffin et al. 1997; King et al. 2012).

Various SIVmac strains with different properties such as virulence, attenuation, coreceptor usage, or neutralization resistance are used in biomedical and translational research. Some of the strains consist of cloned viruses, while others are swarms with genetic variabilities. There are pathogenic differences between viruses propagated *in vitro* by culturing in suitable human or nonhuman primate cells and passaged *in vivo* in NHP. Serial passage in rhesus macaques can increase the pathogenicity of SIVmac. Accordingly, virus with low pathogenicity from clinically healthy monkeys resulted via serial passage at the NEPRC in highly pathogenic SIVmac251 (a swarm generated after four viral passages in rhesus macaques) and SIVmac239 (a clone produced after seven viral passages) (Voevodin and Marx 2009). Another pathogenic clone derived from SIVmac251 is SIVmac32H (Voevodin and Marx 2009). Less pathogenic, highly attenuated clones such as SIVmac142 are infrequently used. Similar to the SIVmac lineage discovery at the

NEPRC, another lineage called SIVb670/H4/H9 group was isolated after unintended transmission of the virus from sooty mangabeys to rhesus macaques in a leprosy-related experiment at the Tulane National Primate Research Center (TNPRC) (Voevodin and Marx 2009). This group of viruses includes SIVsmE660 that is moderately sensitive to neutralization, in contrast to the highly neutralization-resistant SIVmac239 clone (Voevodin and Marx 2009; Pancino et al. 2011; Sui et al. 2013).

An example of an attenuated SIVmac is SIV delta *nef* (SIV Δ *nef*), which lacks the accessory gene *nef* and shows a significantly reduced pathogenicity in rhesus macaques. SIV Δ *nef*-infected adult rhesus macaques are usually able to control infection, have low viral loads, and typically do not progress to simian AIDS, comparable to human HIV-1 long-term non-progressors who control HIV-1 infection without progressing to AIDS (Coffin et al. 1997). However, SIV Δ *nef* is by far more pathogenic in infant rhesus macaques within which infection regularly results in simian AIDS (Coffin et al. 1997).

In order to study clinically relevant HIV-1 gp120 envelope responses in rhesus macaques, simian/human immunodeficiency virus (SHIV) strains have been molecular-biologically engineered. SHIV is a chimera consisting of an SIV backbone with an HIV envelope insert. Since primate lentiviruses are species specific and HIV-1 does not cause infection in macaques, challenges with SHIV in rhesus macaques allow investigating HIV vaccines in the animal model while addressing HIV clade variances as well as antigenic differences between SIV and HIV (Abee et al. 2012).

Routes of SIVmac Transmission

Intravenous inoculation of SIVmac was the classical route for challenge studies in rhesus macaques resulting in a very predictable course of infection in 100% of inoculated animals. However, the probability of transmission of HIV to humans is much lower since most of the infections occur via mucosal route. Therefore, infection

studies in NHP, in particular vaccine challenge studies, have been adapted to mucosal challenge studies, a more relevant clinical setting. Mucosal administration of cell-free or also cell-associated virus commonly utilizes genital such as vaginal or penile (foreskin or urethra), rectal (or gastrointestinal), or oral routes. Transmission of cell-free virus across the intact mucosal epithelial cell layer may occur by transcytosis of the virus particles. This process is possibly enhanced via the neonatal Fc receptor (FcRn) that can transport immunoglobulin G (IgG) across epithelial cells from an acidic pH at the luminal surface to release at a more neutral pH (Gupta et al. 2013). In addition, mononuclear cells such as dendritic cells are considered to facilitate transport of virus through the mucosal tissues toward activated T cells (Voevodin and Marx 2009). Examples of cell-mediated virus are challenges with SIV-infected peripheral blood mononuclear cells (PBMC), infected isolated cells, or semen from infected monkeys. Typically much higher virus doses are necessary for atraumatic mucosal administration than for intravenous challenges because, in contrast to blood, mucous membranes provide protective antimicrobial epithelial barriers that impede viral penetration. As HIV has a low per-sexual-contact transmission rate and only a single or few variants get transmitted (so-called transmitted founder viruses), repeated challenges with low SIV doses are most suitable to test vaccine efficacy (Friedman et al. 2006; Voevodin and Marx 2009; Shaw and Hunter 2012; Sui et al. 2013).

To increase susceptibility to SIV transmission by vaginal route, hormonal treatment with progesterone or progestin can be used to thin the vaginal epithelium and reduce the barrier. In contrast, estrogen thickens the epithelium and hence fortifies the barrier (Voevodin and Marx 2009; Sui et al. 2013). However, hormonal treatment also has immunomodulatory effects. High progesterone level (occurring in the luteal or premenstrual phase of the hormonal cycle in female macaques and humans, during pregnancy, or during progesterone therapy) is associated with a decrease in IgG and IgA production and secretion in cervical and vaginal mucosa and a decrease of cytotoxic

T lymphocytes (CTL) in the female genital tract (Hel et al. 2010). Thinning of the vaginal stratified epithelium decreases the barrier and might facilitate the access of SIV to CD4(+) lymphocytes, macrophages, and Langerhans cells. The latter cells may play a role in transporting membrane-associated SIV to suitable CD4(+) target cells (Hel et al. 2010).

An oral transmission occurs mainly when an infant acquires an SIVmac infection by drinking breast milk from its mother (Voevodin and Marx 2009; Abee et al. 2012). Apart from infection via nursing, the offspring can also acquire SIVmac by blood contact during delivery or less frequently through the placenta. In contrast to transmission within the species (e.g., by sexual contact), the emergence of SIVmac infections in rhesus macaques and HIV-2 in humans highlights the risk of cross-species transmissions with the possibility of genetic diversity by recombination (Gardner 2003; Abee et al. 2012).

Primate Host Determinants and Virus-Host Interactions

Higher viral loads of pathogenic SIVmac during acute infection often result in more rapid progression to simian AIDS. In contrast, infection with less pathogenic viruses may cause a delayed and less severe disease development. Certain host factors including major histocompatibility complex (MHC) genes are associated with an enhanced CD8(+) T-cell-specific control of SIVmac viremia. Rhesus macaques of Indian origin expressing the MHC class I allele *Mamu A*01* are typically less susceptible to SIV infection than macaques lacking these genes who progress more quickly to symptomatic disease. Distinct other MHC-I alleles such as *Mamu B*08* and *B*17* also have some protective effects against SIVmac infection (Pancino et al. 2011; Sui et al. 2013). Some macaques suppress infection over many years to lifelong, so-called elite long-term non-progressors, and are of interest as study models for human HIV-1 controllers (Abee et al. 2012).

The particular monkey species and virus origin also matter for pathogenicity and course of

disease. Indian rhesus macaques infected with commonly used pathogenic SIV variants (e.g., SIVmac251 or SIVmac239 that originated from Indian rhesus macaques) often progress faster to clinical symptoms than Chinese rhesus macaques. The latter frequently have lower SIVmac viral loads with a more attenuated disease course than rhesus macaques of Indian origin. However, viral loads increase in Chinese rhesus macaques when the challenge virus was grown in Chinese rhesus macaques (Sui et al. 2013). This observation of a species-specific adaptation of the virus is also true for SIV infection in natural hosts: While the SIV infection does not lead to pathogenicity, the SIV strain endemically present in vervet African green monkeys results in a higher viremia than experimental infection of sabaeus African green monkeys. Similarly, sabaeus African green monkeys experience a higher viremia than vervet African green monkeys that are experimentally infected with sabaeus SIV (Pancino et al. 2011).

CCR5-tropic SIVmac strains encounter difficulties recognizing CXCR4 host receptors; the reverse applies to CXCR4-tropic viruses. Alternative coreceptors include chemokines such as CCR (C-C motif receptor)1, CCR2b, CCR3, CCR8, and CXCR6 (or STRL33/Bonzo) or various G-protein coupled receptors, e.g., GPR1, GPR15/Bob, and ChemR23 (chemoattractant receptor 23) (Pancino et al. 2011).

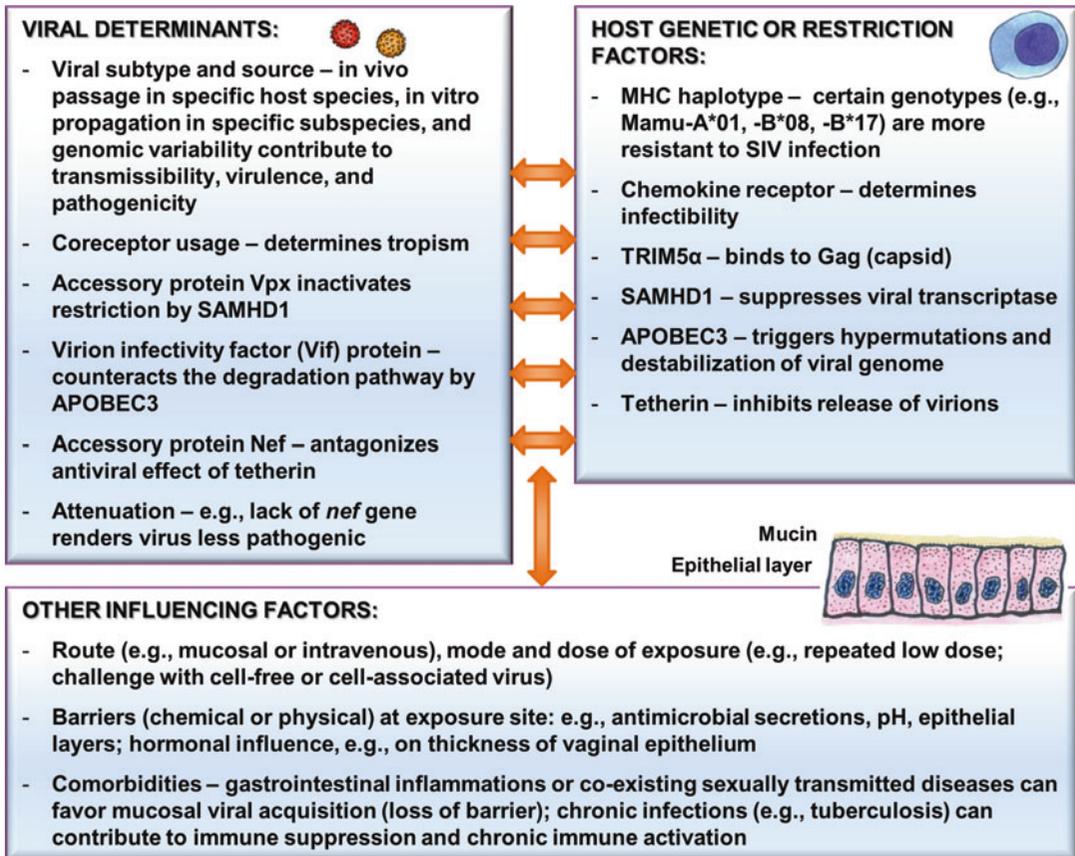
As part of the intrinsic cellular immunity, primate cells express various restriction factors that need to be overcome by SIVmac in order to enable productive infection (Fig. 2). The tripartite interaction motif 5 (TRIM5 α) with or without the insertion of cyclophilin A (CypA) pseudogene exhibits virus isolate-specific capsid-binding activities in rhesus macaques that interrupt uncoating events necessary for reverse transcription (Bieniasz 2004; Pancino et al. 2011; Sui et al. 2013). While sterile- α -motif-and-histidine-aspartic-domain-containing protein-1 (SAMHD1) acts limiting on viral replication by suppressing viral transcriptase activity, the lentiviral accessory protein Vpx (viral protein X) inactivates the restriction by SAMHD1. The cellular cytidine deaminase apolipoprotein

B mRNA-editing catalytic polypeptide-like 3 (APOBEC3) triggers a degradation pathway resulting in hypermutations and destabilization of the viral genome that counteracts the virion infectivity factor (Vif) protein so that SIV/HIV virions deleted of *vif* (SIV/HIV Δ *vif*) have reduced infectivity. The transmembrane protein tetherin has an inhibitive effect on the release of viral particles. While the antiviral activity of tetherin is antagonized by the accessory protein Vpu in HIV infection, Nef has an equivalent function in SIVmac (Pancino et al. 2011). Taken together, the restriction factors have an immunomodulatory suppressive effect on SIV infection, but they are incapable of controlling viral replication.

Innate Immune Responses

The host defense by innate immune responses consisting of physical and chemical barriers at portals of entry (e.g., antimicrobial mucin produced by epithelial cells in the distal colon), cellular components (e.g., neutrophils, macrophages, natural killer (NK) cells), and noncellular components (e.g., cytokines, complement system, other mediators of inflammation) act immediately as the first line of defense against an exposure to SIVmac and do not require prior exposure to SIVmac, but they act unspecifically against any foreign substance or antigen.

Unspecific innate immune responses to pathogenic SIVmac exposure in rhesus macaques include an increased secretion of type I interferons (IFN α and β) and induction of proinflammatory cytokines and chemokines by plasmacytoid dendritic cells, e.g., tumor necrosis factor alpha (TNF α), IL-6, C-C motif ligand 5 (CCL5/RANTES – regulated on activation, normal T cell expressed and secreted), macrophage inflammatory protein (MIP)-1 α or CCL3, MIP-1 β or CCL4, and C-X-C motif chemokine 10 (CXCL10); upregulation of interferon stimulating genes, viral defense by macrophages, NK cells, and gamma delta ($\gamma\delta$) T cells; production of host defense peptides or defensins; and several others (Coffin et al. 1997; Friedman et al. 2006; Bosinger et al. 2011; Pancino et al. 2011). To



SIVmac Infection of Macaques, Immunopathogenesis of, Fig. 2 Interaction between viral determinants of SIV and host genetic or restriction factors. “Viral determinants” influencing virulence, pathogenicity, and transmissibility of SIV are countered by “host genetic

or restriction factors.” Apart from major involvement by the host’s acquired and/or innate immune system (not shown in figure), the interplay between these determinants and additional “other influencing factors” affect viral acquisition, viral control, and/or disease progression

determine the level of immune activation, increased plasma levels of neopterin and beta globulin can serve as biomarkers (Pancino et al. 2011).

NK cells that express various inhibitory or activating killer cell immunoglobulin-like receptors (KIR) on their surface eliminate infected targets either directly by cytotoxicity or indirectly via antigen-dependent cellular cytotoxicity (ADCC) (Pancino et al. 2011). The relative activity of NK cells is dependent on the expression profile of particular KIR molecules (Hellmann et al. 2013). ADCC-inducing non-neutralizing antibodies that occur early after acute infection have been attributed a potential protective role in SIV/HIV infection by possibly delaying

progression to AIDS/simian AIDS (Ackerman et al. 2012). The role of antibody-dependent cellular phagocytosis (ADCP) in SIV/HIV infection, an immune mechanism by which non-neutralizing antibodies recruit phagocytes including monocytes, macrophages, and neutrophils to antibody-opsionized (or antibody marked for phagocytosis) viral particles, still remains unclear (Ackerman et al. 2012). Antibody-dependent complement-dependent cytotoxicity (ADCC), an immune function by which antibody-opsionized viral particles and other antigenic materials are directed through the complement cascade with subsequent lysis, may contribute to some control of viral replication during early SIV infection (Ackerman et al. 2012).

Adaptive Immune Responses

Following acquisition, SIVmac massively replicates in rhesus macaques with peak viral levels between 10^5 and 10^9 RNA copies/ml plasma typically detected after 2 weeks of infection (Pancino et al. 2011). The viral peak coincides with a massive CD4(+) T lymphocyte loss in peripheral blood. For decades, the enhanced programmed cell death of CD4(+) T cells in SIV/HIV infection was attributed to apoptosis. However, caspase-3-mediated apoptosis has been shown to only induce the death of the small fraction (~5%) of activated and productively infected CD4(+) T cells, whereas the remaining vast majority of quiescent CD4(+) bystander cells die by caspase-1-mediated pyroptosis, an extremely inflammatory form of programmed cell death in which the dying cells release large quantities of pro-inflammatory cytokines like interleukin 1 β (IL-1 β) that attract even more cells to die and spur inflammation (Doitsh et al. 2014).

Immediately after the SIVmac peak, the plasma viral load typically sharply decreases by a few log steps (Pancino et al. 2011). This decrease of viremia is temporally associated with a robust rise of CD8(+)CTL. The importance of the cellular immune responses in partial viral containment was demonstrated by experimental depletion of CD8(+) T lymphocytes by antibodies in the primary infection: SIVmac-challenged rhesus macaques are incapable of controlling viremia and undergo a rapid progress to simian AIDS when CD8(+) T-cell responses are inhibited (Schmitz et al. 1999; Friedman et al. 2006; Pancino et al. 2011).

By the third week, the number of circulating CD4(+) T lymphocytes recovers slightly, but is eventually followed by a gradual progressive decline, especially of the memory pool, throughout the course of infection. While the first SIV-antibodies can usually be detected by 2 weeks following infection, seroconversion is completed by a month after exposure, marking the end of primary infection (Pancino et al. 2011). Within the first 10 weeks after SIVmac infection, viremia reaches a plateau or set-point level (Pancino et al. 2011; Abee

et al. 2012). Early on, virus is systemically spread from the initial local site of acquisition to various lymphoid tissues where the virus gets persistently replicated. Alternatively, SIVmac virions can remain dormant inside suitable host cells over longer periods of time by establishing hidden viral reservoirs in latently infected long-lived T memory cells; hence protected from counterattacks by the host immune system, SIVmac evades immune recognition by the host (Picker et al. 2012). While only a fraction of the virions circulates in the peripheral blood, easily accessible for virologic assays, the vast majority of viral particles is produced and stored in lymphoid tissues including the gut that contains the majority of activated memory CD4(+) T cells in the body. In contrast to nonpathogenic SIV infection in African NHP that restore an initial CD4(+) T-cell loss after primary infection, chronic pathogenic SIVmac infection of Asian NHP is characterized by a progradient loss of the CD4(+) T-cell memory population. As the disease progresses, CD4(+)/CCR5(+) T lymphocytes get destroyed and are not sufficiently restored contributing to an immunologic impairment that permits microbial translocation in the gut and inflammation (Pancino et al. 2011; Estes 2013). While the destruction predominantly affects the later-staged CD4(+)/CCR5(+)effector memory T cells, less differentiated CD4(+) central memory T-cell precursors that are capable of producing new effector memory T cells are initially largely spared from loss due to limited co-expression of CCR5. The regeneration of the CD4(+) effector memory cell pool, the major target cells of SIV infection, guarantees viral replication for prolonged time periods. However, due to the hyperimmune activation, with the onset of simian AIDS, the entire CD4(+) memory cell pool gets basically depleted (Picker et al. 2012).

Depletion of the host's CD4(+) T helper lymphocytes further diminishes the host immune control. Since retroviruses utilize error-prone reverse transcriptase for their replication, SIVmac virions show huge mutation rates, replacing the original SIV transmitted founder virus(es) by swarms of escape variants. Thus, the NHP's cellular and humoral immune system is confronted with a

staggering number of viral mutants. Genomically and functionally diverse mutants obtain selective advantage because they are not recognized by the host immune system. The huge level of viral sequence diversity makes it an overwhelming and impossible task for CTL to detect virally infected cells. Moreover, the envelope glycoprotein Env of SIVmac undergoes extensive conformational changes due to errors in the reverse transcription and resulting mutations of proviral DNA. Although B lymphocytes are initially hyperactivated and generate large amounts of SIV-specific antibodies, they have little effect on the magnitude of the primary viremia. As the viral variants continuously select for new variants that are not easily neutralized or deactivated by anti-SIV-specific antibody responses, most antibodies remain inefficient against the next generation of novel viral mutants. Ultimately, the host's adaptive immune responses fail to catch up with the tricks of the intruding virus so that a lifelong viral replication and infection are established (Pancino et al. 2011; Picker et al. 2012; Mascola and Haynes 2013).

Immune Activation in Pathogenic SIVmac Infection

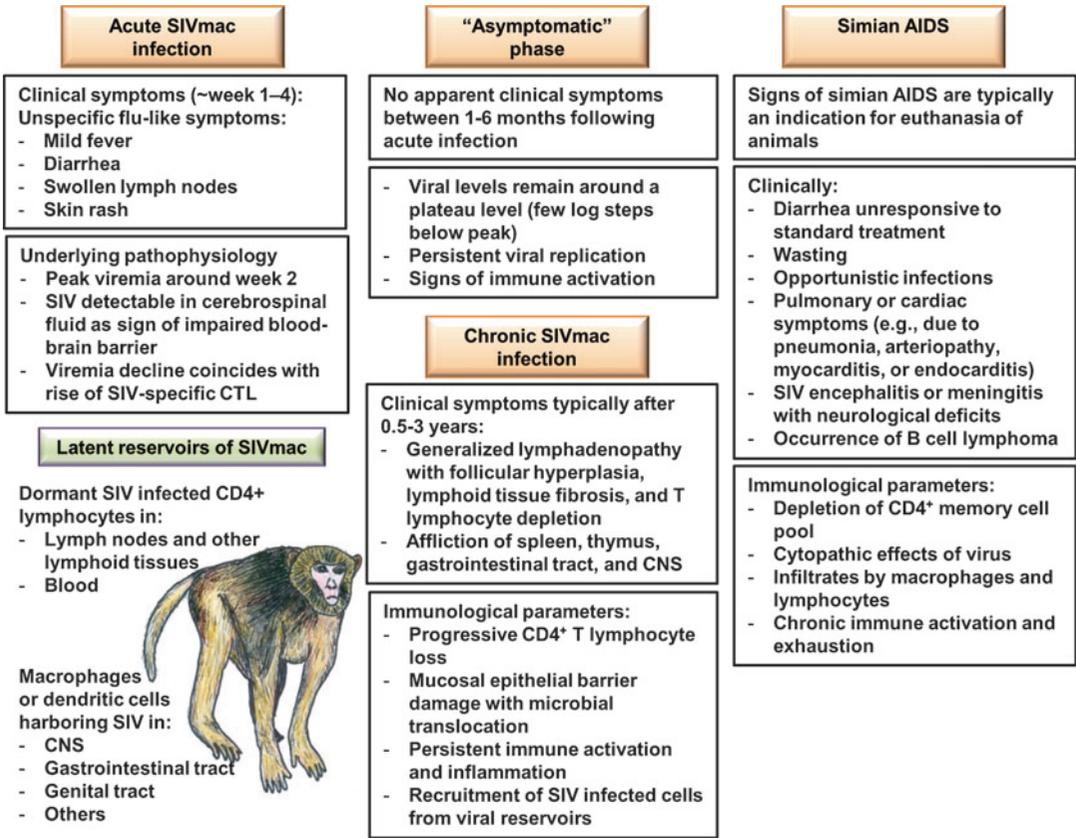
Pathogenic SIVmac infection of Asian NHP causes strong innate immune responses similar to nonpathogenic SIV infection of African NHP. However, SIV-infected African natural hosts present an enhanced immune activation only during acute SIV infection, but this increased immune activation is resolved in the transition to chronic infection. In contrast, chronic hyperimmune activation is a hallmark of pathogenic SIVmac infection. The lack of pathogenicity of SIV infection in African natural hosts is believed to be indicative for specific host adaptations, evolved during extended coevolution of host and virus and not for an effective immune control (Bosinger et al. 2011; Picker et al. 2012). Both adaptive and innate immune responses contribute to keeping the SIVmac virus infection temporally in check, permitting a partial viral containment. However, ultimately these immune responses fail

to eliminate the virus and the vast majority of humans and nonnatural host NHP develop full-blown AIDS.

Clinical Pathological Changes in SIVmac-Infected Rhesus Macaques

Initial clinical symptoms during primary SIVmac infection are unspecific and last from approximately 1–4 weeks (Fig. 3). They can include mild fever, diarrhea, swollen lymph nodes, and malaise. A skin rash with disseminated papules at the trunk, face, and thighs is characterized by a perivascular lymphocytic dermatitis with cytotoxic activity directed against epidermal Langerhans cells. In some macaques, no apparent symptoms occur during the first few weeks following infection. The acute phase of infection is followed by an asymptomatic phase, which can last from months to years until clinical disease becomes apparent (Coffin et al. 1997; Abee et al. 2012).

Clinical signs of simian AIDS typically develop within 1–3 years (compared to 7–10 years in untreated humans), depending on the pathogenicity of the virus, the defense mechanisms of the primate host (i.e., the genetic background), and comorbidities. During the chronic disease stage, various lymph nodes become swollen. In macaques with more prolonged disease course, generalized lymphadenopathy develops with follicular hyperplasia and confluent germinal centers that may be missing in animals with rapid disease progression (Coffin et al. 1997; Friedman et al. 2006). Early after infection, pathogenic SIVmac is detected in the paracortex of lymph nodes, in periarteriolar lymphoid sheaths of the spleen, in medulla of the thymus, to varying degree in the gastrointestinal tract, in the CNS, and in other lymphoid tissues (Letvin et al. 1994). Persistent immune activation leads to chronic inflammation and tissue injury. Attempting to heal the damage, fibroblasts constituting the structural framework of the lymphoid tissue react upon the inflammation by excessive collagen and fibronectin depositions into the extracellular matrix, replacing normal tissue by scar-like



SIVmac Infection of Macaques, Immunopathogenesis of, Fig. 3 Different phases of pathogenic SIV infection. Acute SIV infection is distinguished from chronic pathogenic SIV infection that gradually progresses to simian AIDS. Between the acute and chronic phase, some distinguish an additional "asymptomatic" phase. However, since this phase shows persistent viral

replication and signs of immune activation, the "asymptomatic" phase can also be allotted to the chronic SIV infection. Typical signs and symptoms with underlying pathophysiology are listed. Already starting during acute SIV infection, latent reservoirs of SIV are established that evade immune responses and/or retroviral treatment and contribute to viral persistence

structural elements. Besides providing a supportive infrastructure for lymphocyte populations, fibroblastic reticular cells are crucial for the immunological function by providing lymphocytes with IL-7 (Estes 2013). As naive T cells depend on IL-7 for survival, lymphoid tissue fibrosis causes lymphocytic depletion through programmed cell death, especially of naive CD4(+) T lymphocytes. Since fibroblastic reticular cells require lymphotoxin-beta (LT-β) produced by T lymphocytes for proliferation, the reciprocal dependency of T lymphocytes and fibroblastic reticular cell network perpetually intensifies the progressive fibrosis and CD4(+) T lymphocyte depletion, causing not only

progressive remodeling of the lymphoid architecture but also loss of function (Estes 2013).

A hallmark for pathogenic SIVmac infection is a CD4(+) T lymphocyte loss with a decline of the absolute and relative numbers of CD4(+) T cells and a decreased CD4(+)/CD8(+) T-cell ratio (Letvin et al. 1994; Friedman et al. 2006; Voevodin and Marx 2009; Estes 2013). SIVmac infection is associated with gastrointestinal mucosal epithelial barrier damage that leads to microbial translocation contributing to persistent immune activation and inflammation (Bosinger et al. 2011; Pancino et al. 2011; Estes 2013). Hence, lipopolysaccharide (LPS) produced by bacteria leaking through the gut are elevated in

the plasma and can be used as biomarkers for microbial translocation. Due to the gut barrier damage, chronically SIVmac-infected animals frequently develop persistent diarrhea, occasionally bloody, that is unresponsive to standard treatment and results in dehydration of the monkey. Moribund macaques develop wasting with massive weight loss so that eventually only minimal fat reserves remain, and hip bones and ribs become prominent (Voevodin and Marx 2009; Abee et al. 2012).

Due to its suppression of the immune system, SIVmac triggers opportunistic infections such as generalized cytomegalovirus infection, cryptosporidiosis, *Pneumocystis carinii* pneumonia, candidiasis, atypical tuberculosis by mycobacterium avium, and other, often unusual, infectious diseases (Abee et al. 2012). Furthermore, recent investigations have shown that SIV-infected rhesus macaques suffer from a large number of different gastrointestinal virus infections that contribute to the pathogenicity of the SIV infection (Handley et al. 2012).

Increased cytokine production and elevated numbers of proinflammatory Th17 cells, a lineage of CD4(+) T helper cells producing IL-17 for antimicrobial immune defense at mucosal barriers, as well as SIVmac-infected macrophages in the lung may also cause disorders of the lungs, heart, and blood vessels (Pancino et al. 2011). Chronically SIVmac-infected macaques occasionally develop granulomatous multinucleated giant cell pneumonia, pulmonary arteriopathy that may be associated with thrombosis of vessels and can lead to infarction of lung parenchyma. Pulmonary symptoms clinically manifest as difficulties in breathing or heavy breathing through the open mouth. Cardiac symptoms can occur as a result of an SIV-induced myocarditis and cardiomyopathy. Arteriopathies are caused by infiltrates of moderate numbers of macrophages and lymphocytes causing proliferation of the intimal and medial layer of blood vessels. Similar lesions combined with inflammations develop in other organ systems such as the kidney, pancreas, genital tract, and others causing a multitude of symptoms depending on the organs affected (Coffin et al. 1997; Voevodin and Marx 2009; Abee et al. 2012).

Chronic inflammation and immune activation by SIVmac may also cause disease of the eyes. Ocular pathology in rhesus macaques, e.g., demonstrating as granulomatous lesions of the retina, can be utilized as animal model for retinal pathology in HIV patients (Abee et al. 2012).

SIVmac can be detected in the macaque brain as early as 3 days following primary infection and consistently during peak viremia in perivascular cuffs, the meninges, and the choroid plexus that enables virus detection in cerebrospinal fluid (Williams and Burdo 2012). After crossing the blood-brain barrier, the virus cannot be efficiently reached by many drugs used for antiretroviral therapy and has the ability to hide out for prolonged periods, unrecognized by the immune system, contributing to viral persistence. Thus, the central nervous system (CNS) is regarded as potential latent viral reservoir for proviral DNA (Dang et al. 2012). Following an asymptomatic phase, typically about 25% of SIVmac251-infected rhesus macaques develop SIV encephalitis (SIVE) or meningitis after 1–3 years (Dang et al. 2012; Williams and Burdo 2012). SIVE occurs at a similar rate like CNS manifestations in HIV-1 infected patients without antiretroviral treatment, although neurological symptoms usually progress faster in macaques than in humans. After experimental persistent CD8(+) T-cell depletion of macaques with depleting antibodies, the SIVE risk increases to over 75% with rapid disease progression (Strickland et al. 2012). SIVE is characterized by multinucleated giant cells without inclusion bodies and accumulations of lymphocytes and macrophages perivascular (i.e., around blood vessels) in the CNS. As a marker for cell proliferation and indicator for enhanced immune stimulation in SIVE, increased expression of Ki-67(+) by CD4(+) and CD8(+) memory T lymphocytes is correlated to SIV viral loads in cerebrospinal fluid, but not necessarily SIV plasma viral levels (Dang et al. 2012). Progressive neurological signs manifest as various clinical symptoms, e.g., head tilt, balance difficulties, psychomotoric dysfunction (with overall reduced activities), ataxia (with lack of coordinated muscle movement), or tremor (Dang et al. 2012).

Analogous to HIV-infected human individuals who carry an increased risk of developing non-Hodgkin lymphoma (NHL) or Kaposi's sarcoma (especially in patients without antiretroviral treatment), SIV-infected macaques have a higher likelihood to develop neoplasms, particularly B cell-derived NHL (Voevodin and Marx 2009). While human NHL in HIV patients has been associated with Epstein-Barr virus (EBV), NHP SIV-related B cell lymphomas occur in the presence of rhesus lymphocryptovirus (the macaque equivalent of EBV), and primate rhadinovirus is the correspondent to the human rhadinovirus Kaposi's sarcoma-associated herpesvirus (Abee et al. 2012). SIV is not oncogenic and therefore does not directly cause formation of neoplasms. However, the induced immunosuppression enhances the chance for malignancies (Coffin et al. 1997).

HIV Vaccines

In the most ideal scenario, a successful HIV vaccine would provide sterile protection by completely blocking the virus infection. A similar beneficial vaccine effect may be observed if the vaccine could turn a virus infection into an abortive infection that would permit a local temporal infection without systemic dissemination. If infection could not be prevented, an HIV vaccine should at least slow disease progression. Broadly neutralizing antibodies are the most promising mode of action to provide sterile protection. However, due to a large number of issues, the induction of these kinds of antibodies by an HIV vaccine has been notoriously difficult. Cellular immune responses can only attack the virus once it starts to propagate in vivo and, therefore, at best restrict virus replication at the level of an abortive infection. However, it is commonly accepted that an effective HIV vaccine should induce multiple layers of protection utilizing antibodies and cellular immune responses.

As an effective HIV-1 vaccine still remains elusive, the SIV-macaque animal model serves as a valuable tool to investigate correlates of protection and to evaluate novel treatment modalities and

preclinical HIV vaccine candidates. Vaccination of rhesus macaques with live-attenuated SIV, generated by deletion of the *nef* gene from replication-competent SIVmac239 (SIVmac Δ *nef*), has proven to be the most efficient means of protection against challenge with wild-type pathogenic SIVmac (Coffin et al. 1997). However, due to considerable safety concerns, the live-attenuated HIV vaccine concept could not be advanced into humans: In some vaccinated macaques, the *nef* deletion gets restored due to high retroviral mutation rates and recombination tendencies. The resulting wild-type revertants constitute a major safety concern. Moreover, some SIVmac Δ *nef*-infected infant macaques developed symptoms of simian AIDS so that live-attenuated HIV vaccines based on replication-competent HIV-1 are not suitable as human vaccine (Coffin et al. 1997).

HIV vaccine candidates in recent preclinical trials in macaques have included recombinant viral vectors engineered to express SIV/HIV genes, proteins, synthetic peptides, and recombinant DNA. Various combinations of prime-boost strategies have been applied (Sui et al. 2013). Viral vectors tested in rhesus macaques have been based on poxviruses, adenovirus, vesicular stomatitis virus, cytomegalovirus (CMV), and others, either replication incompetent or replication competent. Frequently inserted genes include *gag*, *env*, and others, but there has not been conclusive agreement which gene inserts are necessary to achieve protection or which genes might even enhance pathogenicity (Sui et al. 2013). Although several HIV vaccine candidates have stimulated enhanced cellular or humoral immune responses, the majority have failed to permanently diminish viral replication and functional viral escape, especially when challenged with heterologous or more neutralization-resistant SIV or SHIV strains.

A number of HIV vaccine trials based on eliciting cellular immune responses were able to slow disease progression or even delay acquisition of infection. However so far, HIV vaccines based on the elicitation of cellular immune responses were not successful in humans and in some cases may have even increased the likelihood of infection. A promising new vaccine concept has recently emerged with the generation of

an SIV protein-expressing replication-competent rhesus CMV vaccine. This approach has elicited robust and durable effector memory CD8(+) T cell capable to effectively control SIV replication in about 50% of the animals (Picker et al. 2012; Sui et al. 2013).

Some of the most promising vaccine candidates build on top of the discovery of very potent neutralizing antibodies either by providing a passive immunization (Barouch et al. 2013) or generation of these types of antibodies via an adenovirus-associated viral vector (Balazs et al. 2012). Moreover, the observation that the protection provided by the first successful AIDS vaccine (Thai Phase III clinical trial RV144) in humans was likely mediated by antibody responses has invigorated the entire field (Rerks-Ngarm et al. 2009; Haynes et al. 2012). A large number of vaccine trials are ongoing that focus on eliciting humoral immune responses against the four most important antibody targets to inhibit viral entry ((1) V3 region of HIV gp120, (2) V1/V2 region of HIV gp120, (3) CD4 binding site on HIV gp120, and (4) membrane-proximal region of HIV gp41).

Conclusions

The SIVmac/rhesus macaque model is an elegant preclinical model to study the immunopathogenesis of HIV infection in NHP. Investigation of viral determinants of SIVmac and their interactions in the NHP host system provides insight into the transmission of SIV, the early steps of infection that cannot be feasibly studied in humans, consecutive viral spread, establishment of viral reservoirs, immune defense mechanisms, and viral evasion from the host immune system. Thorough understanding of the immunopathogenesis of the SIV infection is crucial for the development of treatment modalities and the generation of a successful prophylactic HIV vaccine.

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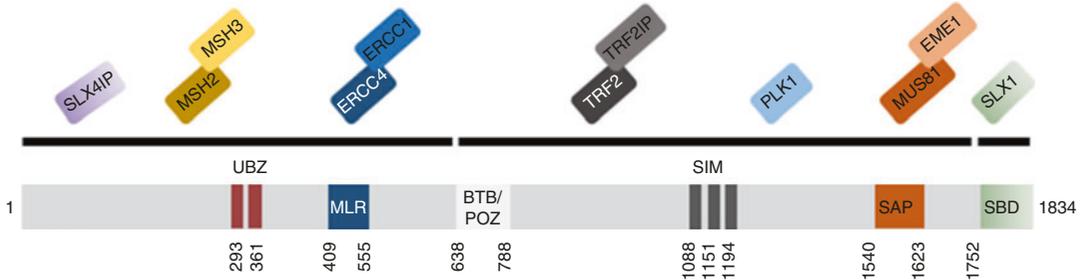
SLX4 Complex and HIV Replication

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Definition

SLX4, also known as BTBD12 or FANCP, is a Fanconi anemia (FA) complementation group protein. Mutations in *SLX4*, or any of the additional 18 FA genes, lead to FA, which is a rare, autosomal recessive, genetic disorder. Clinical features of FA patients include congenital physical anomaly, elevated cancer susceptibility, and



SLX4 Complex and HIV Replication, Fig. 1 Schematic representation of SLX4 scaffold protein and binding partners. SLX4 domains are presented with their binding

partners. Broad interaction domains are indicated by a thick black line

evolution toward aplastic anemia. FA is the most frequent inherited bone marrow failure syndrome. Collectively, FA proteins compose the FA DNA repair pathway that is involved in the repair of double strand breaks by homologous recombination (HR). SLX4 plays important roles in the final steps of HR by facilitating the resolutions of four-way DNA structures called Holliday junctions (HJ). Indeed, SLX4 is a molecular scaffold to which several proteins with nucleic acid binding and processing activity are recruited, forming the SLX4 complex (SLX4com). Additional reported functions of SLX4com include nucleolytic resolution of HJs, telomere maintenance, facilitation of the HIV life cycle, and regulation of spontaneous and HIV-induced innate immune responses.

Introduction

SLX4 is involved in the repair of stalled/collapsed replication forks and DNA interstrand cross-links (ICL) by HR. HR allows accurate repair of DNA lesions by using the sister chromatid as a template and leads to the formation of HJ that must be removed prior to chromosome segregation. The SLX4com is also involved in telomere maintenance through prevention of telomere damage and in negative regulation of pro-inflammatory responses in patient-derived cells, indicating that the FA pathway may also regulate the production of pro-inflammatory cytokines (for review (Kim 2014; Bregnard et al. 2014)). Biallelic inactivating mutations in *SLX4* lead to FA. In

this inherited syndrome, cells are susceptible to chromosomal instability and present hypersensitivity to DNA cross-linking agents, such as mitomycin C (MMC). FA patients are predisposed to various cancers, including leukemia, breast cancer, and head and neck squamous cell carcinoma. Another major clinical feature shared by FA patients is evolution toward aplastic anemia. The reason for bone marrow defects in FA most likely resides in combined molecular features of FA. Indeed, elevated production of pro-inflammatory cytokines, together with DNA repair defects, contributes to compromise bone marrow progenitors of FA patients laying the ground for aplastic anemia.

SLX4 is an 1834 amino acid protein that possesses a BTB/POZ structural domain, at position 638-788. BTB/POZ structural domains promote homomeric or heteromeric dimerization of proteins. SLX4 interacts with several proteins, including proteins involved in nucleic acid processing or binding (Fig. 1). This includes interaction with (i) structure-specific endonuclease modules. ERCC4^{XPB}-ERCC1 (5' endonuclease) interacts with the MUS312-ME19 interaction-like region (MLR) of SLX4, while MUS81-EME1 (3' endonuclease) interacts with its SAF-A/B, Acinus and PIAS (SAP) domain whereas SLX1 (5' endonuclease) with the SLX1 binding domain (SBD). Interaction of SLX4 with these structure-specific endonucleases is key to its role in the resolution of HJs. In addition, SLX4, via its N-terminal region, also interacts with (ii) the MSH2-MSH3 complex involved in DNA mismatch repair and (iii) the SLX4IP^{C200R}

protein of yet-to-be defined function, while its C-terminal region allows interaction with (iv) the PLK1 cell cycle-dependent regulatory kinase sheltering complex and with (v) the TRF2-TRF2IP components of the telomere sheltering complex. The latter interaction is important for SLX4-dependent telomere maintenance (Kim 2014). Interaction with PLK1 has important regulatory roles with regard to SLX4-associated endonuclease activities by ensuring activation of MUS81-EME1 through EME1 hyperphosphorylation.

SLX4 also contains a tandem of ubiquitin-binding motifs (UBZ – Fig. 1), which are essential for its role in the repair of DNA lesions. A cluster of three SUMO-interacting motifs (SIMs) at positions 1088-1091, 1151-1155, and 1194-1194 of SLX4 has been identified to promote SLX4 function in DNA repair processes. Finally, SLX4 and/or SLX4com appears to possess intrinsic SUMO-ligase activity, through binding to the SUMO-loaded SUMO E2 conjugating enzyme UBC9. SLX4-associated SUMO-ligase activity targets at least SLX4 and ERCC4 within the SLX4 complex. SLX4 SUMOylation would contribute to targeting SLX4 to DNA lesions (Gibbs-Seymour and Mailand 2015).

SLX4 and the Fanconi Anemia DNA Repair Pathway

The FA DNA repair pathway functions essentially during the S phase of the cell cycle when sister chromatids are present, allowing repair by HR. Repair of ICLs and collapsed/damaged replication forks by the FA pathway is initiated by recognition of lesions by the FA anchor complex based on FANCM that binds chromatin at damage sites. This triggers the recruitment of the core E3-ligase complex comprised of FANCA, FANCB, FANCC, FANCE, FANCF, FANCG and FANCL where FANCL bears the RING (Really Interesting New Gene) domain that confers E3-ligase activity. The FA core complex monoubiquitinates FANCD2–FANCI, providing the signal for downstream activation of effectors

of the pathway. Among the latter are SLX4, FANCO^{ERCC4}, and additional DNA repair proteins (FANCF^{BRIP}, FANCN^{PALB2}, FANCD1^{BRCA2}, and FANCO^{RAD51C}).

In somatic cells, the favored pathway to remove HJ relies on Bloom (BLM)-related helicases that promote “dissolution” rather than “resolution.” Resolution is a process that may promote sister chromatid exchanges, thus leading to potential cancer-predisposing loss of heterozygosity. For this reason, it is broadly accepted that HJ resolution pathways mostly act as a backup mechanism in cases – absence of BLM or overload of the dissolution pathway – where dissolution does not suffice. Resolution of HJ is mediated by two major pathways in eukaryotic cells: resolvase activity resulting from (i) interaction of SLX1 and MUS81-EME1 with SLX4 or (ii) mediated by GEN1 (for review (Sarbjana and West 2014)). Resolution of HJ by the SLX4com has been shown to occur in a sequential way, by a coordinated mechanism with SLX1 catalyzing the initial rate-limiting incision into an intact HJ and MUS81 cleaving the nicked HJ. Activation of SLX4-associated MUS81-EME1 is cell cycle dependent and is mostly confined to the Gap 2 (G2) phase of the cell cycle and early mitosis (M) after synthesis of bulk DNA (during the S phase) has been completed. This prevents inappropriate processing of healthy replication forks during S phase and subsequent replication stress. Temporal regulation of MUS81-EME1 is achieved in mammalian cells through hyperphosphorylation of EME1 by PLK1.

Although ERCC4-ERCC1 have been shown to interact with SLX4 and to harbor 3' endonuclease activity, the role played by this endonuclease module in HJ resolution remains unclear. Importantly, this structure-specific endonuclease module has been shown to play crucial roles in nucleotide excision repair and to be involved in telomere maintenance through interaction with TRF2. Whether this involves SLX4com-engaged ERCC4-ERCC1 remains to be explored, particularly in the light of SLX4com binding to telomeres.

HIV Vpr and DNA Repair Pathways During HIV Infection

The HIV life cycle interferes with pathways involved in DNA repair, in particular through the Vpr accessory protein. Vpr is a virally encoded 14 kDa protein that exerts many molecular functions resulting in a major cellular phenotype: cell cycle arrest at the G2/M boundary of the cell cycle.

Cell Cycle in the Absence of Vpr

The cell cycle of mammalian cells is divided in four phases: (i) the Gap 1 (G1) phase that corresponds to the interval between M and (ii) the S phase, and (iii) the G2 phase, which precedes the (iv) M phase – where segregation of chromosomes occurs. In healthy cells, the transition through the different phases of the cell cycle is controlled by cyclin-dependent kinases (CDK) and cyclin (CCN) complexes. Cell cycle checkpoints serve as control mechanisms at each of these steps to ensure that the genetic material is correctly transmitted to daughter cells. Halting the cell cycle at these transition steps provides an opportunity to repair lesions or, if the extent of incurred damage is too high, leads the cell to apoptosis.

The G2/M transition is controlled by CDK1:CCNB1. During G2, CDK1:CCNB1 is activated through phosphorylation of CCNB1 by CDK1 and PLK1. This leads to nuclear sequestration of CDK1:CCNB1. Full activation of CDK1:CCNB1 requires dephosphorylation by CDC25C. Once the G2/M boundary is crossed, the complex is inactivated by ubiquitination of CCNB1 by the anaphase-promoting complex (APC). When genotoxic stress is incurred, entry into M can be prevented through inactivation of CDC25C (Stark and Taylor 2006). This response is regulated through a signaling cascade that involves detection of DNA lesions by the key DNA damage response regulators ataxia-telangiectasia-mutated kinase (ATM), ATM and Rad3-related kinase (ATR), and DNA-dependent protein kinase (DNA-PK) and downstream activation of CHK1 or CHK2.

These phosphorylate CDC25C. Consequently, the CDK1:CCNB1 complex remains inactive and the cell cycle stops at the G2/M transition.

Vpr and the G2/M Boundary

Expression of the HIV-1 Vpr accessory protein activates ATR, ATM, CHK1, and CHK2 kinases, causing inactivation of CDK1:CCNB1. In agreement, treatment of Vpr-expressing cells with caffeine, which inhibits ATR and ATM, relieves the Vpr-mediated cell cycle block. Several mechanisms have been proposed to explain checkpoint activation in the presence of Vpr.

Mobilization onto subregions of the chromatin of DNA repair proteins, such as breast cancer susceptibility protein 1 (BRCA1) and γ H2ax proteins, has been reported following expression of Vpr, forming foci that are downstream activation hallmarks of the ATM/ATR pathway. Indeed, factors involved in the repair of broken DNA are recruited to these foci. However, it remains unclear whether Vpr expression leads to genomic lesions. Furthermore, the link between genomic lesions and cell cycle arrest in this context is not established.

Interaction of Vpr with several cellular proteins has been reported, but the only direct partners are VPRBP and SLX4. Vpr interacts with the C-terminus of VPRBP. The interaction of Vpr with the VPRBP-DDB1-CUL4 E3-ligase complex is required, but not sufficient, for G2/M arrest. Vpr can directly bind the carboxy-terminal region of SLX4. Vpr induces the recruitment of VPRBP and kinase-active PLK1 to SLX4. This leads to VPRBP-dependent ubiquitination of MUS81 and hyperphosphorylation of EME1 (Laguet et al. 2014). Thus, SLX4-associated MUS81-EME1 is activated outside of the physiological time frame, i.e., during the S phase. Consequently, Vpr-induced activation of SLX4-bound MUS81-EME1 results in cleavage of healthy replication forks, which leads to replication stress and to G2/M cell cycle arrest. Thus, as supported by previous work (Li et al. 2010), Vpr causes cell cycle arrest through a S phase-dependent mechanism.

SLX4 has also been identified as an ATR substrate, and in yeast, phosphorylation of Eme1 requires Rad53^{ATR} activation (Dehe et al. 2013). Thus, aberrant processing of stalled replication forks by Vpr-activated SLX4-associated MUS81-EME1 would cause replication stress, ATR-CHK1 pathway activation, resulting in inhibition of CDC25C. This signaling cascade ultimately leads to inability of CDC25C to activate CCNB1:CDK1 and thus results in G2/M arrest (Bregnard et al. 2014) (Fig. 2).

Vpr and Genomic Instability

MUS81-EME1 are important for the removal of ultrafine DNA bridges (UFBs) that arise from regions of the genome that replicate at a slower rate, such as centromeres and common fragile sites. UFBs are formed during the S phase and can be visualized after chromosome condensation in mitotic cells as bridges between sister chromatids that must be processed to allow chromosome segregation. Absence of MUS81-EME1 leads to accumulation of unprocessed UFBs and subsequent chromosomal instability and mitotic catastrophe. In the absence of Vpr, accumulation of UFBs is usually translated into cell cycle arrest at the G1/S transition.

Vpr targets MUS81 for ubiquitination by VPRBP, leading to decreased levels of MUS81. Since Vpr expression leads to accumulation of UFBs, it is likely that decreased MUS81 levels in Vpr-expressing cells may be sufficient to impair UFB processing prior to M. However, Vpr-associated replication stress that prevents completion of G2 likely impedes mitotic catastrophe that would otherwise occur. In addition, steric hindrance imposed by UFBs by linking sister chromatids may also contribute to the extent of G2/M arrest witnessed in Vpr-expressing cells (for review (Bregnard et al. 2014)) (Fig. 2).

SLX4 and HIV Escape from Innate Immune Sensing

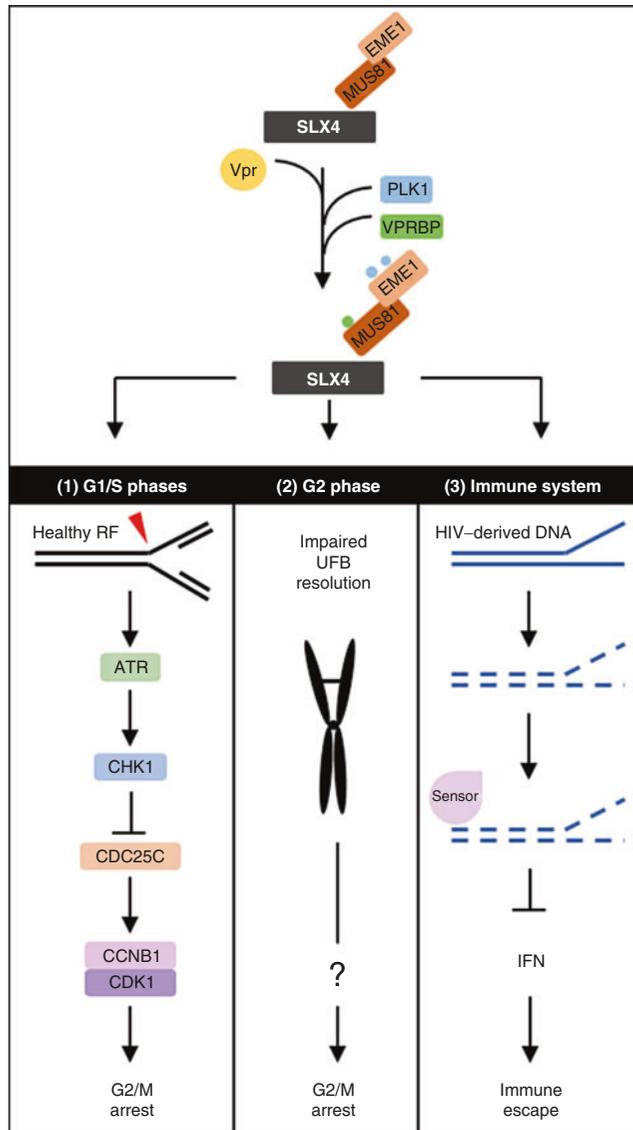
HIV Escape from Innate Immune Recognition

Efficient HIV replication in target cells relies on both the availability of cellular dependency

factors and the ability to escape cellular blocks. One of the most critical steps of HIV life cycle is the delivery of virus-derived nucleic acids in the host cell. This obligate step of the viral life cycle is targeted by several cellular factors, including cellular sensors that are specialized in the recognition of noncanonical nucleic acid species. These sensors belong to the pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) to trigger a signaling cascade that leads to production of pro-inflammatory cytokines, including antiviral interferon (IFN).

PRRs are capable of recognizing a broad range of substrates, including nucleic acids. HIV-derived nucleic acid species include the ssRNA genome and the intermediates generated during the course of its reverse transcription into dsDNA, including DNA/RNA hybrids and DNA flap structures. These intermediates may be recognized by several cellular sensors. Indeed, viral RNA is detected by TLR7 in endosomes and activates IFN production (Beignon et al. 2005). IFI16 has been identified as involved in the detection of nonself, virus-derived DNA in the cytoplasm of lymphoid quiescent CD4 T cells (Monroe et al. 2014). Another cytosolic sensor was also identified to be a specific sensor of HIV and other retroviruses, the cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS), which recognizes viral DNA (Gao et al. 2013). These sensors are the first factors to trigger IFN production and thus establish an antiviral response to infections.

HIV-1 has developed different mechanisms to escape the innate immune response and establish productive infection. To prevent recognition of reverse-transcription intermediates, viral genomes are protected within the capsid core. If decapsidation is correctly orchestrated, fully reverse-transcribed viral genomes are delivered into the nucleus and integrated into the host cell genome. Otherwise, reverse-transcription intermediates may be released in the host cell cytoplasm where they can be detected by innate immune sensors and have adverse effects on the viral life cycle. In this scenario, several mechanisms coexist to prevent innate immune signaling



SLX4 Complex and HIV Replication, Fig. 2 Consequences of premature SLX4 activation by Vpr. Vpr expression leads to the recruitment of PLK1 and VPRBP on SLX4. PLK1 phosphorylates EME1 while the VPRBP E3-ligase complex ubiquitinates MUS81 leading to premature activation of the SLX4com. (1) Premature activation of SLX4com leads to the processing of healthy replication forks (RF) and to ATR activation. CHK1 is activated and inhibits CDC25C by phosphorylation. Thus, CKNB1/CDK1 is inhibited, leading to cell cycle

arrest at the G2/M transition. (2) UFBs formed during S phase cannot be removed during G2 because of premature degradation of MUS81-EME1 complex after VPRBP-dependent ubiquitination. This could contribute to G2/M arrest after HIV infection. (3) SLX4com binds to HIV DNA. SLX4com cleaves dsDNA, which is no longer recognized by a cytoplasmic immune sensor resulting in the block of interferon (IFN) production. This likely contributes to the immune escape of HIV

resulting from exposure of HIV-derived nucleic acid species. These mechanisms include either a direct action on the IFN signaling cascade (inhibition of IFN synthesis, IFN receptor decoy

and inhibition of IFN signaling, or degradation of abortive HIV-derived nucleic acid species, through cellular exonucleases (ribonuclease H2 (RNaseH2) and three-prime repair exonuclease 1

(TREX-1)) or the SLX4com (Laguette et al. 2014).

SLX4 in HIV Escape from Innate Immune Sensing

Infection with an HIV-1 molecular clone harboring a deletion of the Vpr open reading frame causes an increase of IFN production as compared to infection with wild-type HIV-1 (Doehle et al. 2009; Laguette et al. 2014; Okumura et al. 2008). This HIV-induced IFN production is increased following SLX4com subunit (SLX4, VPRBP, and MUS81) knockdown, indicating that the SLX4 complex is required for inhibition of HIV-dependent pro-inflammatory cytokine production (Laguette et al. 2014). *SLX4* or *MUS81* deficiency leads to activation of pro-inflammatory pathways, resulting in the establishment of an antiviral state through upregulation of IFN-stimulated genes rendering the cells unable to support efficient HIV replication. Furthermore, the SLX4com binds HIV-1-derived reverse-transcribed DNA only in the presence of Vpr, and in the absence of SLX4, there is an increase of HIV DNA accumulation in infected cells. This suggests that the SLX4com is required to degrade excess HIV-derived nucleic acids susceptible of triggering innate immune responses, a process for which the MUS81-EME1 endonuclease module appears to be required. Of note, Vpr also compromises the establishment of adaptive immune responses. Vpr-mediated impairment of innate immune recognition may contribute to this process (Fig. 2).

Conclusion

The SLX4 protein, through recruitment of structure-specific endonucleases, is an actor of DNA repair. The HIV-1 Vpr protein takes advantage of SLX4com-associated endonuclease activities, inducing the processing of viral reverse-transcription intermediates that, if present in excess in the cytoplasm, would activate immune responses. In addition, SLX4 also recruits MSH2-MSH3 and TRF2-TRF2IP. The latter is known to account for the protective role played by SLX4 at

telomeres. This function may therefore be altered in the presence of Vpr. This is of particular importance in the light of telomeric damage inducing pro-inflammatory signaling. In addition, the contribution of SLX4 activation to additional Vpr-associated functions remains to be explored. Indeed, Vpr contributes to fidelity of reverse transcription and to the nuclear transport of the pre-integration complex and promotes the trans-activation of LTR promoter and induction of apoptosis. It is clear that HIV-1 exploits the cellular environment to establish a productive and effective infection. The SLX4 complex is an example of how the virus uses a component of the cellular machinery, usually involved in the DNA repair pathway to favor its life cycle.

Cross-References

- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [HIV “Auxiliary” Proteins](#)
- ▶ [HIV Life Cycle: Overview](#)
- ▶ [Identification and Validation of HIV Cofactors](#)
- ▶ [Inflammatory Cytokines](#)

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South, Southeast, and East Asia-Specific Characteristics of HIV/AIDS Epidemic

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Definition

An estimated five million people are living with HIV/AIDS (PLHIV) in Asia as of early 2015. While the characteristics of the HIV epidemic in Asia vary from country to country, there are four major transmission routes, including commercial sex work, unprotected sex with commercial sex workers, needle sharing among persons who inject drugs (PWID), and unprotected sex among men who have sex with men (MSM) (AVERT 2015).

Overview

Compared to Africa, the Americas, and Europe, the HIV epidemic in Asian countries started a decade later. In the early to mid-1980s, while other parts of the world began to tackle the severe HIV epidemic, Asian prevalence rates were exceedingly low, though this changed quickly in the early 1990s. Although the overall HIV prevalence is still much lower compared to countries in southern Africa, Asia's huge population makes the absolute number of PLHIV large.

With declining rates of transmission among PWID due to expanded needle/syringe exchange and, in some venues, drug treatment opportunities, unprotected sex is the major route of transmission, notably linked to sex work and male-to-male sex. It is estimated that from 0.5% to 15% of men buy sex from commercial sex workers in any given year (WHO/UNAIDS/UNICEF 2011). These clients of sex workers can then transmit the HIV that they contracted from sex workers to their low-risk partners (e.g., wives, girlfriends). An increasingly prominent subgroup accounts for the rapid increasing epidemic in many Asian nations, namely, those MSM who do not use condoms often and have multiple partners. More than their Western peers, Asian MSM are more likely to hide their sexual preferences due to the cultural norms and prevalent homophobia. They may get married and have offspring to carry their family names and satisfy family expectations. Thus, MSM can act as a “bridging population,” transmitting HIV both among MSM and also to a broader population (van Griensven and de Lind van Wijngaarden 2010; Wirtz et al. 2013). The second route of transmission is through sharing needles among PWID. Having declined in frequency in nations that have had an aggressive prevention intervention response, such as China, India, Indonesia, Malaysia, Thailand, and Vietnam, HIV transmission continues in nations that have not yet fully embraced prevention on a mass scale, including Burma, Pakistan, and some central Asian nations that are still within the Russian sphere of influence. (Russia is hostile to needle and syringe exchange and, as a consequence, has one of the world's worst HIV epidemics related to

injection drug use [IDU].) In addition, the overlap between IDU and commercial sex makes epidemic dynamics complex. PWID may sell sex to support their drug use habit, or they may turn to drug use after their sex work is initiated (Tucker et al. 2011; UNAIDS 2013).

Region-Specific Characteristics

East Asia (Table 1)

East Asia encompasses a population of over 1.7 billion, including China and Taiwan, Japan, North and South Korea, and Mongolia, among others. Although the HIV prevalence in East Asia is relatively low (<0.1% in the age group between 15 and 49) compared to other regions in the world, the large size of population means that there are hundreds of thousands of PLHIV needing care. Sexual transmission has surpassed IDU as the dominant route of HIV spread. Although heterosexual transmission is most often reported, male homosexual contact is increasing rapidly since 2000, posing a challenge for HIV prevention in East Asia that is reminiscent of challenges in Europe and the Americas.

China, the country with largest population size, has an estimated 437,000 PLHIV (NHFPC 2014). Heterosexual transmission is the dominant route which accounts for 52% of newly infected HIV from 2013 surveillance statistics. An estimated 4–10 million of female sex workers (FSW) work either part- or full time in China. While they have been blamed as a main source of HIV infection, their historic prevalence rates have typically been very low, with the exception of women who are also injection drugs. An estimated 6% of Chinese men have had sex with FSW. The large absolute number of commercial sex consumers combined with low condom use can result in men who are a bridging population, acquiring HIV from high-risk FSW to low-risk intimate partners of the FSW clients (Qian et al. 2005). Since male-to-male sexual contact accounted for 13.7% of HIV infections in 2012, an increase from 2.5% in 2006, this is an increasing proportion of cases. MSM, too, can serve as bridges when they are bisexual with contacts with

low-risk women or with FSW. The traditionally dominant role of HIV transmission among PWID has kept decreasing since 2000 as a result of effects by the Chinese government, community-based organizations, and support from the international community (AVERT 2015; NHFPC 2014; Wu et al. 2015, 2013). A unique and massive outbreak of HIV among former plasma donors in central China was caused by illegal and unhygienic blood donation practices where pooled red blood cells were reinfused post-donation to reduce anemia among donors. This was halted by legal and police action by the mid-1990s, but not until hundreds of thousands of rural farmers and family members were infected (Qian et al. 2006).

Japan is an island nation in East Asia and one of ten most populous nations in the world. By 2011, only 11,146 HIV-positive patients and 5,158 AIDS patients had been reported. The number of new HIV infections reached a record high in 2010 with a total of 1075 new cases of PLHIV. MSM have accounted for more than half of PLHIV in Japan since 2006. Meanwhile, the style of drug use has switched from Japanese to Western style in the past a few years, and PWID accounted for 0.3–0.4% of HIV infections in 2011. About one third of HIV cases were identified among unregistered migrant workers in Japan (AVERT 2015). Despite high numbers of FSW in Japan, HIV has been kept to very low levels by near-universal condom use.

Mongolia is a landlocked country border with Russia to the north and China to the south, east, and west with a total population of 2.8 million in east Central Asia. HIV prevalence is very low in Mongolia with only 62 HIV cases having been reported by the year of 2009. About 80% of cases were among MSM practicing condomless anal intercourse. Bisexual men serve as a bridging population to wives, girlfriends, and/or FSW. Although the HIV prevalence among FSW is relatively low compared to MSM, high rates of sexually transmitted infections suggest potential for further spread of HIV (Munkhbaatar et al. 2014; Parcesepe et al. 2015; Hagan and Dulmaa 2007; Schwebke et al. 1998). In addition, due to poverty and limited employment opportunities, a

South, Southeast, and East Asia-Specific Characteristics of HIV/AIDS Epidemic, Table 1 Characteristics of HIV epidemic in East Asia

Countries	Epidemics				Risk populations				
	# of PLWHA in 2014	Prevalence (0.046–0.070%)	Categorization	Characteristics of the epidemic	Sex workers	MSM	Drug users	Mom to children	Other risk populations
China	437,000	0.058% (0.046–0.070%)	Low-prevalence country	Heterosexual transmission surpassing other routes becomes the dominant transmission route; the number of PLHIV keeps increasing but new infections have been retained at a low level (2012 UNAIDS report)	52% of newly infected HIV is attributable to heterosexual transmission. An estimate of 4–10 million FSW actively work in China	Homosexual contact accounted for 13.7% of HIV infection, compared to 2.5% in 2006	HIV prevalence is 6.4% among this population in 2012, and it keeps decreasing	1.1% of HIV infection was through the mother-to-child transmission in 2011. 75% of HIV-positive pregnant women received ART to prevent MTCT	Former plasma donors: account for 10% of HIV infection in 2005
Japan	11,146 in 2011	<0.1%	Low prevalence	Homosexual transmission is the dominant route, which accounted for more than half of the HIV infection	Sex industry gets increased although it is illegal	In cumulative number, homosexual transmission accounted for 63% of the 11,146 HIV-positive patients and 40% of 5,158 AIDS patients as of December 30, 2011	IDU accounted for 0.3–0.4% of HIV infection in 2011. The pattern of drug use has been changed from Japanese style to Western style (Wada 2013)		Unregistered migrant workers accounted for 1/3 of all cases in 2004. HIV tainted-blood scandal among hemophiliac patients in late 1980s
Mongolia	150 reported case in 2013		Very low prevalence	Early stage with only 5 cases registered during 1992–2005, 20 registered during 2005–2006. With only 150 cases reported as of December 31, 2013		7.5% among MSM in a cross-sectional study conducted in 2012, compared with 6.9% in 2009; more than 80% of cases of HIV were attributable to MSM			
South Korea	10,425 cases by 2013		Low prevalence	Men infection was outnumbered 14 times higher than women, and sex transmission is the major route					

significant proportion (14% in 2005, 5% in 2007) of young women (15–24 years) had reported having sex for money or gifts. Few were self-identified as FSW. With the opening of foreign travel opportunities, an increasing number of Mongolian women have migrated for work as FSW in either China or Russia in the 2000s (AVERT 2015).

South Korea: Since the first HIV case was reported in 1985, a total of 10,425 cases had been reported through 2013 with a high male-to-female ratio of 14.0. Condomless sex, particularly among MSM, is the principal route of transmission. Due to strong stigma and discrimination against HIV and homosexuality, HIV prevention and treatment has been challenging (AVERT 2015).

South Asia (Table 2)

The HIV epidemic in South Asia has been driven by PWID and sex work, though the roles of migrant men and MSM are also substantial. The relative importance of different subpopulations and transmission routes vary by country and even within regions of a country significantly. Poor public health hygiene (e.g., rampant use of therapeutic injections), the often low status of women, the large transvestite/transgender community (*hijras*), and men who migrate for work and often frequent female or male sex workers, MSM, and focal IDU drive the complex South Asian epidemic. Coinfection of HIV and other infectious diseases (e.g., hepatitis C virus [HCV] and tuberculosis [TB]) complicates the treatment regimens and care for patients. India plays an important role in South Asia due to the prevalence of vulnerable populations, a diverse set of epidemic drivers, high HIV prevalence in the urban south, and proximity to many contiguous nations. Heroin from opium is readily available, given the magnitude of poppy farming in Afghanistan. To make drug use more affordable and psychoactive, many PWID choose injection, and needle sharing is common. In addition, migrants include truck drivers, seasonal workers, refugees, and displaced people; all can bridge HIV from high- to low-risk populations within and across countries in this region (AVERT 2015).

Afghanistan is a landlocked country located in Central Asia and South Asia. It is one of the most impoverished countries in the world and has experienced over three decades of war. It is estimated that 4,300 Afghans were living with HIV by the year 2012, mainly PWID. As one of the major opium-producing countries, an estimated one million people aged between 15 and 64 are opioid users. With the reduction of availability of heroin potent enough to be smoked, drug users are more likely to turn to a more cost-effective option— injection. The use of non-sterile shared needles, syringes, and equipment (i.e., other drug paraphernalia) can lead to a rapid HIV transmission among PWID in needle/syringe sharing networks. In addition to PWID, there are a large number of refugees and displaced people in this country; survival sex work and sexual violence is common. Afghanistan's high level of illiteracy and other urgent health priorities (e.g., maternal and infant mortality, other infectious diseases) have contributed to a lack of focus on HIV prevention. Hence, HIV prevalence doubled in the 6 years since 2006 due to the combination of prevalent drug use and unsafe paid sex in the context of low HIV knowledge, lack of HIV testing, and promiscuous use of contaminated needles/syringes (AVERT 2015; UNAIDS 2013).

Bangladesh ranked as the world's eighth most populous country with 169 million people in 2015. The HIV epidemic is concentrated in high-risk populations including PWID, sex workers, MSM, and international returned migrant workers (Alam et al. 2013; Shariful Islam et al. 2015; Urmi et al. 2015). Needle sharing practices are very common among PWID, and approximately 10% of PWID have ever had paid sex with a small proportion of them use condom consistently. For international returned migrant workers, more than half identified HIV cases came from this group and their spouses. In addition, other factors contributing to Bangladesh's HIV/AIDS vulnerability include cross-border interaction with high-prevalence regions like Burma in India and low HIV knowledge among general population, which played an important role of HIV epidemic in this country. Furthermore, an epidemic with different HIV strains may emerge among FSW working in

South, Southeast, and East Asia-Specific Characteristics of HIV/AIDS Epidemic, Table 2 Characteristics of HIV epidemic in South Asia

Countries	Epidemics		Risk populations					Other risk populations
	# of PLHIV	Prevalence	Categorization	Characteristics of the epidemic	Sex workers	MSM	Drug users	
Afghanistan	4,300 [1,600–14,000]	<0.1% [$<0.1\%$ – $<0.1\%$]	Concentrated epidemic among IDUs	HIV prevalence has doubled since 2006. Mainly dominated among IDU	Drug use with unsafe sex made the epidemic even worse		A dramatic increase over the last 4 years	
Bangladesh	9,500 [4,100–97,000]	<0.1%	Low prevalence	focus on MSM, IDU and FSW as well as migrant workers			1.4% to 4.9% in 2005–2006; needle sharing is common practice; 10% IDU buying sex with only less than 10% using condoms	It was estimated that 67% of identified HIV-positive cases in the country were returnee migrant workers and their spouses
India	2,100,000	0.3% in 2013	It is predicted that India will be experiencing “generalized” epidemic	Most affected groups included injecting drug users, sex workers, truck drivers, migrant workers, and men who have sex with men	# of SW is 868,000 with 2.7% HIV prevalence	427,000 MSM with 4.4% of HIV prevalence; MSM had sexual relationship with females in the past 3 m	177,000 IDU with 7.1% of HIV prevalence which has been stabilized since 2007	In 2013–2014, 9.7 million pregnant women accessed HIV testing against a target of 13.2 million – coverage of 74%

(continued)

South, Southeast, and East Asia-Specific Characteristics of HIV/AIDS Epidemic, Table 2 (continued)

Countries	Risk populations											
	Epidemics		Characteristics of the epidemic			Sex workers	MSM	Drug users	Mom to children	Other risk populations		
Nepal	# of PLHIV	Prevalence	Categorization	Characteristics of the epidemic			Sex workers	MSM	Drug users	Mom to children	Other risk populations	
	50,200 in 2011	0.3% [0.2-0.4%]	HIV epidemic Nepal is highly heterogeneous and concentrated among most at-risk population including FSW, IDU, MSM and migrants	HIV in Nepal is extremely heterogeneous; unprotected sex is the main drive, followed by IDU, and MSM			24,649-28,359 FSW with 1.7% of HIV;	They are between 65,864 and 82,330 male sex workers, transgendered and their clients (MTC) in Nepal 2011.	An estimate of 30,155 and 33,742 IDU with 939 were PLHIV in 2011		Nationally, clients of FSWs have account for 4.4% of total estimated HIV infections. Nearly 8% HIV positive among migrants returning from Mumbai.	
Pakistan	97,000(46,000-210,000)	0.10%	Pakistan has a concentrated epidemic among injection drug users in most cities and among male sex workers in a few cities				HIV among female sex workers has remained negligible; the overall HIV prevalence among MSWs remains low at 3.1% (95%CI: 2.8,3.4)		HIV prevalence of 21% among IDUs in 2011		Unsafe injections account for 62% of Hepatitis B, 84% of Hepatitis C, and 3% of new HIV cases	

towns bordering India. Meanwhile, Bangladesh has a high burden of tuberculosis, and the coinfections complicate treatment regime and care for both diseases. All these factors make Bangladesh as the only country in South Asia where new infections keep rising (AVERT 2015; UNAIDS 2013).

Nepal is a landlocked country with a population of 27 million. There were an estimated 50,200 people living with HIV as of 2011, and more than half of them were unaware of their serostatus. The HIV epidemic in Nepal is highly heterogeneous and concentrated in vulnerable populations including FSW, PWID, MSM, and migrants. Nepal estimated that the number of PWID was around 33,000 by 2011. Injecting pharmaceutical drugs and poly-drug use are common. In addition, IDU is linked to commercial sex. HIV prevalence among FSW is about 1.7%. Many women from Nepal are recruited or are trafficked to Mumbai and elsewhere in India to work in brothels. Up to 50% of Nepalese sex workers working in brothels of Mumbai have been estimated to be HIV positive. In order to achieve economic survival, 1.5–2 million people work as internal or external migrants for seasonal and long-term labor. The removal of traditional structure of households promotes unsafe sexual practice including multiple sexual partners and engagement in commercial sex. As of 2011, male labor migrants comprised of 27% of HIV-infected persons in Nepal. Around 4.4% of HIV cases are among clients of sex workers, and 14.4% are MSM. Only 24% PLHIV were deemed eligible to receive ART by the end of 2011, and Nepal's HIV prevention program coverage is limited (AVERT 2015; UNAIDS 2013).

India is the world's second most populous country with over 1.2 billion people. In 2013, the Indian government estimated that 2.4 million Indians were living with HIV. HIV prevalence in India varies geographically, and the main risk groups include FSW, MSM, PWID, transgender/transvestite *hijras*, migrants (particularly those in the south and northeast), and long-distance truck drivers. It is estimated that 836,000 FSW have a prevalence of 2.7%. Government estimates of 427,000 MSM living in India are surely underestimates, and needs are great for both prevention and care (Patel et al. 2012; Thomas et al. 2011).

Due to prevalent stigma and discrimination, many MSM hide their sexual orientation through marriage or sexual relationships with women. An estimated 42% of MSM were married, and 50% have reported sexual relations with a woman in the past 3 months; half reported condom use in their last sexual encounter. Hijras (transgender women and/or transvestite men) often sell sex and represent a subgroup of high risk for HIV and bridging infection to their clients. The HIV prevalence stabilized among PWID and is now falling. An estimated 7.1% of 177,000 PWID in India are HIV infected. While needle/syringe exchange programs have been expanded with good results, opiate substitution therapy is not widely available (Reid et al. 2014). An HIV prevalence of 1% is estimated among 7.2 million migrant workers and 2.6% among long-distance truck drivers. Migrants also act as bridging populations between risk groups and general population (AVERT 2015; UNAIDS 2013).

Pakistan has an estimated population of 192 million in 2015, with an estimated 97,000 PLHIV. Early cases were among returning migrant workers who acquired infection in the Gulf emirates or elsewhere (Shah et al. 1999). The epidemic is concentrated among PWID, but also MSM, *hijras*, FSW, and prisoners (often PWID). The social norms of a conservative Muslim society oppose nonmarital sex, and efforts to prevent HIV are limited, with only modest risk reduction programs targeting MSM, *hijras*, and FSW (Thompson et al. 2013; Kazi et al. 2010; Siddiqui et al. 2011). With IDU as common in urban areas, the epidemic among PWID exploded in 2002 such that over 30% infection rates are seen in major urban areas and incidence rates are among the world's highest among PWID (Samo et al. 2013). Given low literacy rates, high levels of poverty, and prevalent risk behaviors among PWID and sex workers, the further spread of HIV is inevitable unless improved interventions are implemented (AVERT 2015; UNAIDS 2013; Abdullah and Shaikh 2015).

Southeast Asia (Table 3)

The epidemic in Southeast Asia is mainly driven by sexual transmission and IDU. Some countries,

South, Southeast, and East Asia-Specific Characteristics of HIV/AIDS Epidemic, Table 3 Characteristics of HIV epidemic in Southeast Asia

Countries	Risk populations								
	Epidemics	Prevalence	Categorization	Characteristics of the epidemic	Sex workers	MSM	Drug users	Mother to child	Other risk populations
Cambodia	# of PLHIV 76,000 [59,000–120,000]	0.8% [0.5–1.5%] for aged 18–49	Decreased HIV epidemic	Heterosexual transmission driven	15% infected with HIV	Low rate of condom use among MSM		About one- third of new infections occurred through MTCT	Sexual partners and clients of sex workers
Indonesia	610,000 [390,000–940,000] by 2012	Generalized epidemic with 2.4% HIV prevalence in general population ages 15–49	Rapidly increase	Fastest-growing epidemic in Asia. 59% of HIV infections is attributable to IDU, 41% due to heterosexual transmission	Extensive sex industry	High-risk behaviors: 5.2% among MSM (2007 survey)	36% of IDU were HIV infected in 2012		Sexual partners and clients of sex workers
Laos	6,230 reported cases of HIV by the end of 2013	0.29% among 15–49 years old in 2013	Low prevalence	Unsafe sexual activity is the primary mode of transmission	1.00%	3.10%	1.50%		Clients of FSW was estimated as 98,660 in 2010
Malaysia	86,000 [66,000–120,000]	0.4% [0.3–0.6%] for 15–49 years	Concentrated among males	The epidemic concentrated injecting drug users (IDUs), female sex workers and the transgender population. In 2013,	60,000 sex workers comprising of 40,000 female sex workers (FSW) and 20,000 transgender (TG)	There were 2,406 (2.5%) out of 94,841 cumulative number of HIV reported cases among this category	It is estimated that there are about 170,000 IDU in the country	Children and young people ≤19 years made up 3.5% (120) of 3,479 new reported HIV cases, out of which 65 (54%) were <13 years (vertical)	Transgendered: approximately HIV prevalence of 9.7% among them by a 2009 survey
Burma	220,000	0.6% [0.5–0.7%]	one of the highest prevalence countries in HIV in Asia	key risk groups disproportionately are affected by HIV infection (FSW, clients of SW, MSM, IDU)	11.2% (9.2–13.6%); about 60,000 (40,000–80,000) active fsw	22.3% (18.2–26.4%); ≈1.5% of male population is MSM (224,000) in 2008. Syphilis 14.1% among MSM in 2008	34.6% (31.6–37.7%). An estimate of 75,000 (60,000–90,000) are PWID		clients of FSW was estimated as 880,000 in 2007 (5.6% of general population); about 2.5% among military

Philippines	10,514 HIV positive cases in 2013; 15,000 [11,000–23,000] in 2012	<0.1%	Low prevalence	over 90% from sexual contact: from 2007 there has been a shift in the predominant trend of sexual transmission from heterosexual contact (20%) to males having sex with other males (80%)	Low prevalence of condom use	HIV infection increased rapidly among MSM, doubled since 2005	Composed of 4% of PLHIV; high prevalence of needle sharing practice	< 1% cases; Only 5% of HIV-positive pregnant women have received anti-retrovirals to prevent mother-to-child transmission
Singapore	4374	<0.1%	Low prevalence	Predominantly males infected with a M to F ratio of 10:1	0.00%	3.14% in 2013	Very low	
Thailand	490,000	1.20%	Thailand has the highest prevalence of HIV in Asia	An example of a country where a strong national commitment to tackling the HIV and AIDS epidemic has paid off; with an admirable history of HIV prevention efforts	1.8% of brothel-based FSW are HIV positive	Increases in HIV prevalence among MSM is particularly marked in Bangkok, where HIV prevalence among this group has risen from 17.3% in 2003 to 31.3% in 2009;	Among IDU, 22% were infected with HIV	ART has been offered to 90% of infected pregnant women, which significantly reduced the MTC transmission
Vietnam	280,000	0.47%	Concentrated epidemic	Vietnam's epidemic is still in a concentrated phase; Of the 0.47% prevalence, within which 5,670 are children – it indicates the trend moving from high risk to general population	Female sex workers (FSW) are the second highest infected risk population with national rates slightly increasing from 9% in 2007 to 9.3% in 2012	national prevalence for MSM will remain 2% in the 2007–2012 period	IDU accounts for 65% of PLHIV, and the HIV prevalence among male IDU is estimated to be 23.1%	It is reported that more than 1% of pregnant women in some provinces are found HIV positive

notably Thailand, have shown significant improvement as the result of strong government commitment toward HIV prevention. Other nations like Vietnam face concentrated epidemics that are not well controlled in defined subgroups. As with the South Asia, coinfections with HCV, TB, and malaria present major regional challenges (AVERT 2015; UNAIDS 2012).

Cambodia is a country with an estimated population of 15.9 million in 2015 located in the southern Indochina Peninsula. The HIV epidemic is primarily driven by the heterosexual route and the nation experienced high incidence in the 1990s, particularly among FSW. Cambodia has had an aggressive nationwide scale-up HIV prevention campaigns including “100% condom use” among high-risk populations, such as FSW. HIV prevalence decreased from 1.2% in 2003 to 0.6% in 2011, thought to be linked to successes in their prevention efforts. However, the low levels of condom use among MSM as well as continuing mother-to-child transmission underscores the ongoing challenges (Vun et al. 2014). Male clients of FSW have played a bridging role high-risk sex workers to their low-risk wives or girlfriends (AVERT 2015).

Indonesia: Ranking as the fourth most populous country in Asia, Indonesia has an estimated 252 million people. The Indonesian HIV epidemic is understudied, in comparison with other Asian nations (Dokubo et al. 2013). Nevertheless, data suggest that the HIV epidemic has been spreading with an estimated 610,000 PLHIV by 2012. The number of PLHIV has risen sharply in recent years among a few vulnerable groups including PWID, FSW and their clients, and MSM/transgender women. Contributing factors may include a highly mobile population, a growing IDU problem, denial of sexual health service to unmarried people, and the challenges created by both economic and natural crises. PLHIV have had suboptimal access (estimated 24%) to ARV treatment, even lower among children with HIV (11%) (AVERT 2015).

Laos is a landlocked country with a total 2014 population of 6.8 million. The HIV epidemic in Laos is about 0.1% with an estimated 6230

PLHIV by 2013. The epidemic is concentrated among PWID, FSW, and MSM. Among PWID, 5% were seropositive in 2005. Although the HIV prevalence is relatively low among FSW, due to their low literacy and disempowered status, these women are highly vulnerable to HIV infection (AVERT 2015). The high TB incidence rates in Laos pose the HIV-TB coinfection challenges, also a concern in the rest of Asia (Vermund and Yamamoto 2007).

Malaysia is a multiethnic and multicultural nation of 28 million persons. The main HIV transmission route is heterosexual, followed by PWID, and homosexual. Men represented 90% of all reported cases in 2002, a male-to-female ratio of 9:1, but the proportion of women is increasing, and in 2014, the ratio was only 4:1. Social factors contributing to the rapid increase among women likely include gender inequities and denial of sexual health services to unmarried young persons (AVERT 2015).

Myanmar (or *Burma*) is experiencing a serious HIV epidemic with an estimated 220,000 PLHIV within a total population of 55 million persons in 2015. With police crackdowns on drug production, many heroin users have switched to injection as a more cost-effective way of using drugs. Only a few locations provide needle change programs. Similarly, opiate substitution therapy using methadone has been initiated in a small number of government locations. In addition to high prevalence among PWID, HIV rates are rising among MSM in Burma, with MSM HIV prevalence of 23.5% in the city of Yangon and 35% in Mandalay. FSW have high HIV infection rates, but recent condom campaigns seem to be having benefits, analogous to successes in Thailand, Cambodia, and China (Swe and Rashid 2013; Aung et al. 2014). Poverty, poor border control, violence against ethnic and religious minorities, migration, and a lack of investment from the government and international sponsors all complicate prevention and HIV care efforts, including access to antiretroviral therapy (AVERT 2015).

Philippines is considered to be part of Southeast Asia, situated in the western Pacific Ocean. A low-HIV prevalence country, Philippines

estimated 10,514 PLHIV in 2013, less than 0.1% HIV prevalence among adults. About 41% of total infections are attributable to male homosexual contact, and MSM have a rising incidence rate, raising the kinds of concerns noted for China. High population mobility within and outside the country, a conservative culture that suppresses sexual health education, high levels of sex work, low condom use rates, and prevalent IDU all contribute to concerns for an expansion of the HIV epidemic (AVERT 2015; UNAIDS 2013).

Singapore serves as a hub of international travel and business but has a low-prevalence of HIV infection. However, there are high numbers of infections found in countries nearby, which makes it plausible that Singapore will experience an increasing epidemic in the coming decades. About 3% of MSM in Singapore were HIV infected from estimates published in 2010 (AVERT 2015; van Griensven and de Lind van Wijngaarden 2010).

Thailand is in the heart of Southeast Asia with a population of 66 million persons. Since the first HIV case was reported in 1984, more than one million people have been infected with HIV by the year 2008 and half had died. The adult prevalence was as high as 1.3% in 2009, and it ranked as the highest prevalence country in Asia. HIV incidence has been substantial among FSW, PWID, and MSM at different stage of the Thai epidemic timeline. Among MSM, HIV prevalence has expanded in recent years to 20% in 2010, and 41% of all HIV infections were attributable to MSM. This population has experienced a substantially rising prevalence over time (van Griensven et al. 2015). On the other hand, the HIV prevalence among transgender persons has been lower than 10% for reasons that are not clear. The HIV prevalence among venue-based FSW was 1.8%, but the prevalence was as high as 19% among freelance sex workers in past years. HIV prevalence among PWID was more than 20%, but has declined. Due to the strong government-operated crackdown on drug trafficking in 2003, drug users have been labeled as “criminals,” making HIV prevention campaigns targeting PWID quite difficult. There are about five million migrant workers

living in Thailand. Due to the language barriers, high mobility, and exploitative working condition, migrant workers have very limited access to health care to HIV/AIDS, which make them vulnerable to HIV infection. About 7.6% of TB patients are coinfecting with HIV. The government of Thailand has had a strong national commitment to curbing the HIV epidemic and its successes with 100% condom campaigns for brothel-based sex work are legend (Hanenberg et al. 1994; Celentano et al. 1998; Nelson et al. 2002). A total of 490,000 PLHIV lived in Thailand in 2012, half of the peak prevalence (AVERT 2015; Wirtz et al. 2013).

Vietnam is the easternmost country on the Indochina Peninsula in Southeast Asia with a population of 90.5 million as of 2014. By 2012, an estimated 280,000 PLHIV accounted for 0.47% of the adult population. The epidemic is concentrated among high-risk groups including PWID (65% of cases), FSW, and MSM (Do et al. 2012; Le et al. 2015b; Pham et al. 2015). Needle sharing and unprotected sex are common among PWID; Vietnamese PWID have been shown to initiate drug use at earlier ages, and their time between non-injecting drug use to IDU has become shorter in recent years (Do et al. 2012). Among FSW and MSM, inconsistent condom use is the norm. The marginalized status of MSM makes them hard to have access to healthcare and HIV testing (AVERT 2015; UNAIDS 2012). Complex interactions between risk groups are seen worldwide, and Vietnam is no exception. Investigators have examined risk among MSM who are sex workers (Colby et al. 2015) and FSW who are PWID (Le et al. 2015a). Coinfections are complex in this tropical nation, including hepatitis, TB, and malaria.

In summary, Asia is an immense region. We have not discussed central Asia, beyond Mongolia, since this is addressed elsewhere, but in the East Asia, South Asia, and Southeast Asian context, the HIV epidemic is wrapped into particular higher-risk populations. High background population sizes and persons who are behaviorally vulnerable to HIV infection contribute to ongoing HIV spread.

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Stem Cell Transplantation

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Definition

Stem Cell Transplantation (SCT), also referred to as bone marrow transplantation or hematopoietic stem cell transplantation is a treatment in which hematopoietic stem cells are administered to a patient, usually as treatment for hematologic cancer or bone marrow failure. Prior to infusion of stem cells, patients receive fully myeloablative or reduced-intensity chemotherapy both to reduce tumor burden and to allow engraftment of the stem cells. There are several potential sources of the hematopoietic stem cells in clinical use. In autologous SCT, hematopoietic stem cells are collected from the patient prior to chemotherapy and infused after treatment to rescue the hematopoietic system. In allogeneic SCT, hematopoietic stem cells are donated by a close relative or genetically matched unrelated individual and infused after conditioning chemotherapy. Availability of suitably matched donors remains a major limitation to SCT and new advances now allow for more flexibility in matching human leukocyte antigens (HLA) between donors and recipients. Alternative

stem cell sources include the use of partially-matched relatives, partially-matched unrelated donors, and umbilical cord fetal blood.

Introduction

SCT is the standard treatment for a variety of hematologic malignancies for which human immunodeficiency virus (HIV)-infected patients are at increased risk, including primary-refractory lymphoma, relapsed lymphoma, and aggressive leukemia. Early in the epidemic, HIV infection was considered a contraindication to high-dose chemotherapy and SCT. With effective ► [antiretroviral therapy](#) (ART) the morbidity and mortality of HIV infection have been dramatically reduced. In addition, substantial improvements in SCT have occurred, such as prophylaxis against opportunistic infections and the use of leukocyte growth factors. With these advances, HIV-infected individuals are now being offered curative SCT therapy for treatment of hematologic malignancy. Outcomes of autologous SCT in HIV-infected individuals are now considered similar to the general population. In allogeneic SCT, there are a growing number of successful case reports and series. Close attention to interactions between antiretroviral medications to treat HIV, conditioning chemotherapy administered pre-transplant, and immunosuppressants administered post-transplant is required. Of note, there has been a long-standing interest in the possibility that SCT could lead to the cure of HIV infection.

Hematologic Malignancies Associated with HIV-Infection

Many types of hematologic malignancies are associated with HIV infection and acquired immunodeficiency syndrome (AIDS). Aggressive B cell lymphoma was among the “AIDS-defining” conditions that could lead to a clinical diagnosis of AIDS early in the epidemic, even before the virus had been identified. With the widespread institution of ART, the epidemiology of cancer in the HIV/AIDS population has

changed, but hematologic malignancies remain a major cause of morbidity and mortality (Shiels et al. 2011). In the United States, hematologic cancers still account for more than one third of all cancers diagnosed in individuals with HIV/AIDS (Shiels et al. 2011). In the era of ART, the absolute numbers of AIDS-related malignancies such as non-Hodgkin lymphoma (NHL) have decreased but NHL represents a greater proportion of deaths in individuals with AIDS, as a result of substantial success in reducing infection-related mortality (Shiels et al. 2011).

NHL is the most common hematologic cancer in individuals with AIDS (Shiels et al. 2011). Whereas some types of NHL have almost disappeared with ART, such as ► [primary central nervous system lymphoma](#), and others have substantially decreased, such as ► [diffuse large B cell lymphoma](#), ► [Burkitt lymphoma](#) seems unaffected. Similarly ► [Hodgkin lymphoma](#) (HL) has not decreased and may be on the rise (Shiels et al. 2011). Finally, rates of myeloma and certain types of leukemia, such as acute myelogenous leukemia (AML), are also increased in this population.

Role of AutoSCT in the Treatment of Malignancy in HIV- Infected Individuals

In the early years, aggressive chemotherapy in HIV-infected individuals with lymphoma resulted in an unacceptably high risk of treatment-related death with no clear survival benefit (Gates and Kaplan 2002). These early studies made reduced-dose treatment regimens the standard of care and SCT was not considered in this patient population. After implementation of effective ART in 1997, several studies showed that HIV-infected patients could tolerate standard dose regimens (Gates and Kaplan 2002). These improvements were attributed to ART as well as to the institution of routine prophylaxis against pneumocystis pneumonia and herpes virus reactivation. These successes opened the door to explore high-dose therapy with stem cell rescue in the form of autologous SCT, which was the standard treatment for relapsed or primary-refractory lymphoma in the general population.

Autologous SCT to treat lymphoma in HIV-infected individuals has been instituted at several centers. It has also been studied in small cooperative group trials (Ambinder 2009). In these cohorts, the rate of infectious deaths has not been significantly increased and mortality has primarily been attributed to cancer progression or treatment-related organ toxicities such as veno-occlusive disease (Ambinder 2009). The current consensus is that outcomes of autologous SCT in HIV-infected individuals with relatively well-controlled HIV disease are essentially equivalent to outcomes in the general population. There has also been no clear deleterious impact of autologous SCT on the long-term management of HIV infection in these patients. Amounts of HIV virus in the plasma are not significantly increased and immunologic recovery after autologous SCT also appears to be comparable to the general population (Ambinder 2009).

Role of AlloSCT in the Treatment of Malignancy in HIV-Infected Individuals

The favorable outcomes of autologous SCT in HIV-infected individuals have encouraged consideration of allogeneic SCT in HIV-infected individuals with aggressive hematologic malignancies such as leukemia, and in those who require salvage therapy for unresponsive lymphoma. As in autologous SCT, the use of ART to control HIV disease is critical to outcomes in allogeneic SCT. Early in the AIDS epidemic, survival in HIV-infected patients receiving allogeneic SCT was less than 15% (Gupta et al. 2009; Hütter and Zaia 2011). With effective ART and other advances, survival with allogeneic SCT is now greater than 50% (Gupta et al. 2009; Hütter and Zaia 2011).

In addition to concerns regarding increased mortality, there has been speculation that HIV-infected individuals might be at risk for SCT complications such as graft failure and/or graft-versus-host-disease (GVHD). This potential risk is hypothesized to be a result of underlying immunologic dysfunction caused by HIV, which is thought to persist even with effective ART. For

example, in the solid organ transplant setting, although overall survival and organ/graft survival rates are generally comparable between the HIV-infected and uninfected transplant recipients, higher than expected episodes of organ rejection were observed (Stock et al. 2010). Based on the small studies to date in HIV-infected individuals receiving allogeneic SCT, disproportionate rates of graft failure and/or GVHD have not been observed (Gupta et al. 2009; Hütter and Zaia 2011).

ART during SCT in HIV-Infected Individuals

The extraordinary success of ART in controlling and reversing clinical disease due to HIV infection has allowed for curative SCT therapies to be extended to HIV-infected individuals. At the same time, the complexity of ART combinations necessitates focused approaches related to drug interactions, drug toxicities, and drug interruptions. There are more than 20 approved antiretroviral medications. The major classes of drugs include nucleoside or nucleotide analogue reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors, a fusion inhibitor, and a CCR5 chemokine receptor antagonist. Unique challenges associated with specific drugs or drug classes exist as well as more general challenges to maintenance of any antiretroviral regimen during SCT.

Overlapping organ toxicities can occur with antiretrovirals and chemotherapeutic agents (Rudek et al. 2011). Zidovudine or AZT is an NRTI and was the first approved antiretroviral drug. Prior to the development of combination drug regimens, AZT monotherapy was used in HIV-infected individuals during SCT (Hütter and Zaia 2011). With the development of less toxic alternatives, AZT is now contraindicated during SCT as it can cause myelosuppression (Rudek et al. 2011). Other overlapping organ toxicities for which monitoring is required during SCT and concurrent ART, include kidney toxicity, which can occur with the NRTI tenofovir, and peripheral

nerve toxicity which occurs with older NRTIs such as stavudine (d4T) and didanosine (ddI) (Rudek et al. 2011).

Drug interactions between ART, antineoplastic agents, and immunosuppressants used to prevent GVHD are another management challenge in SCT for HIV-infected individuals (Rudek et al. 2011). Interactions due to antiretrovirals in the PI class are the most common. In most cases, two PIs are used in combination; one PI is used at a dose designed to inhibit HIV replication and a second PI, ritonavir, is used at a lower dose designed to inhibit the cytochrome P450 enzyme system in order to increase the concentration and therapeutic efficacy of the first PI. This approach is known as “ritonavir-boosting” and is critical to the efficacy of this drug class. Other drugs are now being developed to affect PI boosting in place of ritonavir. The strategy has been extremely effective and ritonavir-boosted PIs are components in certain recommended first-line ART regimens. Many drugs, including chemotherapeutic agents and immunosuppressants are also metabolized by the cytochrome P450 system and in the presence of PIs, serious interactions can occur (Rudek et al. 2011). In some cases, these interactions can be managed with close monitoring of drug levels though concerns remain that the levels of critical cancer drugs or immunosuppressants may be compromised. In other instances – for example, with myeloablative conditioning regimens and/or the use of high-dose cyclophosphamide, interruption of PI-containing regimens is mandatory for at least a few days (Rudek et al. 2011). Another antiretroviral drug implicated in drug interactions during SCT is the NNRTI efavirenz. Efavirenz is an alternative to the use of PIs in the first-line ART regimens but induces, rather than inhibits, the cytochrome P450 enzyme system. During SCT, efavirenz use also requires close monitoring of levels of several critical medications (Rudek et al. 2011).

Mucositis, nausea, and vomiting are common side effects of cancer chemotherapy and may prevent patients from taking any medications by mouth for extended periods of time. Unfortunately, nonoral formulations of ART have not been widely developed (Swindells et al. 2011).

The only non-oral formulations of antiretrovirals that are currently available include intravenous AZT and the fusion inhibitor enfuvirtide (T20) which is administered as a subcutaneous injection (Swindells et al. 2011). AZT is contraindicated during SCT due to bone marrow suppression. T20 is rarely used except as salvage therapy for HIV-infected patients with multidrug resistant virus and can cause painful injection site reactions (Swindells et al. 2011).

With interruption of ART, detectable levels of HIV in the blood typically return within a few weeks. Although rebound viremia can be managed without adverse clinical outcomes in most cases, there have been cases during SCT in which ART interruption and rebound of virus resulted in a febrile syndrome similar to the acute retroviral syndrome described in primary HIV infection (Hütter and Zaia 2011). Interruption can also increase the risk of developing antiretroviral drug resistance particularly when components of the ART regimen have different half-lives. Efavirenz has a half-life of 36–100 h and remains detectable long after levels of other antiretrovirals in a standard regimen have disappeared (Rudek et al. 2011). This discrepancy in rates of drug elimination will result in a period of functional efavirenz monotherapy and a significant risk of developing HIV drug resistance which will compromise the efficacy of future ART. In addition to these clinical implications, avoiding any interruptions in ART may also be important to emerging strategies that propose to use SCT in the pursuit of an HIV cure.

Donor Sources in SCT in HIV-Infected Individuals

Many patients who need allogeneic SCT are unable to find an HLA-matched donor. Approximately 70% of patients do not have an HLA-matched sibling and only half of those patients will find a matched unrelated donor in the national registry. The proportion of individuals who cannot identify a donor is even higher among racial and ethnic minorities, which are also disproportionately affected by HIV infection. Alternative donor sources have been developed and

studied in the general population but have not been investigated in HIV-infected individuals. These newer strategies include the use of partially-matched or haploidentical relatives or cord blood stem cells (Brunstein et al. 2011). In the past, haploidentical SCT was associated with increased mortality due to high rates of GVHD but this has been reduced with the use of high-dose, post-transplantation cyclophosphamide as GVHD prophylaxis (Brunstein et al. 2011). Concerns remain with regard to cord blood transplant as higher rates of nonrelapse mortality have been reported in a recent cooperative group study (Brunstein et al. 2011); however cord blood SCT may also offer unique benefits related to the role of SCT in HIV cure (Petz et al. 2012).

Persistence of HIV in Hematopoietic Cells

HIV preferentially infects activated CD4⁺ T cells, and as part of the retroviral lifecycle, the virus stably integrates into the host cell genome. Activated CD4⁺ T cells only have a lifespan of 1–2 days but a subset of activated CD4⁺ T cells will become resting memory CD4⁺ T cells. Memory CD4⁺ T cells are long-lived cells that are designed to survive for the life of the individual in order to maintain immunologic memory. As a consequence of this process, if an HIV-infected activated CD4⁺ T becomes a resting memory CD4⁺ T cell, HIV will survive indefinitely within that cell (Eisele and Siliciano 2012). Resting memory CD4⁺ T cells are quiescent and do not actively produce virus, but if these cells are reactivated, they can produce HIV and reestablish active infection (Eisele and Siliciano 2012). In all HIV-infected individuals, resting memory CD4⁺ harboring latent HIV can be detected and the frequency of these cells is stable over time despite treatment with ART (Eisele and Siliciano 2012). This reservoir of latently infected resting memory CD4⁺ T cells is the major impediment to viral eradication.

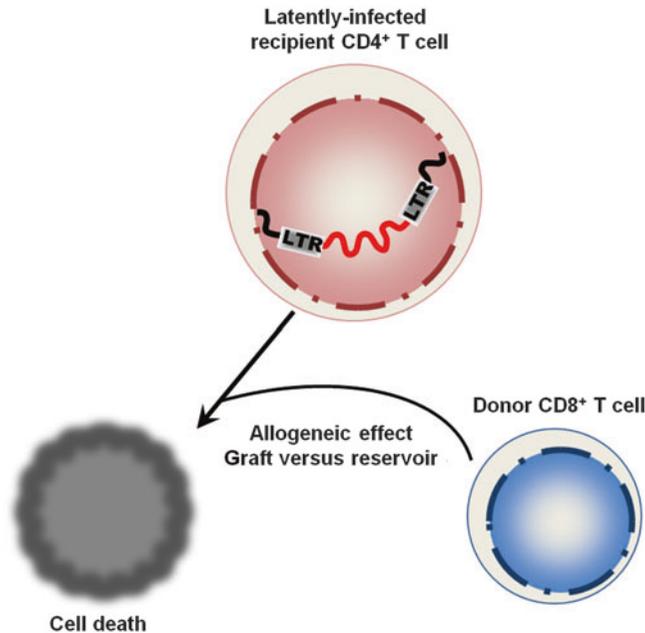
There has been interest in whether hematopoietic progenitor stem cells are directly infected by HIV and whether these cells are an additional

barrier to cure. The majority of studies on this subject in the pre-ART era reported extremely rare detection of HIV in the hematopoietic progenitor/stem cell compartment (McNamara and Collins 2011). The prevailing consensus was that viral detection was due to the presence of CD4⁺ T cells which traffic between the blood and the bone marrow. In the ART era, debate has continued. One study reported evidence of HIV infection in CD34⁺ hematopoietic progenitor cells in patients on ART (McNamara and Collins 2011), but follow-up studies in which CD4⁺ T cells were carefully excluded from samples has not confirmed HIV infection of CD34⁺ cells (Durand et al. 2012a). Thus, consensus remains that latently-infected resting memory CD4⁺ T cells represent the primary obstacle to cure (Eisele and Siliciano 2012).

Role of SCT in HIV Cure

The concept that HIV infection is limited to hematopoietic cells led to efforts in the 1980s to cure AIDS with SCT by replacement of the hematopoietic compartment (Hütter and Zaia 2011). These studies, which occurred prior to the use of effective ART, did not demonstrate success. Decades later, interest in allogeneic SCT as a means to eradicate HIV has been revived. This came to attention with a remarkable report of HIV cure in an HIV-infected patient who was treated for AML with myeloablative chemotherapy and allogeneic SCT (Hütter et al. 2009). The patient was originally known as the Berlin patient, because he received his cancer treatment from a German oncologist, Dr. Gerard Hütter, in Berlin. The patient is now known to the public as Timothy Ray Brown and is a community activist and strong advocate for research efforts focused on an HIV cure.

For treatment of his AML, Brown received myeloablative chemotherapy, total body irradiation, and allogeneic SCT from an “HIV-resistant” donor (Hütter et al. 2009). This unique donor was homozygous for a naturally occurring genetic mutation in one of the cell surface receptors, CCR5. The mutation, known as CCR5Δ32 is a 32-base pair deletion that results in a complete lack of expression of CCR5 on the cell surface.



Stem Cell Transplantation, Fig. 1 Impact of the allogeneic effect on HIV latently-infected cells. The allogeneic effect occurs as a result of donor CD8⁺ lymphocytes which are present within the stem cell transplant product. These donor lymphocytes eliminate host hematopoietic cells. This effect is nonspecific and results in favorable

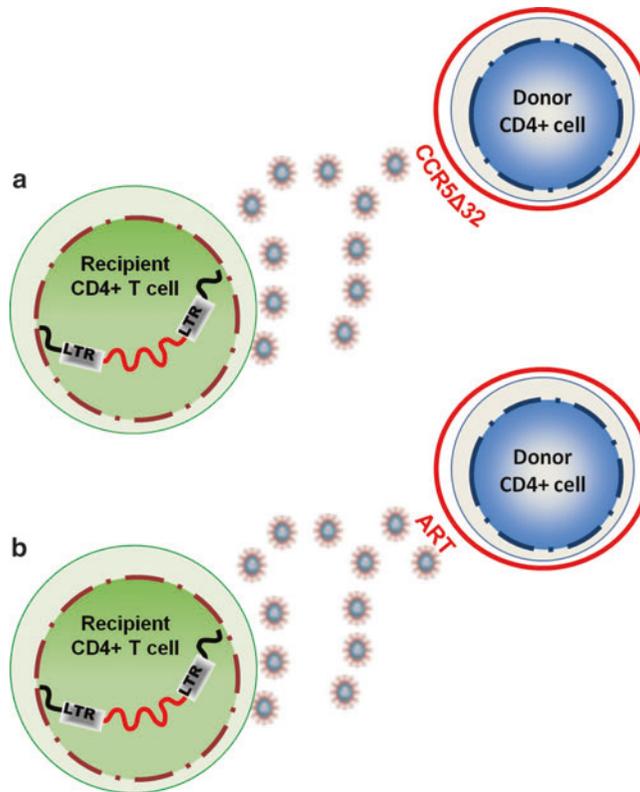
graft-versus-tumor-effects as well as unfavorable graft-versus-host-disease. This allogeneic effect should also eliminate latent HIV infection by killing latently-infected host CD4⁺ T lymphocytes and causing a “graft-versus-viral-reservoir” effect

The majority of HIV variants require binding to the CCR5 receptor to infect cells. These viruses are known as R5-tropic variants. Other strains of HIV use the cell surface receptor, ► **CXCR4** to enter cells, but these X4-tropic variants are rarely transmitted, and typically evolve in late-stage AIDS. Between 5% and 15% of Caucasians of Northern European-descent are CCR5Δ32 heterozygotes and carry one copy of the mutation which does not confer HIV-resistance. Less than 1% of Caucasian individuals homozygotes, and, carrying two copies of the allele, are resistant to HIV-acquisition. Homozygosity for the mutation is even rarer in racial and ethnic minority populations (Petz et al. 2012).

Due to the rarity of this genetic polymorphism, it has been relatively difficult to identify other matched CCR5Δ32 homozygous donors to test this approach. Several strategies have been developed to overcome this challenge (Durand et al. 2012b). First, there are now technologies capable of genetically engineering patient CD4⁺

T cells and/or CD34⁺ hematopoietic progenitor cells to lack CCR5 and CXCR4 (Durand et al. 2012b). This approach has been tested in early phase clinical studies and appears to be safe and well tolerated. A significant impact of this approach on clinical outcomes in patients has not yet been demonstrated. (Durand et al. 2012b). Current challenges of the strategy include optimizing the levels of engraftment and the survival of the genetically modified cells (Durand et al. 2012b). One application of this method that is in preclinical development is a combination of gene therapy tools to mutate CCR5 or CXCR4 in hematopoietic progenitor stem cells from HIV-infected individuals with hematologic malignancies who require autologous SCT (Durand et al. 2012b).

Cord blood SCT may provide another way to overcome the lack of CCR5Δ32 homozygous donors. A program to genetically screen cord blood units for the CCR5Δ32 mutation has been implemented (Petz et al. 2012). This initiative has identified more than 100 CCR5Δ32 homozygous



Stem Cell Transplantation, Fig. 2 (a) HIV-resistant donor cells. The Berlin patient received a SCT from a donor who was homozygous for a naturally occurring 32-base pair deletion in the cell surface receptor CCR5. Homozygosity for this deletion, CCR5 Δ 32, results in a lack of expression of the CCR5, which in addition to CD4 is the co-receptor used by HIV to infect cells. Thus, the transplanted donor hematopoietic system could not be

infected by HIV from the recipient. **(b) Antiretroviral therapy to prevent donor cell infection.** During the process of SCT and the transition from a recipient to donor hematopoietic system, continuous ART may prevent infection of the transplanted cells. If in concert, all host cellular reservoirs of HIV are eliminated by a combination of chemotherapy and the allogeneic effect, this could lead to HIV cure

units, with an ultimate goal of identifying 300 units. Due to less stringent requirements for HLA matching with cord blood SCT, it is predicted that this inventory would translate into a 27% chance of identifying a matched CCR5 Δ 32 homozygous unit for an HIV-infected Caucasian adult who requires SCT (Petz et al. 2012).

The possibility that allogeneic SCT could eradicate HIV infection even without CCR5 Δ 32 donors is also being considered. In the process of allogeneic SCT, donor CD8⁺ T cells in the stem cell product eliminate recipient hematopoietic cells, due to alloreactivity (Fig. 1). This process, known as the allogeneic effect, is

responsible for the graft-versus-tumor-effect as well as GVHD. The transition from a recipient hematopoietic system to a donor hematopoietic system occurs over weeks to months. Once all hematopoietic cells detected in the peripheral blood are donor in origin, the SCT recipient is said to have achieved full donor chimerism. Since all identified HIV reservoirs are in hematopoietic or hematopoietic-derived cells (Eisele and Siliciano 2012), the HIV reservoir in the recipient should disappear once full donor chimerism is complete. The donor cells must be also protected from acquiring HIV during this process for cure to be achieved. In the case of Timothy Ray Brown,

the donor cells were protected by the CCR5Δ32 mutation (Fig. 2a). With allogeneic SCT using standard donors, ART may provide the same protection (Fig. 2b). This latter protective effect might be considered analogous to the ability of ART to prevent HIV acquisition in utero, or after a high-risk needle stick exposure.

Conclusions

SCT is the treatment of choice for several hematologic malignancies which are increased in the HIV-infected population. With continued improvements in HIV therapies and SCT strategies, evidence suggests that SCT can and should be offered to persons living with HIV/AIDS for treatment of cancer. There are special risks to consider in this population related to underlying immunodeficiency as well as the challenges of administering ART, chemotherapy, and immunosuppressants in combination. Prospective trials of autologous SCT have not demonstrated increased mortality or treatment-related complications. Further data is emerging related to the use of allogeneic SCT in this population but there are encouraging reports in the literature. Further investigation in outcomes of SCT using alternative donor sources is needed. Beyond curing malignancy, the prospect of achieving HIV cure with SCT remains an exciting opportunity that promises to bring new insights into the fields of SCT and HIV/AIDS.

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Surveillance Case Definition

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Introduction

Surveillance is the ongoing systematic collection, analysis, and interpretation of data on cases of disease for use in the planning, implementation, and evaluation of public health practice. HIV/AIDS surveillance data are used to monitor the spread of HIV infection, to target HIV prevention and health-care services, and to allocate funding for HIV prevention and care.

AIDS Surveillance

Since the first cases of AIDS were reported in 1981, population-based AIDS surveillance has been used to track the magnitude of the HIV epidemic. State governments are the legal entities responsible for collection of surveillance data on cases of infectious diseases, including AIDS. By the end of 1983, most of the 50 states had enacted laws making AIDS a reportable public health condition (IOM 1986). State and local health departments collect information on AIDS cases from physicians, hospitals, clinics, laboratory reporting, HIV counseling and testing sites, and medical record reviews in a standardized case report form. Before the etiologic agent was identified and diagnostic tests were routinely available, AIDS surveillance focused on monitoring opportunistic illnesses characteristic of severe immunosuppression. The AIDS case definition, reflecting severe morbidity, has been periodically revised to incorporate new understanding of the spectrum of HIV disease, new diagnostics, and changes in medical practice (CDC 1982, 1985, 1986, 1992, 1999).

The reporting of AIDS cases has helped to identify modes of transmission, measure the impact of the epidemic, monitor trends among affected populations, target HIV prevention services, monitor HIV-related outcomes (e.g., death), and allocate resources for HIV treatment and care services (e.g., Ryan White). Understanding AIDS trends and the course of untreated HIV infection allowed the use of back-calculation techniques on AIDS data to reconstruct trends in HIV incidence.

Case Definitions

Following the report of the first cases of AIDS in 1981 (CDC 1981a, b), a case definition of AIDS was published in September 1982 (CDC 1982), and this definition was also adopted worldwide. AIDS was defined as a disease, at least moderately predictive of a defect in cell-mediated immunity (e.g., *Pneumocystis carinii* pneumonia (PCP) and Kaposi's sarcoma (KS)), occurring in a person with no other known cause for immune suppression (CDC 1982). The AIDS indicator diseases met the case definition if the diagnosis was based on reliable methods, such as histology or culture. However, this case definition did not include the full spectrum of AIDS manifestations, which ranged from asymptomatic to non-specific symptoms (e.g., fever, weight loss, generalized, persistent lymphadenopathy) to specific diseases that were insufficiently predictive of cellular immunodeficiency to be included in the AIDS case definition (e.g., tuberculosis (TB), oral candidiasis) and malignancies related to immunodeficiency.

In 1985, the AIDS case definition was revised for the first time, as a result of the discovery of human T-cell lymphotropic virus type II/lymphadenopathy-associated virus (HTLV-III/LAV) (CDC 1985). After HIV was discovered to be the cause of AIDS and highly sensitive and specific HIV antibody tests became available, the spectrum of manifestation of HIV became better defined, and the HIV classification system was developed in 1986 (CDC 1986). By 1986, all 50 states, the District of Columbia, and three US territories had AIDS reporting.

AIDS surveillance case definition was revised again in 1987 to reflect the severe morbidity and spectrum of disease associated with HIV, with inclusion of HIV encephalopathy, HIV wasting syndrome, and a broader range of specific AIDS-indicative diseases for those with laboratory evidence for HIV infection (CDC 1987). As clinicians gained experience with AIDS opportunistic infections (OIs), these conditions were increasingly diagnosed presumptively, up to 10–15% of patients, and no longer met the case definition. To improve the sensitivity and specificity of the AIDS case definition, persons reported with indicator diseases diagnosed presumptively were included if there was laboratory evidence of HIV infection. Exclusions due to other causes of immunodeficiency were eliminated. CDC noted that the effectiveness of the revision would depend on “how extensively HIV antibody tests are used.” Recognizing that pediatric AIDS differed from adults, the pediatric AIDS definition was revised to include multiple or recurrent serious bacterial infections and lymphoid interstitial pneumonia/pulmonary lymphoid hyperplasia as AIDS indicator diseases among children. For children <15 months of age, due to the presence of maternal HIV antibody, laboratory criteria for HIV infection were more stringent.

In 1993, the AIDS case definition for adolescents and adults was expanded to include (1) all HIV-infected persons with severe immunosuppression (a CD4 count of <200 cells/ μ L or a CD4 percentage of <14) and (2) three additional clinical conditions (pulmonary TB, recurrent pneumonia, and invasive cervical cancer), in addition to retaining the 23 clinical conditions in the previous AIDS case definition (CDC 1994). These three conditions, unlike other AIDS OIs, were more common infections, which were recognized to be more severe and frequent among HIV-infected persons with immunosuppression and reflected changes in the epidemiology of HIV infection (e.g., cervical cancer reflected the increasing impact of HIV on women).

In 1993, both the classification system and the AIDS case definition for adults and adolescents were revised to include CD4 as a marker of immunosuppression, to simplify the classification

system, to reflect current standard of medical care that use CD4 to guide both prophylaxis and treatment, and to more accurately categorize HIV-related morbidity. Monitoring of CD4 counts had become of standard of HIV care. Classification of HIV infection and the expanded AIDS surveillance case definition for adults and adolescents were based on both three clinical categories (i.e., A, B, and C) and three categories of CD4 counts (i.e., \geq 500 cells/ μ L, 200–499 cells/ μ L, and <200 cells/ μ L) or CD4 percentages (CDC 1992). Clinical category A included asymptomatic acute or primary HIV infection or persistent generalized lymphadenopathy. Clinical category B includes symptomatic conditions that were not included in clinical categories A or C but are attributed to HIV infection or indicative of a cell-mediated immunity defect or for which the clinical course or management was complicated by HIV infection. Clinical category C included the 23 retained AIDS-defining conditions from the previous AIDS case definition and the three new clinical criteria. Increasing the use of both PCP prophylaxis and treatment had slowed the rate that HIV-infected persons developed AIDS (CDC 1992).

This expansion of the AIDS case definition was made available for both public comment and was discussed at an open meeting in 1992. This increased public interest was related to use of CDC AIDS surveillance definition for defining eligibility of social security benefits. Additionally, at the end of the first decade of AIDS, Congress authorized the funding of outpatient and ambulatory medical and support services for persons with HIV, the Ryan White Comprehensive AIDS resources emergency (CARE) act in August 1990. This included Title I for emergency assistance to metropolitan areas with the highest numbers of reported AIDS cases and Title II which were formula grants to all states that took into account number of reported AIDS cases in most recent years (Bowen et al. 1992; McKinney et al. 1993). Thus, the use of AIDS surveillance data was now expanded to target HIV resources.

The revision to the case definition resulted in a substantial increase in the number of reported cases, most of these met the new case definition

based on severe immunosuppression (CDC 1993). Increased case reporting was greatest among women, racial/ethnic minorities, adolescents, IDUs, and persons infected through heterosexual contact reflecting the changes in the HIV epidemic. Different surveillance methods were used by the states, including laboratory-based reporting of HIV antibody and CD4 counts and using TB and cancer registries to find cases. This increased number of new cases made interpretation of AIDS trends difficult; however, it enabled a representative and complete estimate of the number and distribution of persons with severe HIV-related immunosuppression, facilitating the use of these data to target and evaluate HIV care and treatment programs.

In 1994, the pediatric classification system was revised from the 1987 classification system based on increasing knowledge of the progression of HIV disease in children (CDC 1994). Children were classified into three mutually exclusive categories based on (1) infection status, (2) clinical status, and (3) immunologic status. Diagnosis of HIV infection in children born to HIV-infected mothers was complicated by the presence of maternal anti-HIV IgG antibody which crosses the placenta to the fetus, who are HIV antibody positive at birth, though only 25–30% are infected. In those children who are not infected with HIV, maternal IgG antibody becomes undetectable by 9 months of age and sometimes up to 18 months of age. Thus, HIV IgG antibody tests are not used for HIV diagnosis before 18 months of age. Polymerase chain reaction (PCR) was the most sensitive and specific assay for detecting HIV infection in children born to infected mothers; and positive results on two separate occasions or if the child meets the AIDS case definition defined HIV infection in children <18 months. For those 18 months and older, repeatedly reactive EIA and confirmatory test (Western blot or IFA) were used. Clinical categories were revised to include not symptomatic (N), mildly symptomatic (A), moderately symptomatic (B), and severely symptomatic (C). Immunologic categories included no evidence of suppression, moderate suppression, and severe immunosuppression. CD4+ counts are higher in infants and

young children compared to adults normally and decline over the first few years of life. As a result, children may develop OIs at higher CD4+ counts than adults; thus immune categories were based on age group (<12 months, 1–5 years, 6–12 years).

Impact of Treatment on Surveillance

In the mid-1990s, advances in treatment with combination antiretroviral therapy slowed the progression of HIV disease to AIDS and contributed to first declines in AIDS incidence and mortality (CDC 1997). These advances in treatment decreased the ability of AIDS surveillance data to monitor trends in HIV incidence and to monitor the impact of the epidemic on the health-care system. Antiretroviral treatment of pregnant women and their newborns reduced perinatal HIV transmission and resulted in dramatic declines in the incidence of perinatally acquired AIDS also reducing the usefulness of AIDS data to monitor the impact of new perinatal treatment recommendations.

Therefore, in 1997, the Council of State and Territorial Epidemiologists (CSTE) and CDC recommended that all states and territories conduct HIV case surveillance as an extension of their AIDS surveillance programs (CDC 1997; Council of State and Territorial Epidemiologists, C 1997). In 1999, CDC published guidelines for national HIV case surveillance, including reporting of HIV infection and perinatal HIV exposure to monitor the impact of advances in antiretroviral treatment, implementation of the new HIV treatment and PMTCT guidelines, resources needed for patient care, and the increased need for data on all HIV-infected populations (CDC 1999). At the time of the 1999 recommendations, 24 states and the Virgin Islands had implemented named-based HIV case surveillance in addition to AIDS surveillance; two of these states conducted pediatric surveillance only. Four states and Puerto Rico were reporting cases of HIV infection using a coded identifier.

HIV Surveillance

HIV case surveillance data provide critical information about the current state of the epidemic in

ways that AIDS surveillance data could no longer provide. HIV case surveillance data are more likely to represent new diagnoses and are more useful in assessing the impact of prevention activities. HIV surveillance data can better monitor the extent of the epidemic among youth, where new HIV diagnoses most likely reflect recent infections. States with HIV surveillance have used data on both perinatal HIV-exposed and HIV-infected children to document the decline in perinatal acquired HIV infection and implementation of recommended maternal HIV testing and antiretroviral therapy to prevent perinatal HIV transmission (CDC 1999). By 2007, HIV data were used to calculate funding allocation amounts for Titles I and II, under the Ryan White AIDS program. By April 2008, all 50 states had implemented named-based HIV reporting.

Since the discovery of HIV as the etiologic agent of AIDS and the availability of diagnostic tests to identify HIV infection in 1985, HIV infection reporting was initiated in many states, using the same confidential methods as used for AIDS. Conducting named HIV infection reporting met with great resistance in the community at that time as there was no treatment of HIV infection and there was concern about confidentiality of the information and the potential negative impact on HIV-infected persons such as avoidance in seeking confidential HIV testing and other prevention services. Challenges exist in the interpretation of HIV surveillance relate to the long latency of HIV infection, as characteristics of persons newly diagnosed with HIV infection may represent changes in HIV testing practices and behaviors, rather than changes in incidence. To respond to community concerns, CDC conducted research to assess the impact of named HIV surveillance on person's willingness to seek HIV testing and care, reviewed program practices and legal protections for security and confidentiality of state and local HIV data, and evaluated the performance of coded-identified-based surveillance systems (CDC 1999). Results from an analysis of publicly funded HIV counseling and testing data from six states 12 months before and after HIV reporting found no significant declines in the total number of HIV tests performed in any state (Nakashima

et al. 1998). Other studies, however, documented concerns that name-based reporting of HIV infection was a factor that might deter HIV testing for HIV for some persons with high-risk behaviors and that availability of anonymous testing was associated with higher rates of intention to test. Given these data, CDC strongly supported continued availability of anonymous testing opportunities as part of prevention programs. Based on evaluation of HIV reporting systems using coded identifiers for conducting confidential HIV case reporting, CDC found that confidential name-based HIV/AIDS surveillance systems were most likely to produce quality surveillance data.

Confidentiality and security of HIV surveillance data are of absolute importance. The legal authority to mandate HIV reporting and the methods used for reporting are the responsibility of state governments. HIV surveillance data that are protected by state HIV/AIDS surveillance confidentiality laws and regulations are subject to the same procedures as used for AIDS surveillance. In the 1990s, CDC reviewed state confidentiality laws that protect HIV surveillance data and determined that all states have legal safeguards for the confidentiality of government held health data but that state legal protections varied. To address concerns from the community and to help ensure uniform confidentiality protections, the Georgetown University Law Center developed the *Model State Public Health Privacy Act* (Gostin et al. 2001). The model legislative language protected confidential, identifiable information held by state and local public health departments against unauthorized and inappropriate nonpublic health uses but still allowed public health officials to use surveillance information to accomplish the public health objectives defined by the law. At the federal level, HIV data are protected by federal statutes that prevent release of HIV data for nonpublic health purposes.

As trends in new diagnoses of HIV infection are likely affected by testing practices or behaviors, in 2005, HIV surveillance was expanded in selected surveillance programs to conduct HIV incidence surveillance in conjunction with routine case surveillance to provide a more reliable estimate of newly acquired HIV infections (both

diagnosed and undiagnosed) and to help focus prevention efforts. These programs collect additional data, including testing and antiretroviral use history, and result from additional testing of remnant diagnostic HIV-positive blood specimens. Residual blood specimens are tested for recency using an immunoassay to distinguish between recent and long-standing infections. The resulting data, along with case surveillance data, can be extrapolated to the general population to estimate HIV incidence in the United States (Hall et al. 2008).

HIV surveillance data have been used to more accurately estimate HIV prevalence than AIDS surveillance data alone and have modified existing back-calculation methods (CDC 2011a). The model estimated both overall HIV prevalence and the proportion undiagnosed. More recently, HIV and AIDS surveillance data have been used along with two other surveillance data systems to monitor the continuum of care from HIV diagnosis, linkage and retention in care to receipt of ART, and viral load suppression (CDC 2011b).

Case Definition

In 1999, the revised case definition for HIV infection in adults and children incorporated reporting criteria for HIV infection and AIDS in a single-case definition and new laboratory tests in the laboratory criteria for HIV case reporting (CDC 1999). Four categories of HIV infection for children <18 months were defined: definitively HIV infected, presumptively HIV infected, definitively uninfected with HIV, and presumptively uninfected with HIV. Case definitions were revised to reflect the use of HIV nucleic acid detection tests that can detect HIV infection in almost all infants 1 month of age or older.

In 2008, the adult and adolescent HIV classification system and HIV and AIDS case definition were revised into a single-case definition for HIV infection that includes AIDS and incorporates the HIV infection classification system (CDC 2008). Laboratory-confirmed evidence of HIV infection was required to meet the surveillance case definition for HIV infection, including stage 3 HIV infection (AIDS).

Current HIV/AIDS Surveillance

Monitoring the HIV epidemic in the United States consists of a comprehensive national surveillance system based on standardized methods of data collection. HIV surveillance focuses on key events in the progression of HIV disease, including HIV infection, detected through new HIV diagnoses and, in some areas, is further classified as incident infections. New diagnoses are tracked to identify the degree of immunosuppression and entry into care based on initial CD4 and viral load tests and tracked to determine progression to AIDS and death. To facilitate this, CDC recommends reporting of all HIV-related test results, including CD4+ T-lymphocyte (CD4) results (counts and percentages) and all viral load test results (undetectable and specific values). CD4 and viral load data can be used to identify cases, classify stage of disease at diagnosis, and monitor disease progression. As a result, national HIV/AIDS surveillance data can be used to monitor HIV testing and prevention efforts; determine entry into care; measure viral load suppression, incidence of HIV infection, new diagnoses, AIDS, mortality, and HIV prevalence; and assess unmet health-care needs. However, state reporting laws, regulations, or policies, the level at which results must be reported, vary.

CDC also conducts supplemental surveillance activities, including the Medical Monitoring Project (MMP) and the National HIV Behavioral Surveillance system (NHBS). MMP is a national population-based surveillance system that collects information on clinical outcomes and behaviors of HIV-infected persons receiving care in the United States through abstraction of medical records. Collection of data from interviews with HIV-infected patients provides information on current behaviors that may facilitate HIV transmission; patients' access to, use of, and barriers to HIV-related secondary prevention services, utilization of HIV-related medical services, and adherence to drug regimens (Blair et al. 2014). The NHBS conducts behavioral surveillance among persons at highest risk for HIV infection in the United States. This system involves conducting rotating 12-month cycles of surveillance among the three

groups with the highest HIV burden: men who have sex with men (MSM), injection drug users (IDUs), and heterosexuals at increased risk of HIV infection (CDC 2014a).

Case Definition

The case definition for HIV infection was revised in 2014 (CDC 2014b). The revision addresses new diagnostic multi-test algorithms that do not use Western blot or immunofluorescence HIV antibody assays. This multi-test algorithm consists of a positive (reactive) result from an initial HIV antibody or combination antigen/antibody test and an accompanying or subsequent positive result from a supplemental HIV test different from the initial test. Supplemental HIV tests can now include antibody immunoassays or nucleic acid tests (CDC 2014a). The new definition also includes (1) criteria for differentiating between HIV-1 and HIV-2; (2) recognition of early HIV infection by a negative HIV test within 6 months of HIV diagnosis; (3) revision of criteria for reporting diagnoses without laboratory evidence; (4) simplification of criteria for opportunistic illnesses (OI) indicative of AIDS, eliminating the need to distinguish presumptive from definitive diagnoses of OIs; and (5) consolidation of staging systems for adults, adolescents, and children. A confirmed case can be classified into stage 0 (early HIV infection), to stage 1–3 of HIV infection based on CD4 cell count and stage 3-defining OIs (AIDS). Stage 0 allows monitoring of cases diagnosed within months of their infection when viral loads are high and in their most infectious period when interventions could be most effective to prevent transmission. Staging for children is based on specific age group criteria (<1 year, 1–5 years, ≥6 years). Staging for children aged 6–12 years is treated the same way as in adults and adolescents.

Global HIV/AIDS Surveillance

The World Health Organization (WHO) and the United Nations Program on HIV/AIDS (UNAIDS) oversee global surveillance of HIV. HIV surveillance globally initially relied on

AIDS case surveillance and sentinel studies on HIV prevalence (e.g., antenatal care attendees (ANC)). However, AIDS case reporting was limited in resource-limited settings due to underreporting, delays in reporting, lack of diagnostic testing capacity, and weak health infrastructure. In 2000, WHO/UNAIDS recommended second-generation HIV surveillance strategies which were tailored to the epidemic type in the country. Data sources included HIV and HIV advanced infection or AIDS case reporting but also sentinel surveillance (antenatal care attendees, key populations at higher risk), probability surveys of the general population, STI case reporting and behavioral or biobehavioral surveillance, and immunization clinic surveys for children (WHO 2013). For example, in generalized epidemics, recommended surveillance activities may include HIV or advanced HIV case reporting where case reporting data can be useful for investigating any area where unusually large numbers of cases are reported. However, as with HIV reporting in the United States, to interpret these data correctly, it is critical to understand the underlying pattern of testing, diagnostic capability, and reporting by different facilities. Additionally countries may also conduct general population surveys with behavioral and biological markers, which can provide data on the geographic distribution of HIV prevalence and other risk factors, but these surveys are resource intensive and therefore only conducted every 3–5 years in countries with prevalence over 2% in the general population. ANC sentinel surveillance is the most feasible method to monitor trends in HIV prevalence in the general population and can be used as a proxy for the general population; however the ANC population is young and of course not using condoms.

Case Definitions

WHO developed a clinical AIDS case definition for both adults and children in 1986 for reporting in low- and middle-income countries. This was modified for adults and adolescents to include serological HIV testing and again in 1994 to include the revisions to the 1993 CDC AIDS case definition (WHO 1988, 1994).

With the scale-up of ART, data were needed on persons diagnosed with HIV infection to estimate those in need of care and treatment and assess impact of care interventions. In 2007, WHO recommended reporting of newly diagnosed cases of HIV infection in adults and children and revised the case definitions for HIV infection and AIDS as well as the clinical staging system (WHO 2007). The case definitions for HIV infection were standardized and coordinated with the clinical staging for HIV infection (stages 1 and 2), advanced HIV disease (stage 3), and AIDS (stage 4) and required laboratory confirmation of HIV infection. The clinical staging system was simplified and harmonized the WHO 2002 three-stage pediatric staging system with the WHO 1990 four-stage adult system, including clinical staging and immunologic staging criteria. Although there were some differences between the WHO and CDC definitions and staging systems, the CDC and WHO stages could still be compared (CDC 2008).

Summary

A comprehensive, national HIV/AIDS surveillance system based on standardized methods of data collection continues to provide critical information about the current state of the epidemic both in the United States and, globally, and is critical for HIV prevention, care, and treatment program planning and evaluation (Table 1).

Appendix: 2014 CDC Revised Surveillance Case Definition for HIV Infection (CDC 2014b)

Criteria for a Confirmed Case

Criteria for a confirmed case can be met by either laboratory evidence or clinical evidence. Laboratory evidence is preferred over clinical evidence.

Persons Aged ≥ 18 Months and Children Aged < 18 Months Whose Mothers Were Not Infected

Laboratory Evidence Laboratory criteria require reporting of the date of the specimen

collection for positive test results in multi-test algorithms or stand-alone virologic tests and enough information about the tests to determine that they meet any of the following criteria:

- A multi-test algorithm consisting of
 - A positive (reactive) result from an initial HIV antibody or combination antigen/antibody test
 - An accompanying or subsequent positive result from a supplemental HIV test different from the initial test (CDC 1981b)

The initial HIV antibody or antigen/antibody test and the supplemental HIV test that is used to verify the result from the initial test can be of any type used as an aid to diagnose HIV infection. For surveillance purposes, supplemental tests can include some not approved by the Food and Drug Administration (FDA) for diagnosis (e.g., HIV-1 viral load test, HIV-2 Western blot/immunoblot antibody test, and HIV-2 NAT). However, the initial and supplemental tests must be “orthogonal” (i.e., have different antigenic constituents or use different principles) to minimize the possibility of concurrent nonspecific reactivity. Because the antigenic constituents and test principles are proprietary information that might not be publicly available for some tests, tests will be assumed to be orthogonal if they are of different types. For example:

- One test is a combination antigen/antibody test and the other an antibody-only test.
- One test is an antibody test and the other a NAT.
- One test is a rapid immunoassay (a single-use analytical device that produces results in < 30 min) and the other a conventional immunoassay.
- One test is able to differentiate between HIV-1 and HIV-2 antibodies and the other is not.

Tests also will be assumed to be orthogonal if they are of the same type (e.g., two conventional immunoassays) but made by different manufacturers. The type of HIV antibody test that verifies the initial test might be one formerly used only as

Surveillance Case Definition, Table 1 Changes in the case definition and classification system used for surveillance of HIV/AIDS, CDC, and WHO, over the course of the epidemic, 1981–2014

Year (reference)	Adult/pediatric	Case definition/classification	WHO/CDC	Main change in the case definition
CDC 1981a, b				First cases of Kaposi's sarcoma and PCP in young homosexual men
CDC 1982	All	First AIDS case definition	CDC/WHO	Persons with a disease moderately predictive of a defect in cell-mediated immunity and no known cause for diminished resistance to that disease (e.g., KS, PCP, and serious OI)
CDC 1985	All	Revised AIDS case definition	CDC/WHO	Expanded to add cases with a positive serologic test for human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) and with other severe opportunistic infections and chronic lymphoid interstitial pneumonitis for children <13 Excluded cases with negative serologic tests, no other type of HTLV-III/LAV test with positive result, and do not have low number of CD4 counts or low ratio of CD4–CD8 lymphocytes
CDC 1986	All	First HIV classification system	CDC	Definition of HTLV-III/LAV infection: repeatedly reactive screening tests for HTLV-II antibody (e.g., enzyme-linked immunosorbent assay) and antibody identified in supplemental test (e.g., Western blot, immunofluorescence assay); direct identification of virus in host tissue by virus isolation (not widely available) Classification into groups and subclassified based on CD4 counts and types of opportunistic infection Group 1: acute HTLV-III/LAV infection Group 2: asymptomatic HTLV-II/LAV infection Group 3: persistent generalized lymphadenopathy Group 4: other HTLV-III/LAV disease
CDC 1987	All	Revised AIDS case definition	CDC	Inclusion of HIV encephalopathy, HIV wasting syndrome, and a broader range of specific AIDS indicator diseases, inclusion of presumptively diagnosed indicator diseases, and exclusion of cases with other causes of immunodeficiency. Multiple or recurrent serious bacterial infections and LIP are AIDS indicator diseases among children but not adults
CDC 1987	Pediatric	HIV classification system in children <13 years of age	CDC	Definition of HIV infection in children Classification based on clinical symptoms Class P-O perinatally exposed infants with indeterminate HIV status Class P-1; asymptomatic infection; subclassified based on immunologic testing Class P-2: symptomatic infection; subclassified based on types of symptoms
CDC 1993	Adult and adolescent	Revision of the classification system and AIDS case definition	CDC	Expanded to include (1) all HIV-infected persons with severe immunosuppression (a CD4 count of <200 cells/ μ L or a CD4 percentage of <14) and (2) three additional clinical conditions (pulmonary TB, recurrent pneumonia, and invasive cervical cancer)

(continued)

Surveillance Case Definition, Table 1 (continued)

Year (reference)	Adult/pediatric	Case definition/classification	WHO/CDC	Main change in the case definition
CDC 1994	Pediatric	Revised classification system for children <13 years of age	CDC	The pediatric classification system based on increasing knowledge of the progression of HIV disease in children. Children were classified into three mutually exclusive categories based on (1) infection status, (2) clinical status, and (3) immunologic status
CDC 1999	All	Updated HIV case definition and recommendations for national HIV reporting	CDC	Recommend that all states and territories conduct case surveillance for HIV infection as an extension of current AIDs surveillance activities
CDC 2007			WHO	Recommends reporting cases of HIV infection as HIV infection or advanced HIV disease, including AIDS; four clinical stages for disease classification to reflect the WHO ART treatment guidelines: (1) no symptoms, (2) mild symptoms, (3) advanced symptoms, and (4) severe symptoms; and requires laboratory confirmation of HIV infection
CDC 2008	All	Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged <18 months and for HIV infection and AIDS among children aged 18 months to <13 years – United States, 2008	CDC	HIV classification system and the surveillance case definitions of HIV infection and AIDS were revised into a single-case definition for HIV infection that includes AIDS. Laboratory-confirmed evidence of HIV infection is required to meet the surveillance case definition for HIV infection, including stage 3 (AIDS)
CDC 2014	All	Revised surveillance case definition for HIV infection – United States, 2014	CDC	The new definition includes (1) new diagnostic multi-test algorithms that do not use Western blot or immunofluorescence HIV antibody assays; (2) criteria for differentiating between HIV-1 and HIV-2; (3) recognition of early HIV infection; (4) revision of criteria for reporting diagnoses without laboratory evidence; (5) simplification of criteria for opportunistic illnesses (OI) indicative of AIDS, eliminating the need to distinguish presumptive from definitive diagnoses of OIs; and (6) consolidation of staging systems for adults, adolescents, and children

an initial test (e.g., conventional or rapid immunoassay, HIV-1/HIV-2 type-differentiating immunoassay), or it might be one traditionally used as a supplemental test for confirmation (e.g., Western blot, immunofluorescence assay).

- A positive result of a multi-test HIV antibody algorithm from which only the final result was reported, including a single positive result on a

test used only as a supplemental test (e.g., HIV Western blot, immunofluorescence assay) or on a test that might be used as either an initial test or a supplemental test (e.g., HIV-1/HIV-2 type-differentiating rapid antibody immunoassay) when it might reasonably be assumed to have been used as a supplemental test (e.g., because the algorithm customarily used by the reporting laboratory is known).

- A positive result or report of a detectable quantity (i.e., within the established limits of the laboratory test) from any of the following HIV virologic (i.e., nonantibody) tests:
 - Qualitative HIV NAT (DNA or RNA)
 - Quantitative HIV NAT (viral load assay)
 - HIV-1 p24 antigen test
 - HIV isolation (viral culture)
 - HIV nucleotide sequence (genotype)

Clinical (Nonlaboratory) Evidence Clinical criteria for a confirmed case (i.e., a “physician-documented” diagnosis for which the surveillance staff have not found sufficient laboratory evidence described above) are met by the combination of:

- A note in a medical record by a physician or other qualified medical-care provider that states that the patient has HIV infection
- One or both of the following:
 - The laboratory criteria for a case were met based on tests done after the physician’s note was written (validating the note retrospectively).
 - Presumptive evidence of HIV infection (e.g., receipt of HIV antiretroviral therapy or prophylaxis for an opportunistic infection), an otherwise unexplained low CD4+ T-lymphocyte count, or an otherwise unexplained diagnosis of an opportunistic illness.

Children Aged <18 Months Born to Mothers Who Have an Unknown Infection Status or Were Known to Be Infected

Laboratory Evidence A child aged <18 months is categorized for surveillance purposes as HIV infected if all of the following criteria are met:

- Positive results on at least one specimen (not including cord blood) from any of following HIV virologic tests:
 - HIV-1 NAT (DNA or RNA)
 - HIV-1 p24 antigen test, including neutralization assay for a child aged >1 month
 - HIV isolation (viral culture)

- HIV nucleotide sequence (genotype)
- The test date (at least the month and year) is known.

One or both of the following:

- Confirmation of the first positive result by another positive result on one of the above virologic tests from a specimen obtained on a different date
- No subsequent negative result on an HIV antibody test and no subsequent negative result on an HIV NAT before age 18 months

Clinical Evidence

- The same criteria as in section “[Clinical \(Nonlaboratory\) Evidence](#)”
- All three of the following alternative criteria:
 - Evidence of perinatal exposure to HIV infection before age 18 months
 - A mother with documented HIV infection
 - A confirmed positive test for HIV antibody (e.g., a positive initial antibody test or antigen/antibody test, confirmed by a supplemental antibody test) and a mother whose infection status is unknown or undocumented
 - Diagnosis of an opportunistic illness indicative of stage 3
 - No subsequent negative result on an HIV antibody test

Definition for Date of Diagnosis of a Confirmed Case for All Ages

Laboratory Criteria If the diagnosis is based on laboratory evidence, the diagnosis date is defined as the earliest date on which the specimen was obtained for a positive HIV test result.

Clinical Criteria If the diagnosis was based on clinical evidence (“physician documented”) rather than laboratory evidence, the diagnosis date is defined as the date (at least the year) of diagnosis reported in the content of the medical record. If the diagnosis date was not reported in the note, the date when the note was written can be used as a proxy.

Section 2: Criteria for Classifying the HIV Type as HIV-2

All HIV infections in the United States should be assumed to be type 1 (HIV-1) unless laboratory test results are sufficient to classify the infection as type 2 (HIV-2), dual HIV-1 and HIV-2 infections, or undifferentiated HIV infection, as described below. Clinical or epidemiologic evidence might lead to laboratory testing for HIV-2 but is insufficient for classifying the HIV type as HIV-2.

Persons Aged ≥ 18 Months and Children Aged < 18 Months Not Perinatally Exposed

HIV-2 Infection

For HIV-2 infection, one or more of the following laboratory criteria are necessary and sufficient:

- FDA-approved HIV-1/HIV-2 type-differentiating antibody test result positive for HIV-2 and negative for HIV-1
- Positive HIV-2 Western blot (WB) (or immunoblot or line assay) result and negative or indeterminate HIV-1 WB result
- Positive qualitative HIV-2 NAT result
- Detectable quantitative HIV-2 NAT (viral load)
- Laboratory results interpreted as consistent with HIV-2 infection by a laboratory expert experienced in differentiating HIV-2 from HIV-1 if laboratory evidence for HIV-2 is ambiguous

Dual Infection with HIV-1 and HIV-2

The HIV type is classified as “dual” infection (both HIV-1 and HIV-2) if both an HIV-1 NAT and an HIV-2 NAT are positive.

Undifferentiated HIV Type

The HIV type is classified as “undifferentiated” if there is no positive or detectable result from an HIV-1 NAT and a laboratory expert cannot resolve ambiguous evidence for HIV-2, such as:

- HIV-2 WB is positive and HIV-1 WB is HIV positive.
- HIV-1/HIV-2 type-differentiating antibody test result interpretation is “undifferentiated” (positive for both HIV-1 and HIV-2).

Difficulty of Diagnosing HIV-2 Infection in Children Aged < 18 Months Born to Mothers Known to Be HIV Infected or Whose HIV Infection Status Is Unknown

In perinatally exposed children aged < 18 months, antibody tests are not used to diagnose HIV infection because of the expectation that they might be false indicators of infection in the child due to passive transfer of maternal antibody. The HIV-1 NAT routinely used to diagnose HIV-1 infection in children of this age is likely to be negative in an HIV-2-infected child because it is insensitive to HIV-2. A positive HIV-2 NAT result would satisfy the criteria for a case. Otherwise, the diagnosis of HIV-2 infection in a child will need to wait until the child is aged 18 months, when it can be based on antibody test results.

Section 3: Criteria for Uninfected and Indeterminate HIV Infection Status of Perinatally Exposed Children Aged < 18 Months

Uninfected

A child aged < 18 months who was born to an HIV-infected mother or had a positive HIV antibody test result is classified for surveillance purposes as not infected with HIV if all three of the following criteria are met:

- Laboratory criteria for HIV infection are not met (see section “[Laboratory Evidence](#)”).
- No diagnosis of a stage-3-defining opportunistic illness attributed to HIV infection.
- Either laboratory or clinical evidence of the absence of HIV infection as described below.

Laboratory Evidence

Definitively Uninfected

- No positive HIV NAT (RNA or DNA)
- At least one of the following criteria:
 - At least two negative HIV NATs from specimens obtained on different dates, both of which were at age ≥ 1 month and one of which was at age ≥ 4 months

- At least two negative HIV antibody tests from specimens obtained on different dates at age ≥ 6 months

Presumptively Uninfected

- Criteria for definitively uninfected with HIV are not met.
- At least one of the following four laboratory criteria are met:
 - At least two negative NATs from specimens obtained on different dates, both of which were at age ≥ 2 weeks and one of which was at age ≥ 4 weeks.
 - One negative NAT (RNA or DNA) from a specimen obtained at age ≥ 8 weeks.
 - One negative HIV antibody test from a specimen obtained at age ≥ 6 months.
 - If criteria for HIV infection had initially been met by one positive HIV NAT test, then it must have been followed by at least two negative test results from specimens obtained on different dates, one of which is:
 - A NAT test from a specimen obtained at age ≥ 8 weeks
 - An HIV antibody test from a specimen obtained at age ≥ 6 months
- No subsequent positive NAT.

Clinical Evidence

A note in a medical record by a physician or other qualified medical-care provider states that the patient is not infected with HIV.

Indeterminate HIV Infection Status

A child aged < 18 months born to an HIV-infected mother is categorized as having perinatal exposure with an indeterminate HIV infection status if neither the criteria for being HIV infected nor the criteria for being uninfected are met.

Section 4: Criteria for Classifying the Stage of HIV Infection

The stages of HIV infection defined in this document are for surveillance staging of disease and might not be appropriate for patient care, clinical research, or other purposes. A confirmed case that

meets the criteria for diagnosis of HIV infection can be classified in one of the five HIV infection stages (0, 1, 2, 3, or unknown). Stage 0 indicates early HIV infection, inferred from a negative or indeterminate HIV test result within 6 months of a confirmed positive result, and these criteria supersede and are independent of the criteria used for later stages. Stages 1, 2, and 3 are based on the CD4+ T-lymphocyte count. If the CD4+ count is missing or unknown, the CD4+ T-lymphocyte percentage of total lymphocytes can be used to assign the stage. Cases with no information on CD4+ T-lymphocyte count or percentage are classified as stage unknown. If a stage 3-defining opportunistic illness has been diagnosed, then the stage is 3 regardless of CD4 T-lymphocyte test results, unless the criteria described below for stage 0 are met. CD4+ T-lymphocyte counts or percentages at the time of diagnosis allow classification of cases by stage at diagnosis. Subsequent CD4+ T-lymphocyte counts or percentages help monitor disease progression and whether the person is receiving ongoing care.

The stage characterizes the status of HIV disease at a particular point in time. Of primary interest to surveillance is the stage at initial diagnosis, but the stage can change in either direction after diagnosis and might be defined with reference to dates of interest such as the most advanced stage recorded through a particular date. The stages are defined as follows:

2014 Classification of Stage of HIV Infection, CDC

Stage 0 consists of a sequence of discordant test results indicative of early HIV infection in which a negative or indeterminate result was within 180 days of a positive result. The criteria for stage 0 supersede and are independent of the criteria used for other stages.

Stage 0 can be established either:

- Based on testing history (previous negative/indeterminate test results): a negative or indeterminate HIV test (antibody, combination antigen/antibody, or nucleic acid test) result within 180 days before the first confirmed positive HIV test result of any type. The first positive test

Surveillance Case Definition, Table 2 HIV infection stage^a based on age-specific CD4+ T-lymphocyte count or CD4+ T-lymphocyte percentage of total lymphocytes

Stage	Age on date of CD4+ T-lymphocyte test					
	<1 year		1–5 years		≥6 years	
	Cells/ μ L	%	Cells/ μ L	%	Cells/ μ L	%
1	≥1,500	≥34	≥1,000	≥30	≥500	≥26
2	750–1,499	26–33	500–999	22–29	200–499	14–25
3	<750	<26	<500	<22	<200	<14

^aThe stage is based primarily on the CD4+ T-lymphocyte count; the CD4+ T-lymphocyte count takes precedence over the CD4 T-lymphocyte percentage, and the percentage is considered only if the count is missing. There are three situations in which the stage is not based on this table: (1) if the criteria for stage 0 are met, the stage is 0 regardless of criteria for other stages (CD4 T-lymphocyte test results and opportunistic illness diagnoses); (2) if the criteria for stage 0 are not met and a stage 3-defining opportunistic illness has been diagnosed, then the stage is 3 regardless of CD4 T-lymphocyte test results; or (3) if the criteria for stage 0 are not met and information on the above criteria for other stages is missing, then the stage is classified as unknown

result could be any time before the positive supplemental test result that confirms it

- Based on a testing algorithm: a sequence of tests performed as part of a laboratory testing algorithm that demonstrate the presence of HIV-specific viral markers such as p24 antigen or nucleic acid (RNA or DNA) 0–180 days before or after an antibody test that had a negative or indeterminate result. Examples of algorithms that would fulfill this requirement include:
 - A positive initial HIV immunoassay result (e.g., antigen/antibody or antibody only) followed by a negative or indeterminate supplemental antibody test result (e.g., HIV-1/HIV-2 antibody differentiation assay or Western blot) and a positive NAT result. All three tests are usually performed as part of the same testing algorithm, but time might elapse between tests if additional specimens must be obtained for definitive supplemental testing.
 - A negative initial HIV immunoassay result followed by a positive NAT result that might have been done to evaluate the presence of acute HIV infection.

Exception

A confirmed case of HIV infection is not in stage 0 if the negative or indeterminate HIV test used as the criterion for it being a recent infection was preceded >60 days by evidence of HIV infection, such as a confirmed positive HIV test result, a clinical (physician-documented) diagnosis of

HIV infection for which the surveillance staff have not found sufficient laboratory evidence, a CD4+ T-lymphocyte test result indicative of stage 3 (Table 2), or an opportunistic illness indicative of stage 3.

Classifying a case as stage 0 depends on documenting negative HIV antibody test results in the specific situations described above. Negative test results from testing algorithms that have concluded that the person is not infected need not be reported to HIV surveillance programs.

Progression of Stage After Initial Diagnosis in Stage 0

Although the stage at diagnosis does not change, if >180 days have elapsed after the stage was 0 at diagnosis, the stage at the later date is classified as 1, 2, 3, or unknown, depending on CD4+ T-lymphocyte test results (Table 2) or whether an opportunistic illness had been diagnosed >180 days after HIV infection diagnosis. (Table 2)

Stages 1, 2, 3, and Unknown

If the criteria for stage 0 are not met, the stage is classified as 1, 2, 3, or unknown, depending on CD4+ T-lymphocyte test results or whether an opportunistic illness was diagnosed (Table 2). Infection among children aged 6–12 years is staged with the same criteria as infection among adults and adolescents, including opportunistic illnesses indicative of stage 3 (see below) that formerly applied only to adults and adolescents



(i.e., pulmonary tuberculosis, recurrent pneumonia, and cervical cancer). Multiple or recurrent bacterial infections (other than recurrent salmonella septicemia), which formerly applied only to children aged <13 years, now apply only to children aged <6 years. Lymphoid interstitial pneumonia is no longer classified as indicative of stage 3 in children because it is associated with moderate rather than severe immunodeficiency (CDC 1986). The diagnosis of any of the opportunistic illnesses, irrespective of diagnostic method used, will meet the criteria for staging, thereby eliminating the requirement in the 2008 case definition for some of them to be “definitively” diagnosed.

2014 Stage 3-Defining Opportunistic Illnesses in HIV Infection

Bacterial infections, multiple or recurrent ^a
Candidiasis of bronchi, trachea, or lungs
Candidiasis of esophagus
Cervical cancer, invasive ^b
Coccidioidomycosis, disseminated or extrapulmonary
Cryptococcosis, extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than the liver, spleen, or nodes, onset at age >1 month)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy attributed to HIV ^c
Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age >1 month)
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi sarcoma
Lymphoma, Burkitt (or equivalent term)
Lymphoma, immunoblastic (or equivalent term)
Lymphoma, primary, of brain
<i>Mycobacterium avium</i> complex or <i>Mycobacterium kansasii</i> , disseminated or extrapulmonary
<i>Mycobacterium tuberculosis</i> of any site, pulmonary ^b , disseminated, or extrapulmonary
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
<i>Pneumocystis jirovecii</i> (previously known as “ <i>Pneumocystis carinii</i> ”) pneumonia
Pneumonia, recurrent ^b
Progressive multifocal leukoencephalopathy

(continued)

<i>Salmonella</i> septicemia, recurrent
Toxoplasmosis of brain, onset at age >1 month
Wasting syndrome attributed to HIV ^c

^aOnly among children aged <6 years

^bOnly among adults, adolescents, and children aged ≥6 years

^cSuggested diagnostic criteria for these illnesses, which might be particularly important for HIV encephalopathy and HIV wasting syndrome, are described in the following references:

CDC (1994)

CDC (1992)

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Systems Biology

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Definition

HIV-1 infects CD4+ T cells and completes its replication cycle in approximately 24 h. During this time, infection profoundly perturbs the cellular physiology and makes extensive use of host factors. Cells react to viral invasion by deploying a defense strategy, including general innate immune mediators and specific antiviral factors. At the organism level, the viral-host interaction involves barriers and innate and acquired immunity and results in physiological changes in response to the infectious process. The study of these complex relationships and the tools for large-scale screening of factors involved in cellular and organism responses is part of the field of systems biology.

Systems Biology: Overview

Key elements that define systems biology are (i) the use of high-throughput quantitative measurements (ii) of a model that can be perturbed (iii) in iterative cycles with (iv) integration and modeling of the resulting data. In the context of HIV infection, an ideal systems biology experiment would include a model that dynamically captures the changes and interactions that occur during infection. One of the outcomes is the identification of host factors necessary for successful infection (sometimes referred to as “dependency factors”) or host factors that limit infection (generally referred to as “restriction factors”). A number of technologies are being used to identify these factors (Table 1), although their use does not necessarily represent a complete systems

Systems Biology, Table 1 Large-scale quantitative measurements used in HIV research

Approach	Technology	Materials	Outcomes	References
Transcriptome studies	Microarrays, RNA deep sequencing	Peripheral blood, individual cell populations, cell lines	Description of features of the infectious process that massively modulate the antiviral defense systems, as well as genes involved in the cell cycle and degradation/ proteasome pathway. Elite controllers have CD4 + T-cell transcriptome profiles that are similar to that from individuals receiving effective treatment and, in many cases, undistinguishable from that of the uninfected individuals. Next-generation sequencing offers an unprecedented opportunity to jointly analyze cellular and viral transcriptional activity without prerequisite knowledge of the nature of the transcripts	Rotger et al. 2010 , 2011 ; Lefebvre et al. 2011 ; Schopman et al. 2012 ; Imbeault et al. 2012
miRNA screens	Microarrays	Peripheral blood, individual cell populations	There are technical issues that result in conflicting literature on the role of host miRNAs in HIV infection. miRNAs may potentially target the conserved Nef-3'-LTR region	Sun et al. 2012
Gain-of-function screens	Recombinant DNA, overexpression	Cell lines	One gain-of-function screen used a cDNA library representing 15,000 unique genes in an infectious HIV-1 system. A second screen expressed a collection of over 300 interferon-stimulated genes	Schoggins et al. 2011
siRNA screens	Silencing siRNA, shRNA technologies	Cell lines	Individually, each screen has identified a few hundred candidate genes. However, there is limited overlap across studies except for a small core of proteins that include the mediator complex, a number of key kinases, and components of the NF- κ B complex	Bushman et al. 2009
Proteome analysis	Various mass spectrometry approaches	T-cell lines and primary cells	Analyses of 2,000 to 3,200 proteins by various groups resulted in limited overlap. This may reflect differences in detection and quantification methods, and in the experimental systems	Kramer et al. 2012 ; Navare et al. 2012

(continued)

Systems Biology, Table 1 (continued)

Approach	Technology	Materials	Outcomes	References
Protein-protein immunoprecipitation	Affinity tagging and purification mass spectrometry	Cell lines	HIV proteins interact with 435 human proteins, of which 11 host factors were reported to inhibit HIV replication	Jager et al. 2012
Evolutionary analysis	Amplification and sequencing of gene orthologs, whole primate genomes	Evolutionary analyses followed by functional analysis	Mainly used in the study of single genes, its use is becoming integral part of large-scale screens. In parallel, sites under positive selection in the viral genome identify escape from host pressure	Snoeck et al. 2011 ; Telenti and Johnson 2012
Genome-wide genetic screens	Genotyping arrays, full exome or genome sequencing	DNA from large patient samples	All genes/proteins identified in the various large-scale approaches can be evaluated for genetic polymorphism in individuals and populations. A number of dataset from genome-wide association studies can be queried for this purpose	Fellay et al. 2010

biology approach as defined above. This chapter describes both the techniques and their application in the field of virology and HIV.

Transcriptome and Proteome Analyses

Among the very first tools of systems biology were technologies that simultaneously measured the expression level of all genes (transcriptome) in a particular cell model or tissue. Such studies generally sought to discover genes and pathways that were differentially expressed after some level of perturbation. In recent years, the analysis of mRNA levels has undergone significant change. Most notably, the techniques have evolved from microarrays to deep sequencing. Microarrays suffered from the limitation of hybridization to a fixed number of probes covering known genes, and in the less than optimal capacity to compare across experiments and laboratories. In contrast, the new generation of analyses using RNA deep sequencing offers absolute counts of all genes expressed – including transcript orientation and sequence and splice variants and alternative RNA species such as lincRNAs, miRNAs, and sRNAs that may also have biologic function of

importance to infectious diseases. Furthermore, there is increasing emphasis on the dynamic process that modulates transcription and on the possibility to investigate specific cellular populations – eventually at the single-cell level.

Microarray transcriptome analysis has been extensively applied to cell lines and more recently to ex-vivo studies of CD4 and CD8 T cells, monocytes, and macrophages from HIV-infected individuals. A recurrent observation is the profound perturbation of the cellular physiology of the infected cell in vitro (Rotger et al. [2010](#)) and of the general signatures of deregulation of the interferon response in vivo. In primate models of pathogenic infection (Rhesus macaque, ► [SIVmac infection of macaques, immunopathogenesis of African green monkey](#), ► [SIV infection of African Green Monkeys](#)) the early interferon response is maintained beyond the seroconversion phase (Bosinger et al. [2009](#); Jacquelin et al. [2009](#)). In contrast, nonpathogenic models of infection (Sooty mangabeys, ► [Non-Pathogenic Infection of Sooty Mangabeys](#)) are characterized by a normalization of the levels of transcription of interferon-stimulated genes after primary infection despite a persistently high level of viral replication. The profile of interferon response in

humans mirrors that of the models of infection in monkeys. Human rapid progressors have persistently high levels of expression of interferon-stimulated genes that increase with increasing levels of viremia (Rotger et al. 2011). The rare HIV-infected individuals that progress very slowly despite high levels of replication have an interferon-stimulated gene expression pattern closer to that of Sooty mangabeys (Rotger et al. 2011). Similarly, elite controllers (individuals maintaining extremely low viral loads in the absence of therapy, ► [Long term non progressors and elite controllers](#)) have been the object of transcriptome analyses. The consistent observation in various cellular subsets is that of minimal transcriptome differences compared to healthy, HIV-negative donors (Rotger et al. 2010; Vigneault et al. 2011). Successful antiretroviral therapy can also normalize transcriptome profile to a substantial degree (Rotger et al. 2010). Importantly, this normalization of cell function may help to alleviate some of the consequences of long-term immune activation resulting in better patient outcomes.

RNA sequencing is progressively applied to the study of HIV infection (Lefebvre et al. 2011; Schopman et al. 2012; Chang et al. 2011). The major benefit of this technique is the apparent unbiased measurement of splice variants and transcripts with non-reference sequence (i.e., variant alleles). The most revealing aspects concerned the analysis of expression of viral sequences in the infected cell that may represent up to 0.7% of all transcripts. Alternative viral RNA splice events, antisense viral transcripts, and numerous small RNAs that correspond to the HIV-1 RNA genome have been identified. The majority of the small viral RNA sequences have a positive polarity and may represent miRNAs; the small portion of viral RNAs of negative polarity is mainly encoded within the 3'-UTR and may represent viral siRNAs – they were shown to potently repress HIV-1 production (Schopman et al. 2012). A number of studies evaluated host miRNA expression differences of CD4 T cells of HIV-1-positive individuals. There is however controversy on the contribution to pathogenesis of selected miRNAs (Sun et al. 2012).

Many studies are hampered by the fact that the study materials are a heterogeneous mix of infected and uninfected cells that may dilute the signal of subtle expression changes in the infected cellular subset. This is particularly an issue in the study of CD4 T cells from peripheral blood of infected individuals. Novel approaches use reporter viruses to allow sorting of the infected cells followed by transcriptome profiling of the infected versus the uninfected cell populations (Imbeault et al. 2012). These have shown that within a population of activated CD4 T cells, HIV-1 has no detectable effect on the transcriptome of bystander cells – at least at the early time points following infection (Imbeault et al. 2012). Improvement in single-cell transcriptome analyses will allow more precise analysis in the future (Tang et al. 2011).

While the transcriptional profiles associated with infection have been extensively studied, the proteome profile has been less well characterized. High-throughput mass spectrometry has been used in the study of T cells infected with HIV to show that the most dynamic changes are observed at the time when intracellular viral production reaches its maximum. More recent studies used the isobaric tag for relative and absolute quantitation (iTRAQ (Navare et al. 2012)) method to reveal early changes in the host proteome affecting the ribosomal proteins and translational machinery, suggesting that HIV might disrupt cellular functions of the infected T cell even before the time of peak intracellular viral protein and virion production. Linking the transcriptomic profile to the proteomic profile is an ongoing process. As expression of specific species of RNA and protein interactions can both modulate viral replications; understanding how these levels of biology interact is a critical next step.

Functional Screens

Technical developments facilitate the large-scale analyses of gene and protein functions and interactions. These generally involve the systematic perturbation of every gene in the genome. The first such series of analyses used gene silencing

through siRNA or shRNA to knock down the expression of each gene individually (Bushman et al. 2009). Although each aimed at determining the full complement of host proteins required for productive HIV replication (albeit in different model systems), the overlap in genes significantly identified as required by the virus for productive infection was limited. Thus, individual screens proved difficult to interpret, probably because of difficulties in standardization, bystander effects on the cell, and false positives and negatives. Although the individual genes identified showed limited overlap, the joint analysis of these data supports evidence of shared pathways that when targeted result in reproducible effect on viral replication (Bushman et al. 2009). These initial screens mainly proposed genes that when silenced brought viral replication down – described as “dependency factors.” More recently, screens aimed at the identification of those genes that when silenced are associated with greater viral replication – so-called candidate restriction factors (Liu et al. 2011). Restriction factors may be more readily identified through gain-of-function screens where a permissive system is rendered nonpermissive by the introduction of a gene or protein product. For example, TRIM5 α was identified by the expression of a DNA library prepared from primary rhesus monkey lung fibroblasts in HeLa cells, rendering them resistant to HIV-1. More recently 380 interferon-stimulated genes were assessed in a large-scale gain-of-function screen (Schoggins et al. 2011). Several effectors were identified with activity against HIV as well as other viruses.

The global landscape of HIV-host protein interactions, as determined by genome-wide screens, has also been reported recently (Jager et al. 2012). Affinity tagging and purification mass spectrometry were used to identify physical interactions of all 18 HIV proteins and polyproteins with host proteins in two different human cell lines. HIV proteins interacted with 435 human proteins, with 40% of the interactions shared by both cell lines. Eleven host proteins were reported to inhibit HIV replication. Similar strategies, using expression of viral open reading frames from viruses other

than HIV, mapped numerous interactions with host factors and revealed different perturbation strategies used by individual viruses and by viral classes (Pichlmair et al. 2012). Certainly, understanding the full landscape of genes that enhance, restrict, or otherwise interact with HIV can lead to the identification of novel targets for intervention.

Evolutionary Genetics

Unique to the host-pathogen relationship is the process of interaction, defense, and escape. At the genetic and genomic level, this translates in specific signals that can be identified by bioinformatic analyses and thereafter tested in functional screens (Telenti and Johnson 2012). The approach using functional evolutionary biology is of particular interest in the study of HIV because of the long association of retroviruses (and lentiviruses) with mammalian genomes. Repeated exogenous viral attacks and episodes of cross-species transmission and successful endogenization contribute a significant load of retroviral material to the host genome. Host-pathogen coevolution resulted in positive selective pressure on genes of innate immunity.

Evolutionary genetics has been used successfully in the study of the TRIM and APOBEC families, as well as in the study of BST2/tetherin and more recently SAMHD1. The virus deploys counteracting factors that are often multifunctional and target host antiviral molecules for proteasomal degradation. In all cases, genetic analyses have used sequences from primate orthologs to identify the domains and specific codons that likely participate in direct interaction with viral products. Experiments then used swaps of domains and site-directed mutagenesis to validate the role of specific sites in the process of host-pathogen interaction.

The field is now moving toward genome-wide assessments of signatures of positive selection and including these measurements as part of the characterization of genes and proteins identified by the genome-wide screens described in previous sections of this entry.

Restriction factors are expected to display features of rapid adaptation, while dependency factors, being part of central cellular processes and not necessarily engaged in direct interaction with the pathogen, are encoded by highly conserved genes.

Systems Vaccinology

High-throughput approaches used in systems biology greatly benefit from study designs where the perturbation is readily controlled. Thus, administration of an immunogen (and adjuvant) to a defined, and in many cases healthy, population can be seen as an optimal setting to apply concepts of systems biology at the organism level. This has been the rationale behind studies on the immunogenicity and signatures of successful vaccination against yellow fever and influenza. This approach has also been identified as promising for the study of vaccines against HIV (Nakaya and Pulendran 2012). Here, the value of systems vaccinology is in the understanding of vaccines that do not have clear correlates of protection or immunogenicity, such as the HIV vaccine tested in the RV144 trial. Understanding cascades of gene/protein expression that are modified in the context of a challenge of a protective vaccine can help to inform design of future studies as these will provide a readout in addition to the more traditional immunological correlates (i.e., antibodies and CTLs).

Currently, this field of research has primarily targeted whole blood or peripheral blood mononuclear cells. This material is widely available; however, the analysis captures both cellular composition and transcript abundance and may not identify a particular cell type of significant function. Additionally, the cellular heterogeneity may mask subtle changes in gene function in important cell types of low abundance. Some advanced bioinformatic tools can deconvolute the signatures as an alternative approach to studying selected cellular subsets. The integration of these approaches into clinical trials may capture enough information to assist rational vaccine design.

Host and Viral Genetics

The wealth of human genetic variation and the ever-increasing accessibility of methods to interrogate the full genome are being used extensively to understand complex traits. Although these techniques are currently being used to study differences in disease outcome after HIV infection (discussed in a separate entry “► [Host Genetics and Genomics](#)”), they exclusively focus on association testing of single variants or single genes (albeit in the genome-wide context). This has proved successful for discovering common variants in large cohorts; however, recent studies demonstrate that the vast majority of human variation is rare which, if tested individually, would require samples of unrealistic size (Keinan and Clark 2012). This situation may be improved by collapsing approaches that combine variation across genes and pathways to enhance power for detection, on the hypothesis that multiple hits in a gene and pathway may result in the same outcome at the population level. In HIV infection, in addition to using canonical biological pathways, analysis could be enhanced by the full understanding of the host systems perturbed by the virus: including dependency factors, restriction factors, and innate immune mediators. These data sets (such as the ones discussed above) could be used to feed back information to genetic studies to perform pathway-wide association analyses as proposed for other complex diseases (Califano et al. 2012).

In the case of the infected patient, the system includes both the host and the pathogen. Although much work has been done on viral sequences to highlight variants that impact viral fitness, this work has mainly focused on selection pressure mediated by HLA genes on viral epitopes. Systematic analysis of the interaction between variant alleles of host genes and viral sequences may be used to uncover other genes that induce selection pressure on the virus, thus identifying novel host/virus interactions. Such evidence exists for alleles of TRIM5 in rhesus macaques that show varying ability to restrict retroviruses (Kirmaier et al. 2010) and has been proposed for KIR in humans (Alter et al. 2011). Such analyses will require the generation of large data sets of

combined host and viral sequences, possibly including time points throughout the course of infection, as well as new analytical methodologies to account for viral and host population structure. As sequence yields and cost and availability of deep sequencing improve, this will become a feasible method by which to study host/virus interactions.

Conclusions

The type of knowledge generated by novel technologies and new conceptual approaches will take some time to mature and deliver. Additionally, the volume of data and the production of statistical and bioinformatics tools that can properly manage and perform integrated analyses are still under development. It is however important to emphasize the interest to compile detailed information on all components and interactions, as no gene acts in isolation from the broader system. Even the most-detailed functional studies of a single protein and single interaction can be facilitated and placed in context by data on the biological setting where such individual events take place.

Among the first series of initiatives to enhance access to such data types have been the creation of repositories and search motors that allow querying of multiple large-scale screens. The Gene Overlapper (<http://microb32.med.upenn.edu/GeneListOverlapper/>) provides output from genome-wide surveys of host-cell genes linked to HIV infection and allows user-configured exploration of overlaps among studies. GPS-Prot (<http://kroganlab-cmp.ucsf.edu/wiki/gpsv103/index.php>) is a tool to display proteins within their landscape of interacting partners. In order to build up a complete network for protein-protein interactions, it is necessary to access multiple databases. GPS-Prot searches publicly available databases and overlays genetic/proteomic profiling data on the physical interaction network. Such initiatives need to be extended to other data types in order to fully realize and harness the power of system-wide information.

As methods mature for acquiring holistic information on genetic variation, gene/protein

expression, and protein-protein interaction, methods to integrate these data to build networks that accurately reflect the complexity of the system will need to be widely accepted. Such analyses will allow for the rational design of experiments to perturb these systems and monitor the resulting changes to detect candidate pathways for drug targeting and intervention. While integration of data may not fulfill all the criteria that define systems biology, it will prove a useful tool for understanding HIV pathogenesis and immunology.

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T Follicular Helper Cells in HIV Infection

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Definition

T follicular helper (TFH) cells are CD4⁺ T helper cells that localize in germinal centers of secondary lymphoid tissues and specialize in supporting B-cell differentiation and maturation, mainly through secretion of IL-21. These cells are phenotypically characterized by constitutive expression of the B-cell follicle homing marker CXCR5, and their developmental program seems to be guided by the transcription factor Bcl6. Increasing data suggest that functional defects of TFH cells during HIV-1 infection contribute to dysregulation of humoral immunity and decreased responsiveness to vaccines in HIV-1-infected persons. In addition, TFH cells seem to be highly susceptible to HIV-1 infection and may play an important role as a cellular reservoir for HIV-1 persistence. Manipulation of TFH cells may be necessary for the induction of HIV-1-specific broadly neutralizing antibodies by preventative vaccine candidates.

This entry provides a summary of the multiple roles of TFH cells in HIV-1 immunopathogenesis.

Introduction

The first line of defense against invading pathogens is the innate immune response. This response is rapid but rather unspecific. If the innate response fails to clear the pathogen, the adaptive or acquired immune response is activated. The adaptive immune response takes more time (days to weeks) to develop but is more specific. The two major arms of adaptive immunity are cellular and humoral responses, which work in synergy to ensure a specific and efficient response to intra- and extracellular pathogens. These responses adapt to the specific antigen on the basis of the hosts' genetic background and differentiate and mature to allow for the maximum affinity and avidity. During the adaptive response, immunological memory is developed which generates a rapid specific response upon reencounter with that same pathogen. The induction of a memory humoral response consisting of B cells capable of secreting highly specific antibodies is dependent upon CD4 T-cell help and specifically a subset of CD4 T cells called T follicular helper (TFH) cells. Understanding differentiation and function of TFH cells is important since most licensed human vaccines function by inducing T-cell-dependent protective antibody responses. This entry will give an overview of TFH cells and

address their functions in initiation and maintenance of potent B-cell responses and how that might be dysregulated in HIV infection.

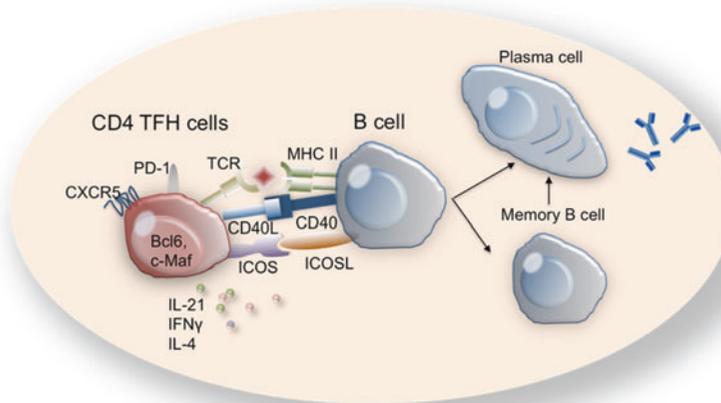
T-Cell-Dependent Induction of B-Cell Responses

The induction of B-cell responses and antibody production occur in secondary lymphoid organs, and with the exception of lipid and polysaccharide antigens, this event is T-cell dependent. During viral infections, dendritic cells take up the antigen and transport it to secondary lymphoid tissues where priming of specific CD4 T cells takes place. These primed CD4 T cells interact with BCR (B-cell receptor)-activated B cells which initially occur at the follicular border (see Fig. 1). Following this initial interaction, B cells can enter distinct pathways, either become extra-follicular, short-lived plasma cells and perifollicular early memory B cells or go through germinal center (GC) reaction to facilitate the establishment of a pool of long-lasting memory B cells. When entering the germinal center

pathway, the B cells undergo (I) somatic hypermutation, which involves random mutations of the B-cell receptor, (II) selection of randomly mutated B cells with the highest affinity for the specific pathogen which ensures specificity of the immune response, and (III) class switching, during which the immunoglobulin undergoes class-switch recombination (CSR). Depending on the extracellular signals induced by the pathogen, activated B cells are able to switch their IgM surface antibodies to IgG, IgA, or IgE, all with different functions adapted to the pathogen. These are all events leading to the generation of high-affinity mature antibody secretion and memory formation. These events are highly dependent on CD4 T-cell help and no formation of GCs can occur in the B-cell follicles without this crosstalk.

Characterization of TFH Cells

The importance of intrafollicular CD4 T-cell help for potent B-cell responses was acknowledged a long time ago, but it was only in the year 2000 that these CD4 T cells started receiving recognition as



T Follicular Helper Cells in HIV Infection, Fig. 1 CD4 TFH cells are critical for the induction of potent B-cell responses. The first interaction between TFH cells and B cells occurs at the follicular border in secondary lymphoid tissue where B cells present a given antigen to these specific T cells and crosstalk between the T cell and activated B cell

occurs through co-stimulatory molecules such as CD40L, ICOS, and cytokines such as IL-21. Stimulated B cells can then enter germinal center reaction where they undergo somatic hypermutation, class switching, selection, and differentiation into antibody secreting plasma cells or memory B cells, events that are all dependent on TFH cells

a separate subset and acquired their name T follicular helper (TFH) cells (Breitfeld et al. 2000; Schaerli et al. 2000). The classification of CD4 T cells into separate lineages is typically based on differential cytokine secretion and expression of various transcription factors shaping their diverse functions. Whereas T-bet and Gata-3 are highly expressed by TH1 and TH2 subsets, respectively, TFH cells express high levels of the transcriptional repressor Bcl6 (Chtanova et al. 2004). In addition to Bcl6, other transcription factors such as B-cell-activating transcription factors (BATF) and musculoaponeurotic fibrosarcoma (MAF) have also more recently been identified for being involved in TFH cell differentiation. Although Bcl6 can be used to define TFH cells, due to their unique localization in B-cell follicles, TFH cells have often been phenotypically characterized by expression of CXCR5 in addition to various other chemokine receptors, inhibitory receptors, and co-stimulatory molecules including high expression of PD-1 and ICOS, but also CXCR4, CD95, SAP, CD154 (CD40L), BTLA, and CD69 (reviewed in Yu and Vinuesa 2010). TFH cells are capable of secreting various cytokines, including typical TH1 (IFN γ , TNF- α), and TH2-like (IL-4) cytokines; however, IL-21 is considered as the cardinal TFH cell cytokine and is used to characterize this distinct T helper cell subpopulation (Chtanova et al. 2004).

TFH Cell Functional Markers

TFH cells provide help to B cells through the expression of multiple surface molecules and cytokine/chemokine secretion. However, the interaction between TFH cells and B cells is not unidirectional. TFH cells provide helper signals to B cells and B cells provide crucial survival signals for TFH cells. The surface molecules expressed on TFH cells are not only involved in direct contact with B cells but also in positioning TFH cells in close proximity to B cells, as it is the case for chemokine receptors such as CXCR5. One of the most important stimuli for B cells comes through CD40-CD40L signaling. Upon stimulation TFH cells can express high levels of CD40L, which

induces B-cell activation, proliferation, and survival when binding to CD40 on B cells. Other molecules involved in the direct contact between TFH cells and B cells are SLAM family receptors. The SLAM receptors are expressed on CD4 T cells and bind SAP expressed on B cells. The interaction between SAP and SLAM receptors prolongs T-cell-B-cell interaction, and their absence results in reduced GC formations. ICOS-ICOSL interaction is also important for GC formation, but so far the benefit has only been proven on the TFH cell side, for which it is important for differentiation and maintenance. In addition to molecules involved in direct contact between the two cell types, cytokines play a crucial role. IL-21 is considered a cardinal cytokine for TFH cells although other CD4 T-cell subsets can secrete it too. IL-21 has several important functions; it is the most potent cytokine for driving plasma cell differentiation and promotes B-cell survival, and like other cytokines such as IL-4 and IFN γ , it drives class switching into certain antibody isotypes (Crotty 2011).

CD4 T Follicular Cell Subsets

As mentioned previously, TFH cells in secondary lymphoid organs are often characterized by high expression of BCL6, CXCR5, PD-1, and ICOS. However, these cells are not a homogenous cell population. Recently, a subset of these T follicular cells expressing FoxP3 has been described. This transcription factor defines T regulatory cells, a CD4 T-cell subset that limit effector T-cell response and maintain peripheral tolerance (Ramiscal and Vinuesa 2013) by modulating the dephosphorylation pathway of ATP (Toth 2013). Mice studies have shown that these T follicular regulatory (TFR) cells localize in the B-cell follicles and can regulate humoral immune responses through repressing TFH cells and GC formation. Studies in HIV-infected individuals indicate significant perturbations of Treg frequency and function depending on the stage of the disease (Schulze zur Wiesch 2011); however, the role of follicular Tregs in HIV-infected lymph nodes remains to be elucidated. Further, emerging data indicate that there are both CXCR3-positive and

CXCR3-negative TFH cells. CXCR3 is expressed on TH1 type of cells, and these cells preferentially secrete IFN γ upon stimulation, whereas CXCR3-negative cells secrete IL-4, a typical TH2 cytokine. It therefore appears that within TFH cells, there is great heterogeneity which is currently investigated.

Peripheral TFH Cells

Owing to their function, TFH cells have mainly been detected in lymphoid organs. However, a subset of peripheral CD4 T cells express CXCR5. The characterization of TFH cells in the periphery will become increasingly important to study the role of TFH cells in human diseases or following vaccinations, since human lymph nodes are challenging to acquire especially with regard to longitudinal studies. Characterization of peripheral TFH (pTFH) cells based on current lymphoid markers has been challenging due to alterations of phenotypic markers on these cells upon memory differentiation and migration out of lymphoid tissue. Individuals deficient in the co-stimulatory molecule ICOS, which have severely reduced GCs and are deficient in CXCR5 expressing CD4 T cells in the periphery, shed light on the potential role of pTFH cells since ICOS is important for survival and maintenance of TFH cells; it is believed that CXCR5-positive CD4 T cells in the periphery stem from TFH cells. CXCR5 expressing CD4 T cells in the periphery express a memory phenotype and are considered peripheral TFH (pTFH) cells. Beyond CXCR5, various markers have been used by different research groups to describe pTFH cells including PD-1, CXCR3, CCR6, and ICOS, and results have been contradictory. It is possible that, depending on the milieu and whether they are recently activated, TFH cells or memory TFH cells have variable B-cell helper capabilities *in vitro* and express various levels of PD-1, CXCR3, and ICOS (Schmitt and Ueno 2013). However, it has to be emphasized that TFH-mediated B-cell maturation and differentiation and the formation of a normal germinal center reaction will primarily depend on the presence of functional intrafollicular TFH cells in secondary lymphoid organs.

Induction of TFH Cells

Naïve CD4 T cells can differentiate into functionally distinct subsets, such as TH1 and TH2 based on the type of APC it interacts with, its TCR avidity for the antigen, and the surrounding cytokine milieu (Tao et al. 1997). These mechanisms are also involved in the differentiation of TFH cells. It has been shown that CD4 T cells with strong TCR affinity preferentially develop into TFH cells *in vivo*. Upon activation of CD4 T cells by dendritic cells, maturation of CD4 T cells into becoming specialized in B-cell help starts with migration to the follicular border. This migration is dependent upon chemotaxis. The pre-TFH cells downregulate the chemokine receptor CCR7, which ligands CCL19 and CCL12 are mainly expressed in the T-cell zone, and instead upregulate CXCR5, which ligand CXCL13 is produced in the B-cell follicles. This shift in chemokine sensitivity is controlled by the transcription factor Bcl6. Without Bcl6 expression, TFH cells do not develop *in vivo*. The role of cytokines for TFH cell development has been demonstrated *in vitro* where TFH cells were generated by CD4 T-cell activation in the presence of IL-6, IL-12, and IL-21. Lack of either IL-6 or IL-21 retains TFH cell frequencies, most likely due to a redundancy between the two cytokines (Eto et al. 2011). High levels of IL-6 are present during viral infections, and increased frequencies of TFH cells during inflammatory conditions have been noted in several autoimmune disorders, in a few cancers, and in chronic viral infections as discussed below.

TFH Cells in Chronic Viral Infections

In 2011, it was first shown in a mouse model for viral infection that virus-specific CD4 T cells preferentially differentiate into TFH cells in lymphoid organs in chronic infection (Fahey et al. 2011). The induction of TFH cell differentiation in this mouse model was driven by the inflammatory cytokine IL-6 which upregulated Bcl6 expression in CD4 T cells. TFH cells were subsequently shown to be expanded in SIV-infected monkeys. These monkeys also showed a substantial increase

in B-cell proliferation and antibody secretion in the lymph node (Hong et al. 2012; (Petrovas et al. 2012). These findings have since then been confirmed in humans. An increase in the relative frequency of TFH cells among CD4 T cells was detected in the lymph nodes of chronic HIV-infected individuals compared to healthy controls (Lindqvist et al. 2012). The accumulation of TFH cells in HIV-infected lymph nodes strongly correlated with the differentiation of germinal center B cells into the plasma cell lineage but not into memory B cells, indicating an important role for TFH cells in the observed skewing of B-cell subsets and subsequent hypergammaglobulinemia in chronic HIV infection (Lindqvist et al. 2012).

Dysregulated TFH Cell- and B-Cell Responses in HIV Infection

One of the hallmarks of HIV infection besides loss of CD4 T cells is defective humoral immune responses with reduced class switching of antibodies, loss of memory B cells, and reduced antibody responses and protection following vaccinations (Moir and Fauci 2009). It was previously believed that the loss of CD4 T cells contributes to dysfunctional B-cell responses in HIV infection, but with recent data showing an expansion of TFH cells in chronic infections, this is now challenged. Under physiological conditions, TFH cells are a limiting factor in the GC, allowing only B cells with high specificity to survive and further differentiate into plasma cells or memory B cells. The GC B-cell selection is a safety checkpoint to prevent the development of autoreactive B-cell clones or less efficient B cells to survive. It is possible that an abnormal expansion of TFH cells in chronic infections leads to loss of that selective pressure on the GC B cells which leads to secretion of high amounts of nonspecific antibodies. A common condition in HIV-infected individuals is called hypergammaglobulinemia, which is manifested as hypersecretion of nonspecific IgG antibodies. It is however not clear how the dysregulation of TFH cells in HIV infection correlates with the loss of neutralizing antibodies. Under physiological conditions, TFH cells expand following infection and start contracting after 5 days, most likely

as a result of antigen clearance (Deenick et al. 2010). However, in chronic viral infection with persistent viremia, the retraction of TFH cells is not observed and the increased frequency could be due to accumulation. In line with this, it was shown that abundant amounts of virions are trapped on the surface of follicular dendritic cells (FDC) and that viral antigens (e.g., p24/gag) are retained on the FDC network despite successful antiretroviral combination therapy (Tenner-Racz et al. 1998; Popovic et al. 2005). Although there is an increase of TFH cells in HIV infection, the crosstalk between TFH cells and B cells is compromised which contributes to dysregulated B-cell responses. Germinal center B cells in HIV-infected individuals express high levels of the inhibitory ligand PD-L1, which reduces TFH cell survival and cytokine secretion through the PD-1 receptor resulting in weakened B-cell help (Cubas et al. 2013). Moreover, alterations in the intrafollicular cytokine milieu are thought to contribute to these changes. The TFH signature cytokine IL-21 plays a pivotal role in mediating a normal germinal center reaction. It has been shown that the addition of exogenous IL-21 could restore B-cell maturation and immunoglobulin secretion in HIV infection *ex vivo* (Cubas et al. 2013). However, the exact mechanisms of disturbed T-cell/B-cell crosstalk in HIV-infected GC remain to be elucidated.

TFH Cells as Viral Reservoirs

Memory CD4 T cells are selectively targeted by HIV, and substantial depletion of these cells occurs throughout AIDS progression. In addition lymphoid organs are the primary sites not only for immune activation but also for viral replication (Tenner-Racz et al. 1998; reviewed in Stellbrink and van Lunzen 2001), making TFH cells with their effector memory phenotype prime targets for HIV. In fact it has been shown that CD4 T cells within germinal centers contain the highest frequency of infected cells and these cells were most efficient in supporting virus replication (Hufert et al. 1997; Perreau et al. 2013). TFH cells have been shown to be preferentially infected even in patients with low viral loads, and replication-competent infectious virions could be readily

recovered indicating the role of TFH cells as potential viral reservoirs (Perreau et al. 2013). The germinal centers are to a large part devoid of CD8 T cells, which may support viral persistence within the TFH cell reservoir. Moreover, activated memory type TFH cells in GC are ideal target cells for de novo infection in the presence of persistent low-level viral replication.

Implications for Vaccine Design

The vast majority of licensed vaccines today imitate immune responses to natural infection and generate long-lived protective antibody responses. However, inducing an immune response against HIV by vaccination resembling natural infection will not be sufficient to provide protection. The knowledge on how to induce potent TFH cell responses and subsequent B-cell and antibody responses by vaccination strategies is scarce. The availability of antigen can regulate the development and function of TFH cells through differential presentation on follicular dendritic cells (FDC) and B cells. The balance between inducing sufficient B-cell help and exacerbated or dysfunctional TFH cell responses needs to be carefully considered and evaluated (Pratama and Vinuesa 2014). The generation of an efficient antibody-based HIV vaccine will require rational design of a combination of antigen, adjuvant (immunomodulator), and delivery system to induce T-cell responses capable of providing optimal B-cell help. Therefore, understanding how to induce and regulate TFH cell responses will be crucial for providing the right B-cell help. Current research thus focuses on the interplay between TFH and B-cell responses in GC, resulting in the formation of high avidity and affinity broadly neutralizing antibodies.

Conclusions

Current studies of TFH cells in chronic HIV infection indicate that TFH cells may have important implications for the dysregulation of B-cell responses in HIV infection. This may be mediated by an abnormal expansion or dysfunction of this highly specialized T helper cell subset or a

combination thereof. The exact mechanisms of perturbations of T-cell-B-cell interactions are not yet fully understood, but recent studies suggest that these alterations are associated with persistence of HIV in the TFH compartment as well as subtle changes in transcription and signaling pathways including dysbalances in the cytokine milieu. A better understanding of the role of TFH cells in health and diseases is crucial to be able to address TFH cell functions as potential therapeutic targets for the induction of proper B-cell responses and against the persistence of HIV infection in this specific cellular reservoir.

Cross-References

- ▶ [Central Memory CD4 T Cells](#)
- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [HIV and SIV, B-Cell Responses to](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [Role of Regulatory T Cells During HIV Infection](#)
- ▶ [Th17 Cells](#)

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T Memory Stem Cells

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Definition

T memory stem cells (T_{SCM}) are a small population of long-lived memory T cells that represent the most undifferentiated stage of all memory T cell subsets. Functionally, T_{SCM} seem to be able to differentiate into all memory T subsets while simultaneously maintaining their own pool size through homeostatic self-renewal. T_{SCM} can persist in the human body for extremely long

periods of time and seem to be responsible for maintaining lifelong cellular immunity to pathogens by generating effector T cells based on demand (Gattinoni et al. 2011; Lugli et al. 2013b). Emerging data suggest that CD4 T_{SCM} can play a major role as a long-term reservoir for HIV persistence despite antiretroviral therapy, and that infection of these cells represents a distinguishing feature of pathogenic SIV infection in nonhuman primates (Buzon et al. 2014b; Cartwright et al. 2014; Klatt et al. 2014). This entry summarizes recent advances in understanding the role of T_{SCM} in HIV/SIV disease pathogenesis.

Introduction

Upon first antigen encounter during an immune response, naive T cells develop into activated effector cells, the majority of which die during the subsequent antimicrobial immune defense process. However, a small proportion of these antigen-experienced cells survives, enters the memory cell pool, and persists long-term to provide antigen-specific immune protection. Such memory cells can be classified according to developmental, maturational, and functional characteristics into central-memory (T_{CM}) and effector-memory (T_{EM}) T cells (Zielinski et al. 2011; Gattinoni et al. 2012; Sallusto et al. 2004; Lanzavecchia and Sallusto 2005). Central-memory T cells represent a long-lasting cell population that express lymphoid tissue homing markers, secrete mostly IL-2 upon TCR stimulation, and can rapidly transition into effector-memory cells upon activation. In contrast, effector-memory T cells are more short-lived, express chemokine receptors and adhesion molecules that allow for extravasation into inflamed tissues, and can rapidly execute effector functions. This classification has led to the assumption of a developmental hierarchy in which central-memory cells represent a more immature version of memory T cells that continuously replenish the effector-memory cell pool in a stem cell-like fashion while preserving their own pool through homeostatic approval (Graef et al. 2014). However, based on a number of experimental and

theoretical considerations, the presence of a defined memory cell population with more pronounced stem cell-like properties has been postulated for many years, and recently, small populations of memory T cells with extraordinary abilities to persist long-term and to repopulate all memory cell populations have been identified in mice, nonhuman primates, and humans (Gattinoni et al. 2009, 2011; Lugli et al. 2013a). These cells, termed “T memory stem cells” (T_{SCM}), were observed both within the CD4 and the CD8 T cell compartment and exhibited a naïve T cell phenotype, except for the surface expression of the memory cell markers CD95 and IL-2R β . Functionally, T_{SCM} were shown to have the greatest proliferative capacity compared to all memory T cell subsets and are characterized by their ability to undergo asymmetric cell divisions that can lead to homeostatic proliferation and self-renewal, as well as to transitional proliferation into more mature central-memory and effector-memory T cell populations. Moreover, when T_{SCM} are serially transplanted in animal models, T_{SCM} can repopulate the full diversity of the memory T cell compartment, such as T_{CM} , T_{EM} , and T_{TD} populations, and efficiently reconstitute cellular immunity in immunocompromised hosts (Gattinoni et al. 2011; Cieri et al. 2013; Lugli et al. 2013a, b). Due to these characteristics, T_{SCM} are likely to persist in the human body for extremely long periods of time and seem to be responsible for maintaining lifelong cellular immunity by generating effector T cells based on demand.

Role of T_{SCM} in Antiviral Immune Defense

Although T_{SCM} represent an immature and quiescent population of memory T cells, they can execute classic lymphocellular effector functions upon stimulation with their cognate antigen. This includes the ability to secrete IFN- γ , IL-2 or TNF- α , and IFN- γ . So far, antigen-specific CD8 T_{SCM} against CMV, flu, tumor antigens, and SIV have been identified (Gattinoni et al. 2011), but it is likely that they contribute to cellular immune

defense against virtually all microbial pathogens that can challenge the host. Virus-specific CD8 T_{SCM} have been studied in most detail in SIV-infected rhesus macaques (Lugli et al. 2013a). These investigations demonstrated that SIV-specific CD8 T_{SCM} cells are generated early after infection and persist throughout the disease process but, in most cases, make only small contributions to a given population of epitope-specific T cells. Interestingly, these studies also revealed that viral mutational escape in CD8 T cell epitopes leads to progressive elimination of central-memory and effector-memory CD8 T cells recognizing the wild-type epitope, indicating that these cell populations require continuous antigenic stimulation to maintain their long-term survival. In contrast, CD8 T_{SCM} specific for the wild-type epitope were continuously detected after viral mutational escape, and their relative contribution within the total population of antigen-specific CD8 T cells disproportionately increased, consistent with prolonged antigen-independent homeostasis of antigen-specific CD8 T_{SCM} (Lugli et al. 2013a). Although the functional role of HIV-specific CD8 T_{SCM} in humans has not yet been explored, a recent correlative study demonstrated that in untreated HIV patients, the frequency of total CD8 T_{SCM} is inversely associated with plasma viral load and with biomarkers of T cell immune activation while being positively correlated to absolute CD4 T cell counts (Ribeiro et al. 2014). If further corroborated in functional studies, CD8 T_{SCM} cells might therefore have an important role for influencing HIV-1 immune defense and natural HIV disease progression.

Susceptibility of CD4 T_{SCM} to HIV-1 Infection

HIV preferentially infects CD4 T cells through the recognition of host cell CD4 molecules and the chemokine receptors CCR5 or CXCR4. However, different CD4 T subpopulations with distinct maturation and activation status differ with regard to their susceptibility to HIV-1 infection *in vitro* (Chomont et al. 2009; Buzon et al. 2014a, b).

Initial investigations on the intrinsic susceptibility of CD4 T_{SCM} cells to HIV-1 indicated that CD4 T_{SCM} are susceptible to *ex vivo* HIV-1 infection, and that HIV-1 mRNA was readily detectable within the CD4 T_{SCM} population in HIV-1 patients who do not receive suppressive antiretroviral therapy, indicating that they are permissive to HIV-1 infection *in vivo* (Buzon et al. 2014b). The susceptibility of CD4 T_{SCM} to R5-tropic infection HIV-1 infection appeared to be similar to CD4 T_{CM} cells, and cytopathic effects associated with HIV-1 infection seemed to be relatively low in CD4 T_{SCM} cells. Interestingly, *ex vivo* infection with a VSV-G-pseudotyped HIV virus that bypasses viral coreceptor-mediated entry processes indicated that among all CD4 T cell subsets, highest levels of viral production were achieved in CD4 T_{SCM} cells (Buzon et al. 2014b), for reasons that are currently unclear. Using an elegant system to discriminate between abortive, latent, or productive infection, recent experiments demonstrated that *ex vivo* infection of CD4 T_{SCM} can result in both productive and latent HIV-1 infection. However, the rate of abortive infection in CD4 T_{SCM} was relatively high in comparison to T_{CM} and T_{EM} cells, possibly due to altered expression or function of the antiviral restriction factor Samhd1 in this cell compartment (Tabler et al. 2014). Of note, expression levels of Trim5a, an HIV restriction factor inhibiting viral uncoating, are significantly reduced in CD4 T_{SCM} compared to alternative CD4 T subsets, which may support their susceptibility to HIV-1 (Buzon et al. 2014b). Collectively, these results suggest that CD4 T_{SCM} are permissive to HIV-1 infection *in vivo* and *in vitro*, highlighting their potential to serve as a putative long-lasting reservoir *in vivo* for HIV infection.

Contribution of T_{SCM} to HIV-1 Long-Term Persistence

The fact that CD4 T_{SCM} are easily infected *in vitro*, harbor HIV RNA *in vivo* in untreated HIV-1 patients, and are long-lived cells might suggest that these cells could serve as an important component of the long-lasting viral reservoir

when active viral replication is effectively suppressed by antiretroviral therapy. In this regard, first studies showed that CD4 T_{SCM} from ART-suppressed HIV-1 patients contain HIV-1 DNA and, importantly, that they have the ability to produce infectious viral particles upon activation (Buzon et al. 2014a, b; Jaafoura et al. 2014). This indicates that these cells can represent a reservoir for replication-competent viral strains in ART-treated subjects. However, due to their low frequency, CD4 T_{SCM} only contribute ~8% to the total viral CD4 T cell reservoir in an average population of ART-treated patients. This contribution seems to critically depend on the duration and timing of antiretroviral treatment initiation: In long-term treated patients who initiated ART early after infection and have a comparatively small total HIV reservoir, CD4 T_{SCM} appeared to make a relatively larger contribution to the total viral reservoir size, as opposed to patients who started treatment during the chronic phase of the infection and had an increased total viral reservoir size (Buzon et al. 2014a, b). Longitudinal studies over more than 10 consecutive years of antiretroviral treatment demonstrated that HIV-1 DNA in CD4 T_{SCM} cells remained stable or only slightly decreased, while decay of HIV-1 DNA in alternative CD4 T cell populations was more pronounced (Buzon et al. 2014b); this led to a disproportionate increase in the contribution of CD4 T_{SCM} to the viral reservoir over time and supports the role of CD4 T_{SCM} as a long-term reservoir for HIV-1 (Buzon et al. 2014b). Moreover, additional studies demonstrated that the half-life of HIV-1 DNA in CD4 T_{SCM} was much longer than in any of the other T cell populations, consistent with CD4 T_{SCM} as a preferential site for viral long-term persistence (Jaafoura et al. 2014). Importantly, phylogenetic studies demonstrated identical HIV-1 sequences in CD4 T_{SCM} collected at the beginning of antiretroviral therapy and several years later; moreover, HIV-1 DNA sequences collected from CD4 T_{SCM} after many years of treatment showed close phylogenetic associations to plasma sequences from early disease. This suggests that viral strains circulating in early infection can persist long-term upon infection of these long-lived memory CD4 T cell subsets (Buzon et al.

2014b). Overall, these studies imply that HIV-1-infected CD4 T_{SCM} serve as a very long-lasting reservoir for HIV-1 in ART-treated patients.

Role of T_{SCM} in SIV and HIV Pathogenesis

Pathogenic SIV infection of rhesus macaques (RM) imitates the disease course of HIV-1-infected untreated humans and leads to progressive loss of CD4 T cells associated with clinical signs of immune deficiency. In contrast, SIV infection of natural hosts for the virus, such as sooty mangabeys (SM), is typically nonpathogenic, despite high levels of virus replication. Several mechanisms have been proposed to explain the nonpathogenic nature of SIV infection in SM, such as a preserved mucosal barrier, reduced expression of the CCR5 SIV coreceptor in the surface of the target CD4 T cells, and downmodulation of the CD4 receptor during the generation of the memory cell population (Chahroudi et al. 2012). In RM, SIV infection is typically associated with high levels of viral replication within the CD4 T_{CM} subset (Paiardini et al. 2011), and a direct link between virus-mediated CD4 T_{CM} killing, CD4 T_{CM} depletion, and the onset of the immunodeficiency has been observed in infected RM (Letvin et al. 2006; Okoye et al. 2007; Brenchley et al. 2012). This represents a striking contrast to SIV-infected SM, where viral infection of T_{CM} seems to be reduced. More recent studies extended these findings by demonstrating even more pronounced differences between SIV infection of CD4 T_{SCM} from SM and RM. While CD4 T_{SCM} from RM are fully susceptible to SIV infection and support high-level SIV replication, SIV infection was in many cases completely undetectable in CD4 T_{SCM} from SM, suggesting the presence of cell-intrinsic restriction mechanisms that protect this particular cell subset against viral infection. These data indicate that the selective resistance of CD4 T_{SCM}, and to a lesser extent of CD4 T_{CM}, to SIV infection represents a distinguishing feature of nonpathogenic SIV infection in natural hosts (Cartwright et al. 2014). Similar observations were recently made in

HIV-1-infected viremic nonprogressors (VNP), an exquisitely rare group of patients who maintain normal CD4 T cell counts for prolonged periods of time in the presence of continuous high-level viral replication and in that sense resemble SIV-infected natural hosts. Interestingly, HIV-1 infection of CD4 T_{SCM} and CD4 T_{CM} from VNP was significantly reduced in comparison to HIV-1 patients with typical disease progression (Klatt et al. 2014). Thus, low levels of SIV and HIV infection within the CD4 T_{SCM} and, to a lesser extent, within the CD4 T_{CM} population seem to be a characteristic feature influencing the disease progression of nonpathogenic SIV and HIV infections. Future studies will be needed to determine possible resistance mechanisms of T_{CM} and T_{SCM} cells to viral infections in SM and viremic nonprogressors.

Conclusions

CD4 T_{SCM} represent a newly discovered T subpopulation that seems to have a key role as a long-lived reservoir for viral persistence despite ART. Future studies will be needed to analyze whether other CD4 T cell populations with stem cell-like functional properties, such as Th17 cells (Kryczek et al. 2011; Muranski et al. 2011), can also serve as a long-lasting viral reservoir for HIV-1 in persons with pharmacologically suppressed viremia. The identification of infected cells with stem cell-like properties as an important component of the viral reservoir may offer novel opportunities for clinical strategies to target the most durable components of the latent viral reservoir. Since stem cell-like transcriptional programs seem to guide long-term maintenance of CD4 T_{SCM}, molecular pathways responsible for stem cell homeostasis may represent attractive targets for reducing HIV-1 persistence in CD4 T_{SCM} in vivo. Moreover, CD4 T cells with stem cell-like properties may represent desirable target cells for creating long-lived populations of CD4 T cells that are resistant to HIV-1 after manipulation of viral coreceptor expression with, e.g., zinc finger nucleases (Kiem et al. 2012).

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Talaromyces (Penicillium) marneffeii and HIV

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Definition

Talaromyces marneffeii, formerly *Penicillium marneffeii*, is a dimorphic fungus. (Samson et al. 2011) *T. marneffeii* infection (TMI) is a common opportunistic infection in HIV-infected patients living in the endemic areas, particularly Southeast Asia, Southern China, and India. However, there have

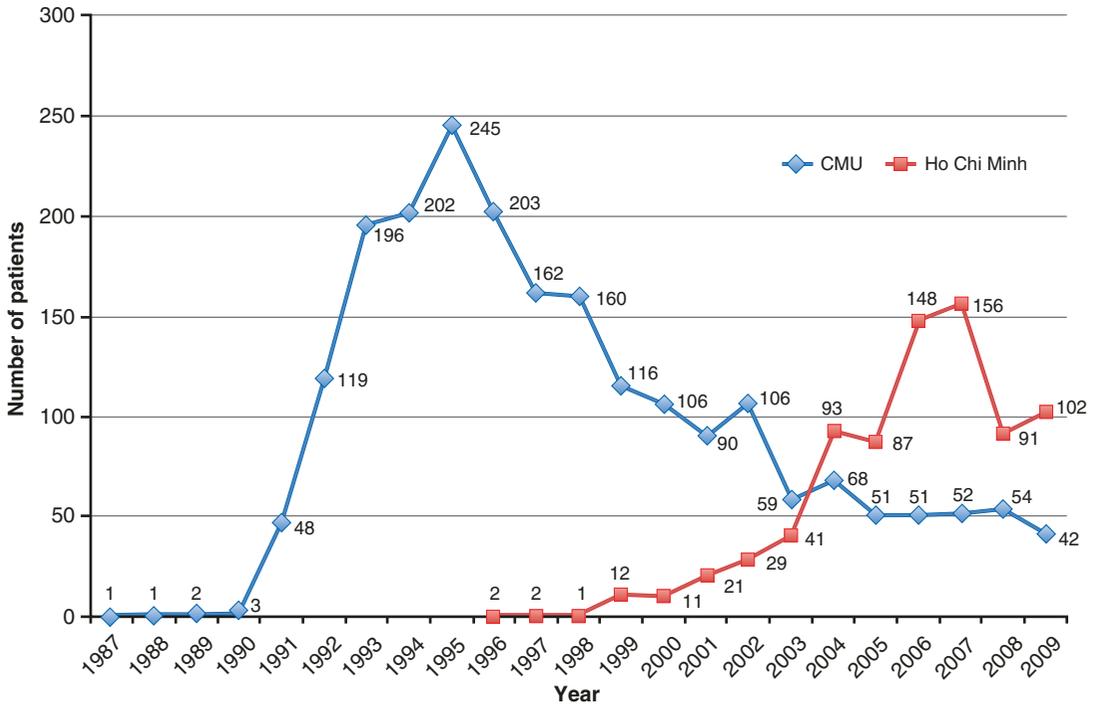
been reports in HIV-infected travelers who have traveled to the endemic areas. (Vanittanakom et al. 2006). The clinical manifestations varied widely from asymptomatic to disseminated infection involving several organs including central nervous system. The epidemiology, clinical manifestations, diagnosis, treatment, and prevention will be described here.

Introduction

T. marneffeii was first isolated in 1956 from the internal organs of bamboo rats (*Rhizomys sinensis*) in Vietnam (Capponi et al. 1956). The first natural human infection was reported in 1973 in an American missionary who had been living in Southeast Asia (DiSalvo et al. 1973). He suffered from non-Hodgkin lymphoma several years after returning to the USA and underwent splenectomy. *T. marneffeii* was isolated from his spleen. Before the HIV/AIDS epidemic, human TMI was uncommon and occurred mostly in immunocompromised patients. The incidence of TMI increases rapidly after HIV/AIDS epidemic hit Southeast Asia in late nineteenth century. The first case of TMI in HIV-infected patients was reported from Thailand in 1988. Since then, there were a number of cases among patients with advanced HIV infection, mostly from Southeast Asia. During 1990s, TMI ranked the third most common opportunistic infection in HIV-infected patients in northern Thailand, following tuberculosis and cryptococcal meningitis (Supparatpinyo et al. 1994). HIV-infected patients who had CD4 cell count < 100 cells/mm³ are at risk of developing TMI (Supparatpinyo et al. 1994). In the era of combination antiretroviral therapy (cART), the incidence of TMI is significantly declined except for those who are unaware of HIV status, unable to access to antiretroviral treatment, or who have suboptimal treatment responses.

Epidemiology

TMI is endemic in Southeast Asia, including but not limited to Thailand, Vietnam, Laos, Myanmar, and Malaysia; Southern China; Taiwan; Hong Kong; and India. The majority of cases were



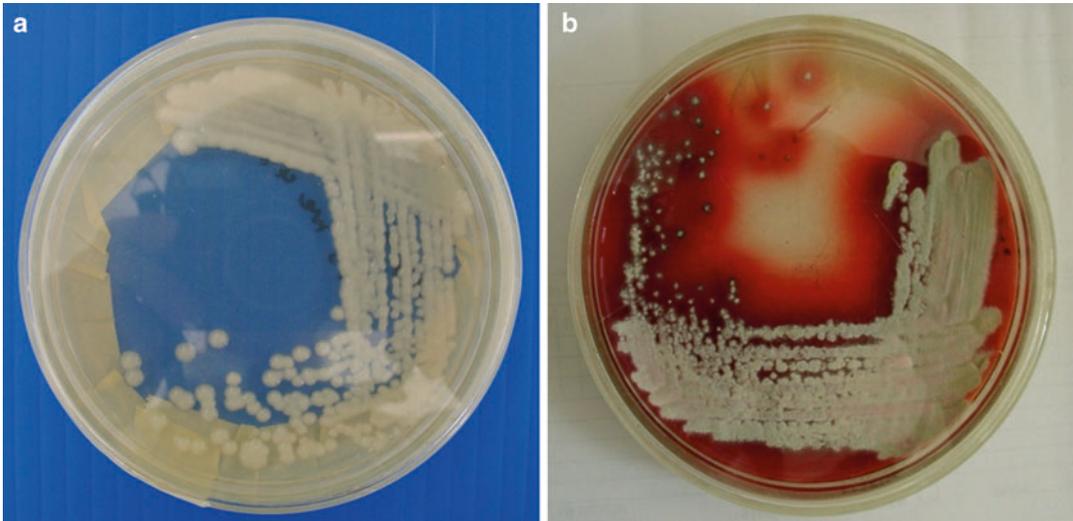
Talaromyces (Penicillium) marneffeii and HIV, Fig. 1 Epidemic curve of patients with *Talaromyces marneffeii* in Thailand and Vietnam

reported from northern Thailand and Vietnam (Supparatpinyo et al. 1994; Le et al. 2011). The increase of patients in these countries corresponds with the spread of HIV transmission into the areas. In northern Thailand, the greatest number of cases was reported during 1990s after the start of HIV/AIDS epidemic in 1984, whereas the number of cases in Vietnam reached its peak in 2007 after the start of HIV/AIDS epidemic in 1996 (Fig. 1) (Supparatpinyo et al. 1994; Le et al. 2011).

Bamboo rat is known to be the natural reservoir of *T. marneffeii* (Deng et al. 1986). A study done in bamboo rat (*Rhizopus* species) in Guangxi showed that *T. marneffeii* can be isolated from 91% of internal organs of *R. pruinosis* and *R. sinensis* (Deng et al. 1986; Li et al. 1989). In Thailand, the prevalence of *T. marneffeii* in internal organs of *R. sumatrensis*, *R. pruinosis*, and *Cannomys badius* were 93%, 75%, and 19%, respectively (Chariyalertsak et al. 1996a). Although *T. marneffeii* can be isolated from this animal, it is still unclear how these animals and humans acquire this fungus. It is believed that bamboo rat may shed

T. marneffeii into the soil and humans acquire the fungus from exposure to contaminated soil or aerosols. A case-control study in northern Thailand comparing 80 HIV-infected patients with TMI and 160 HIV-infected patients without TMI revealed that history of exposure to soil especially during rainy season, but not the exposure or consumption of bamboo rats, was a risk factor of TMI (Chariyalertsak et al. 1997). However, soil sampling from bamboo rat burrows were rarely positive for *T. marneffeii*. The hypothesis of airborne transmission from environmental source has been postulated from a report of undiagnosed HIV-positive physician who contracted *T. marneffeii* while visiting the laboratory culturing *T. marneffeii* on the open bench without direct exposure to the fungus (Hilmarsdottir et al. 1994). The seasonal increase in TMI in rainy season suggests an expansion of environmental reservoirs with favorable growth conditions for the fungus (Le et al. 2011; Chariyalertsak et al. 1996b). A study in Vietnam showed that the number of TMI cases was strongly associated with humidity (Bulterys et al. 2013). Another route of transmission





Talaromyces (*Penicillium*) *marneffe* and HIV, Fig. 2 Characteristics of yeast and mold forms of *Talaromyces marneffe* (a) Yeast colonies (b) Mold colonies

was direct inoculation of *T. marneffe* into the skin, which has been reported in the first human case of TMI, when Segretain accidentally pricked his finger with a needle contaminated with *T. marneffe* (Segretain 1959). The incubation period has not been well defined. A study in Vietnam using the model to estimate the incubation period from the time of exposure to the onset of symptoms estimated that the incubation period was 1 week (95% CI 0, 3 weeks) (Bulterys et al. 2013). However, there was a case report from Australia of an HIV-infected patient who had a 10-year period between probable exposure and clinical onset; this suggested the possibility of a long latency with subsequent reactivation (Jones and See 1992).

Mycology

T. marneffe was named in honor of Hubert Marneffe, the director of Pasteur Institute in Indochina, after its discovery in 1959 (Capponi et al. 1956). It is the only species in this genus that is categorized as dimorphic fungus. At the room temperature, it grows as a mold on Sabouraud dextrose agar, and grows as a yeast on brain-heart infusion (BHI) agar incubation at 37 °C. The analysis of complete genome demonstrates

that *T. marneffe* is closely related to molds than yeasts. The young colonies of *T. marneffe* mycelia have a flat green surface with underlying deep red coloring; aged colonies produce a soluble red pigment that diffuses into the agar. Microscopically, the mycelia show typical structures of *Talaromyces* species, including hyaline septate hyphae and fruiting structures composing of branching metulae and phialides which produce conidia in chains. The demonstration of mold to yeast conversion after incubating at 37 °C is required before concluding that the isolate is *T. marneffe*. The yeast colonies of white to tan color develop in a few days. The yeast cells are round to oval with a central septum (Segretain 1959). Figure 2 shows characteristic of colony of yeast and mold form of *T. marneffe*.

Pathology

Pathology of TMI depends on immune status of the hosts. In immunocompetent host, granulomatous and suppurative reactions are frequently seen (Supparatpinyo et al. 1994; Vanittanakom et al. 2006). The presence of epithelioid granuloma may lead to the diagnosis of tuberculosis which is also common in the same areas. In

immunocompromised host, necrotizing reactions with poor granuloma formation are commonly seen.

Clinical Manifestations

The majority of TMI developed in advanced HIV-infected patients. A study in northern Thailand showed that more than 90% of TMI occurred in patients with CD4+ cell counts of < 100 cells/mm³. Patients with TMI commonly present with systemic symptoms and signs including fever, weight loss, fatigue, and malaise. Skin lesions are present in 70–80% of patients. The lesions are mostly molluscum-like lesions distributing over face, neck, upper extremities, and upper trunk (Fig. 3). Some patients may also present with involvement of one or more organs, e.g., lung, liver, spleen, lymph nodes, bone marrow, and central nervous system. Clinical manifestations of TMI in three large cohorts from northern Thailand, India, and Vietnam are shown in Table 1. Comparing to HIV-uninfected patients, HIV-infected patients were more likely to have fever, umbilicated skin lesions, and splenomegaly (Kawila et al. 2013).

Immune reconstitution inflammatory syndrome (IRIS) after initiation of cART is of concern. Unmasking IRIS diagnosed within 1–3 months after cART initiation has been reported. The



Talaromyces (*Penicillium*) *marneffe*i and HIV, Fig. 3 Skin lesions in patients with *Talaromyces marneffe*i infection

symptoms are not severe in endemic area and mostly present with atypical skin lesions. Instead of umbilicated skin lesion, scaly lesion and erythematous papules and nodules, with or without central necrosis, have been reported (Ho et al. 2010; Saikia et al. 2010). Lymphadenopathy and massive hepatosplenomegaly have been reported in those from nonendemic area traveling to high-risk area (Hall et al. 2013). Up to present, paradoxical IRIS in patients with established TMI have not yet been reported after antiretroviral initiation.

Diagnosis

Definite diagnosis is usually made by isolation of the fungus from clinical specimens. Bone marrow culture gives the highest yield, approaching 100%, followed by skin, lymph nodes, and blood cultures (Table 2). HIV-infected patients were more likely to have fungemia than HIV-uninfected patients (Kawila et al. 2013). The median time to identify *T. marneffe*i from skin and blood cultures was 4 days (IQR 3–5 days) and 5 days (IQR 4–6 days), respectively (Le et al. 2011).

Histopathological diagnosis can also be made on tissue biopsy stained with periodic acid-Schiff (PAS) or Glomormimethenamine silver (GMS) stains. The characteristic yeast-like cells are 3–5 μm in size, round to oval shape, pleomorphic, and frequently contain clear central septum (Drouhet 1993). In contrast, *Histoplasma capsulatum* yeast-like cells are 2–5 μm in size, round to oval, monomorphic, and occasional budding. Granuloma formation may be absent due to poor immune responses.

Presumptive diagnosis could be rapidly made by microscopic examination of Wright-stained clinical specimens showing characteristic yeast-like cells similar to those in histological findings (Fig. 4). In patients with heavy infection, yeast-like cells may be found in peripheral blood smear.

Serological assays to detect antibodies specific to *T. marneffe*i have been studied, e.g., micro-immudiffusion technique using mycelial phase exoantigens, indirect fluorescent antibody test

Talaromyces (Penicillium) marneffeii and HIV, Table 1 Comparisons of clinical manifestations among patients with *Talaromyces marneffeii* infection

	Thailand (Supparatpinyo et al. 1994) (N = 80)	India (Ranjana et al. 2002) (N = 36)	Vietnam (Le et al. 2011) (N = 513)
Demographic data			
Male	74 (92)		421 (82)
Age (years)	32.4 (18–63)		28 (25–32)
Symptoms			
Fever	74 (92)	35 (97)	419 (82)
Anorexia			318 (62)
Fatigue/malaise		31 (86)	239 (47)
Cough	39 (49)		205 (40)
Abdominal pain			160 (32)
Diarrhea	25 (31)	8 (22)	150 (30)
Weight loss	61 (76)	36 (100)	92 (18)
Signs			
Body temperature			38 (37–38.5)
Highest temperature			39.5 (38.5–40)
Body temperature \geq 38.3 °C	79 (95)		
Pallor	62 (78)	31 (86)	286/397 (72)
Thrush	59 (74)	4 (11)	139/496 (28)
Skin lesions	57 (71)	29 (81)	346/487 (71)
Genital ulcer	5 (6)		
Hepatosplenomegaly		14 (39)	286 (56)
Hepatomegaly	41 (51)		
Splenomegaly	13 (16)		
Lymphadenopathy	46 (57)	12 (33)	131 (26)
Laboratory findings			
Hemoglobin (g/dL)	9.6 (5.4–16.7)	9.5 (6.6–12.4)	8 (6–9.8)
White blood cell count (\times 1000 cells/ μ L)	6.2 (1.6–17.2)	5.9 (2–12)	
Absolute lymphocyte count (\times 1000 cells/ μ L)	1.3 (0.2–4.0)		0.4 (0.2–0.7)
Platelet count (\times 1000 cells/ μ L)			82 (42–150)
AST	122 (4–650)		122 (63–230)
ALT	43 (5–194)		60 (34–119)
CD4 cell count (cells/ μ L)	N = 20 9 (1–44)		N = 62 7 (4–24)

Categorical data are presented in number (%), continuous data are presented in means and range for study in Thailand and India, and median and IQR for study in Vietnam

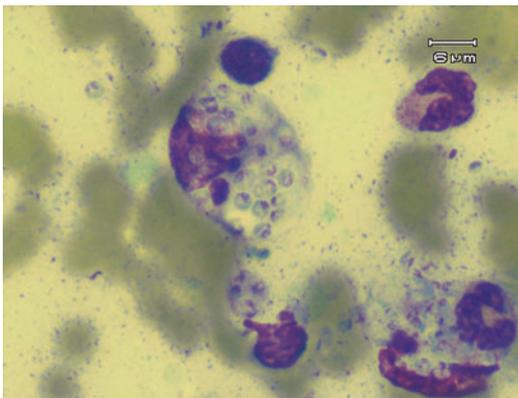
using antigens from germinating conidia and yeast forms, immunoblot assay using 54- and 50-kDa protein antigens produced during the growth phase of the yeast and mycelial forms, and an enzyme-linked immunosorbent assay (ELISA)-based antibody test using a recombinant *T. marneffeii* mannoprotein (Mp1p). In addition, *T. marneffeii* antigen detections have been developed. Monoclonal antibody used to detect

Aspergillus fumigatus galactomannan was found to cross-react with *T. marneffeii* with lower titer of antigen detection. An enzyme immunoassay for quantifying *T. marneffeii* urinary antigen was developed using fluorescein isothiocyanate-labeled purified rabbit hyperimmune IgG. The diagnostic sensitivity and specificity were higher than 95%. The tests have potential role to monitor treatment response to antifungal therapy.

Talaromyces (Penicillium) marneffeii and HIV, Table 2 Isolation of *Talaromyces marneffeii* from clinical specimens

	Thailand (Supparatpinyo et al. 1994)	Vietnam (Le et al. 2011)
Blood	59/78 (75.6)	395/472 (84)
Skin biopsy/ scraping	47/52 (90.4)	186/195 (95)
Lymph node	9/9 (100)	17/20 (85)
Peritoneal fluid	N/A	9/23 (39)
Cerebrospinal fluid	3/20 (15)	21/59 (36)
Bronchoalveolar lavage	N/A	7/9
Bone marrow	26/26 (100)	2/2
Oropharyngeal lesions	N/A	8/13
Sputum	14/41 (34.1)	N/A
Stool	N/A	6/10

N/A: not applicable



Talaromyces (Penicillium) marneffeii and HIV, Fig. 4 Yeast cells of *Talaromyces marneffeii* from skin scraping

A monoclonal antibody-based sandwich ELISA was the other method developed for antigen detection in clinical specimens including urine samples. However, studies in larger number of patients are required before making a conclusion of the role of serologic testing (Vanittanakom et al. 2006).

Lastly, the PCR techniques for detection of *T. marneffeii* have been developed in clinical research. The utility in clinical practice needs further studies.

Treatment

In vitro study demonstrated that the MIC of *T. marneffeii* to azoles is low except for fluconazole (Supparatpinyo et al. 1993). The MIC for amphotericin B and flucytosine varied widely. A study in Thailand between 1990 and 1992 among 30 clinical isolates showed the lowest MIC to miconazole, followed by itraconazole (Table 3). The clinical responses were correlated with the in vitro study. However, clinical responses to amphotericin B, which had geometric mean MIC of 0.976, was 80% (Supparatpinyo et al. 1993). The different form of *T. marneffeii* may show different susceptibility to the same agent. In one study, yeast form was more sensitive to fluconazole and itraconazole than mold, while mold form displayed greater susceptibility to amphotericin B and 5-FC (Sekhon et al. 1993). A new azole, voriconazole, has also demonstrated a good activity against *T. marneffeii* in vitro, comparable to itraconazole (Radford et al. 1997). Echinocandins (micafungin and anidurafungin) also has in vitro activity against *T. marneffeii*, (Nakai et al. 2003; Odabasi et al. 2004) and mold form has lower MIC than yeast form (Nakai et al. 2003).

Amphotericin B with or without flucytosine has been used to treat TMI. However, prolonged treatment and high dosage of amphotericin B is associated with kidney injury, electrolyte imbalance, and infusion-related reactions.

The preferred regimen for TMI is amphotericin B 0.6 mg/kg/day intravenously for 2 weeks, followed by oral itraconazole 400 mg/day for 10 weeks (Sirisanthana et al. 1998). The study showed that 72 of 74 patients (97.3%) responded to treatment with negative blood culture at 12th week as well as resolution of fever and disappearance of skin lesions. Among 65 patients whose blood cultures were initially positive, all have cleared fungus from bloodstream at the 2nd week. Liposomal amphotericin B 3–5 mg/kg/day can be used when renal adverse effects are of concern. In patients with mild disease, oral itraconazole 400 mg/day for 8 weeks followed by 200 mg daily is recommended. Oral solution of itraconazole has better bioavailability than the

Talaromyces (Penicillium) marneffei and HIV, Table 3 In vitro susceptibility of *Talaromyces marneffei* isolates

Antifungal agents	Number of Isolates	MIC ($\mu\text{g/ml}$)	
		Geometric mean	Range
Miconazole	29	0.001	<0.002–0.156
Itraconazole	28	0.009	<0.002–0.19
Ketoconazole	29	0.027	<0.002–0.078
Fluconazole	30	7.937	0.313–20.0
Amphotericin B	29	0.976	0.25–4.0
5-Flucytosine	29	0.248	<0.015–0.46

capsule. Voriconazole 6 mg/kg intravenously every 12 h on day 1 and 4 mg/kg every 12 h for at least 3 days followed by oral voriconazole 200 mg twice daily for a maximum of 12 weeks can be used (Supparatpinyo and Schlamm 2007). Oral treatment with voriconazole from day 1 can be used in patients with mild disease.

Prevention

Prevention of exposure is difficult for people living in the endemic area. However, for those with advanced HIV infection living in nonendemic area, avoidance of travel to endemic areas is recommended. Primary prophylaxis for TMI in HIV-infected individuals with CD4+ cell count of <200 cells/mm³ has been studied in Thailand. Oral itraconazole 200 mg/day can prevent the occurrence of systemic fungal infections, i.e., cryptococcosis and TMI (Chariyalertsak et al. 2002). An observational study found that fluconazole 400 mg/week orally was also effective (Chaiwarith et al. 2011). Currently, primary prophylaxis is recommended for HIV-infected individuals who have CD4+ cell count of <100 cells/mm³ and live in endemic areas (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2013). In patients who are receiving cART and have gained the CD4+ cell count to >100 cells/mm³ for at least 6 months, discontinuation of primary prophylaxis may be considered (Chaiwarith et al. 2007). In HIV-infected patients with TMI who do not receive antiretroviral therapy, half of them may develop relapse within 6 months after discontinuation of successful antifungal treatment (Supparatpinyo et al. 1998).

A double-blind, placebo-controlled trial in Thailand demonstrated that oral itraconazole 200 mg daily reduced relapse rate of TMI from 57% to 0% (Supparatpinyo et al. 1998). Currently, all patients should receive secondary prophylaxis with oral itraconazole 200 mg daily. Again, once the patients receive cART and achieve CD4+ cell count of > 100 cells/mm³ for at least 6 months, secondary prophylaxis can be discontinued (Chaiwarith et al. 2007).

Consideration Regarding Antiretroviral Initiation

Currently, there is no strong evidence to recommend optimal timing to start antiretroviral therapy in HIV-infected patients presenting with TMI. However, as mentioned above, TMI occurring after cART initiation was more commonly an unmasking IRIS than a paradoxical IRIS. Therefore, initiation of cART should not be delayed due to a concern of IRIS. Particularly, in patients with CD4+ cell count of < 50 cells/mm³, cART should be started as soon as possible after initiation of antifungal therapy. For those who have CD4+ cell count of >50 cells/mm³, cART may be delayed until 2 weeks after treatment (Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents 2013).

Prognosis

If left untreated, patients with TMI may have poor clinical outcome. In a study in Vietnam among 513 patients with TMI, the case fatality rate was

17% despite amphotericin B or itraconazole treatment (Le et al. 2011). Among 101 deaths, the median time from admission to death was 2 days (IQR 1–5 days). The prognostic factors for poor clinical outcome included intravenous drug users, shorter history of illness, absence of fever or skin lesions, and dyspnea. The presence of skin lesions was the only factor associated with prompt treatment initiation.

Conclusion

T. marneffeii infection is among the most common opportunistic infections in patients with advanced HIV disease. Although the incidence declined in the era of antiretroviral therapy, some HIV-infected individuals who are unaware of their HIV serostatus, unable to access to antiretroviral treatment, or who have suboptimal HIV treatment responses may suffer from *T. marneffeii* infection. Presumptive diagnosis can be easily made by microscopic examination of the clinical specimens. This disseminated infection has poor prognosis if left untreated. Physician awareness to prompt diagnosis and treatment can reduce morbidity and mortality from this infection.

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Tat Expression and Function

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Definition

HIV-1 hijacks the cellular transcription machinery by expressing the virally encoded transactivator of transcription (Tat) protein. Tat is a 14- to 16-kDa nuclear/nucleolar protein that serves as an essential adaptor for cellular transcription elongation factors and other transcriptional regulators. This RNA-binding protein recognizes a stem-loop structure called transactivation response element (TAR) in the 5' extremity of initiating viral transcripts. The structural plasticity of Tat enables versatile interactions with host proteins, as well as host and viral RNAs, and forms the basis for Tat's critical role in HIV transcription and pathogenesis. No current drug treatment targets Tat, but recent progress in deciphering the structure and modification status of Tat provides novel impetus for drug development that may help tackle HIV infection and latency at the transcriptional level.

Tat Regulates Its Own Expression

When HIV integrates (► [Integration](#)) into the human genome, the proviral cDNA undergoes chromatinization and becomes subject to cellular RNA polymerase II-mediated transcription (► [Transcription \(Initiation, Regulation, Elongation\)](#)), like a human gene. However, basal HIV transcription is not consistently efficient, as the human RNA polymerase II (pol II) complex pauses shortly after the transcription start site and, in the absence of additional elongation signals, yields only so-called short transcripts that are insufficient to support viral replication (Kao et al. 1987). While human genes depend on activation or differentiation signals to recruit transcription elongation factors, HIV encodes its own specialized viral factor called Tat to overcome this block preventing viral transcript elongation. Tat binds avidly to a cellular protein complex, the positive transcription elongation factor b (P-TEFb) (P-TEFb (Cyclin T1, Cdk9), Cofactors and Transcription) – a factor first discovered to play a fundamental role in HIV transcription but now known to regulate a large number of human genes. P-TEFb, composed of cyclin T proteins (cyclin T1, T2A, and T2b with only cyclin T1 supporting Tat transactivation) and the cyclin-dependent kinase 9 (CDK9), promotes transcriptional elongation not only by enhancing the catalytic rate of the polymerase but also by dissociating negative elongation factors that are physically blocking processive transcription. In this way, Tat exploits this host elongation factor by recruiting it to the TAR element for robust stimulation of viral transcription and the completion of a productive viral replication cycle.

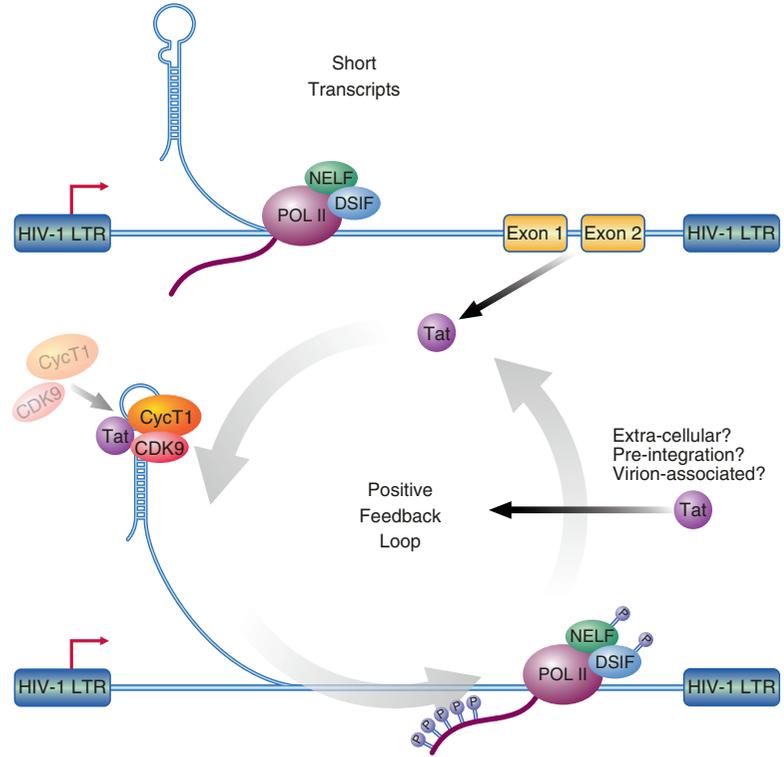
The two phases of HIV-1 transcription have distinct characteristics. The early, Tat-independent phase promotes low-level expression of viral gene products. It is driven by interactions of host transcription factors and *cis*-acting elements located within enhancer and promoter regions in 5' long-terminal repeat (LTR) sequences of the proviral DNA. For example, in the context of activated CD4+ T cells, the preferred target of HIV infection, the cellular transcription factor NF- κ B binds to tandem recognition sites within the HIV promoter

to induce expression of full-length HIV transcripts through Tat-independent recruitment of P-TEFb. However, as this recruitment is transient and less robust than with Tat, this first phase is characterized by a proportionally higher production of short or prematurely terminated transcripts that do not support HIV replication (Fig. 1).

The second Tat-dependent phase promotes high-level viral protein expression. It is driven by adequate levels of the Tat protein generated from full-length viral transcripts produced in the first phase. These transcripts undergo multiple splicing events before being exported to the cytoplasm to produce early viral gene products, such as the viral Nef and Rev (► [HIV-1 Rev Expression and Function](#)) proteins. Importantly, these transcripts also promote production of Tat, which jumpstarts full-length HIV transcript production and fuels persistent expression of Tat in a positive autoregulatory feedback loop (Fig. 1). As a result, the majority of full-length HIV RNA chain assembly, followed by successful viral replication, occurs in the presence of adequate Tat expression and activity. While Tat's role in transcription elongation is well established and the focus of intense studies in the HIV and transcription fields, additional studies point to potential functions of Tat in initiating HIV transcription by recruiting histone-modifying and chromatin-remodeling complexes (► [Histone Acetyl Transferase \(HAT\): CBP/p300, p/CAF, GCN5; and Histone Deacetylase \(HDAC\): YY1](#)) as well as the stimulation of transcription initiation in the absence of TATA-box-binding protein-associated factors (TAFs).

Most models attribute the early expression of Tat to full-length transcript production from integrated proviral DNA as described above, but alternative or complementary models exist. (1) Tat may be expressed before proviral integration from nonintegrated viral DNA, a common feature of HIV and other retroviruses. Tat transcripts have been detected as early as 1-h postinfection in the absence of integration; these may increase the initial pool of Tat proteins fueling early viral transcription. (2) Small amounts of Tat may be incorporated into viral particles and may jumpstart early viral transcription. Support for this model comes from observed defects of Tat-deficient

Tat Expression and Function, Fig. 1 Tat expression is fueled by a positive feedback loop



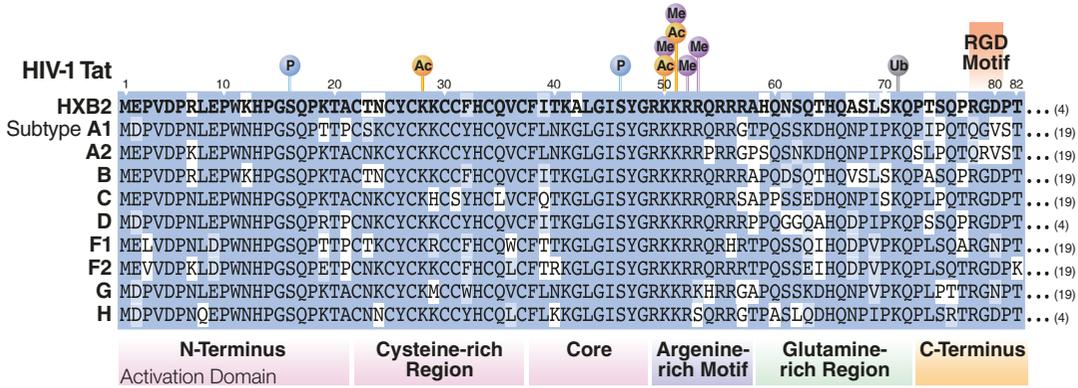
viruses in pre-transcription steps of the viral life cycle, such as reverse transcription (► [Reverse Transcription](#)). Moreover, Tat peptides have been detected by mass spectrometry in highly purified preparations of HIV-1 virions produced by macrophages. However, most studies fail to detect Tat in viral particles. (3) Tat may translocate from neighboring infected cells and activate HIV transcription. Tat contains a protein domain that allows cell membrane penetration, and release of biologically active forms of Tat by infected cells has been reported. While the release and uptake of extracellular Tat has been observed in vitro and may contribute to transcriptional and pathogenic effects of Tat, especially in sanctuaries such as the brain, the quantity and in vivo biological activity of extracellular Tat remain unclear.

Tat Is a Small Versatile Factor with Protein and RNA-Binding Properties

Tat is a small nuclear/nucleolar protein of 72–101 amino acids (aa) that displays a net positive

charge, low hydrophobicity, and low sequence complexity. In the early phases of transcription, a full-length protein (101 aa) is encoded by two *tat* exons and expressed from highly spliced HIV transcripts, whereas later in infection, an additional one-exon form (72 aa) is produced from unspliced transcripts because of a stop codon in the intron after the first *tat* exon. An 86-aa form of Tat is a variant found in the laboratory-adapted strain HXB2 and in subtype D and H Tatts (Fig. 2).

Traditionally, Tat is divided into six conserved regions, based on functional contributions and protein or RNA interactions (Fig. 2). The acidic and proline-rich N-terminus (aa 1–21) is required for LTR transactivation and binds the human transcriptional coactivator and acetyltransferase CREB-binding protein (CBP) (► [Histone Acetyltransferase \(HAT\): CBP/p300, p/CAF, GCN5](#)). The adjacent cysteine-rich region (aa 22–37) features seven clustered cysteine residues, all of which except Cys31 are essential for Tat's transcriptional activity, in a sequence reminiscent to zinc finger motifs common to many human transcription factors. The Tat core (aa 38–48) displays



Tat Expression and Function, Fig. 2 BLOSUM sequence alignment of consensus Tat sequences from HIV-1 group M subtypes A–H, as obtained from

<http://www.hiv.lanl.gov/>. The Tat sequence from the lab-adapted HXB2 isolate is also shown (UniProt ID: P04608)

high sequence conservation among subtypes (Fig. 2) and, with the N-terminus and cysteine-rich region, forms the so-called activation domain (AD, aa 1–48). The Tat AD provides the primary interaction surface for P-TEFb and is essential for the powerful transactivation of the HIV LTR by Tat. In a crystallographic structure of Tat/P-TEFb, the Tat AD extensively interacted with P-TEFb with Tat being almost completely buried into a cleft between the first and second cyclin boxes of cyclin T1 (Tahirov et al 2010). Cyclin T1 is in contact with 88% of the covered Tat surface area; Tat formed more hydrogen bonds with P-TEFb than it did with itself. In addition, the N-terminus of Tat contacted the so-called T loop in CDK9, a part of CDK9 critically regulating its enzymatic activity. These findings support a widely held view that Tat is intrinsically structurally disordered and requires host cofactors for proper folding.

Next to the Tat AD lies the arginine-rich motif (ARM) (aa 49–57), which has general RNA-binding properties and in cooperation with cyclin T1 makes specific contact with TAR RNA. TAR spans positions +1 through +59 of the 5' end of nascent viral transcripts and is thus transcribed irrespective of Tat. TAR spontaneously adopts a stem-loop structure and a three-nucleotide pyrimidine-rich bulge, a unique and invariable secondary structure. Both of these features provide recognition surfaces for the Tat ARM, and cyclin

T1 binds TAR only in complex with Tat. Tat:cyclin T1 binding to TAR is highly cooperative, suggesting that TAR functions to select Tat molecules in complex with P-TEFb. No crystal structure of the HIV Tat ARM in complex with TAR and P-TEFb has been solved so far, but a similar structure of the equine anemia infectious virus (EIAV) Tat protein shows that the retroviral ARM is helical and binds the major groove of the TAR stem-loop (Anand et al. 2008). The N- and C-terminal regions flanking the ARM interact with cyclin T1 to expose the helical ARM for TAR recognition, providing structural basis for cyclin T1 and Tat cooperativity in TAR binding. Importantly, the ARM also serves as a nuclear/nucleolar localization signal and harbors many of the known posttranslational modifications of Tat. Basic residues within the ARM are also essential for the ability of Tat to translocate across intact cell membranes.

The glutamine-rich region of Tat (aa 58–72) is implicated in altering cell fate by inducing T-cell apoptosis, without any specific role in transcription attributed to this region. Similarly, the Tat C-terminus (aa 72–101) has also been implicated in altering T-cell fate and activation. This region is entirely encoded by the second *tat* exon and is thus only present in the full-length form of Tat. Historically, due to extensive studies of the truncated, 86-aa laboratory-strain mutant of Tat, it is likely that the biological activity of the full-length Tat C-terminus is not fully appreciated. The

C-terminus in most strains contains a so-called RGD motif, which can bind surface integrins and, thus, in addition to the ARM, may play a role in the uptake of extracellular Tat.

How Does Tat Deliver Active P-TEFb to Nascent Viral Transcripts?

The tripartite interaction among Tat, P-TEFb, and TAR RNA is at the core of productive HIV transcription (Wei et al. 1998). It is within this trimolecular complex that Tat enacts its leading function – enabling robust elongation of viral transcripts. Owing to the fact that P-TEFb controls transcriptional elongation of many human genes, CDK9 catalytic activity within the cell is tightly regulated. P-TEFb occurs in HeLa cells in roughly equal parts as a small, active complex and as a large, inactive complex, in which CDK9 and cyclin T proteins are sequestered by the small nuclear (sn) RNA 7SK and several accessory proteins termed the 7SK snRNP, which include the CDK9 inhibitors Hexim 1 or Hexim 2, the La-related protein LARP7, and the methylphosphate-capping enzyme MEPCE. CDK9 within this complex is inactive but considered poised for rapid activity after the dissociation of the inhibitory factors. While this mechanism of P-TEFb regulation is well established in tumor cell lines, much less is known about the regulation of P-TEFb activity in non-transformed cells. In resting CD4⁺ T cells, cyclin T1 levels are low due to posttranscriptional regulation via microRNAs (Chiang et al. 2012).

It is mechanistically unclear how Tat delivers active P-TEFb to the site of TAR production. While active P-TEFb associates with the bromodomain-containing protein 4 (BRD4) to activate transcription elongation of cellular genes, BRD4 serves as a cellular competitor of Tat for P-TEFb binding. Tat might extract P-TEFb from its complex with BRD4 to deliver the cyclin T1/CDK9 core complex to the HIV promoter. However, structural similarities between the 7SK snRNP:P-TEFb and the Tat:TAR:P-TEFb complex provide alternative hypotheses. Indeed, a fraction of cellular Tat is found in complex with

7SK snRNA and several snRNP components except Hexim proteins (Sobhian et al. 2010), and the Tat ARM shares remarkable sequence similarity with the RNA-binding domain of Hexim. These findings support a model where Tat dismantles the 7SK snRNP through direct competition with Hexim1 for 7SK snRNA binding. A distinct model proposes that Tat competes with Hexim1 for cyclin T1 binding, as Tat and Hexim1 bind to the same region in cyclin T1 and Tat exerts higher affinity for this site *in vitro*.

Tat may employ one or more of these activation mechanisms with existing or newly forming P-TEFb complexes. Whether inactive P-TEFb complexes engage with chromatin and become locally activated during the transition to productive transcription remains open. Evidence exists that Tat targets the inactive P-TEFb relegated by the 7SK snRNP to the chromatinized provirus, suggesting that Tat liberates P-TEFb at the site of transcription. The synthesis of TAR has a critical role in the ejection of the 7SK snRNP once it is assembled at the viral promoter. In this model, the emergent TAR competitively displaces the 7SK snRNA and snRNP components, thus activating CDK9. The precise role of Tat in this process and the presence of TAR in short transcripts remain open questions. Tat function likely involves a combination of activation mechanisms to adapt HIV transcription to different cellular environments to ensure vigorous high-level HIV transcription.

Tat Binds Cellular Cofactors Other Than P-TEFb

While functional and structural data show that P-TEFb, in conjunction with TAR, is a crucial cofactor for Tat transactivation, several bioinformatic and experimental studies sought to determine the global catalog of human factors that interacts with Tat. Approaches include algorithms designed to extract information from existing literature, large-scale yeast two-hybrid screens, siRNA screens, and tandem affinity purification coupled to mass spectrometry. These studies confirmed reported interaction partners and

revealed novel Tat-interacting factors. The nearly 200 human Tat interaction partners include the ATP-dependent RNA helicase DDX5, the protein deacetylase SIRT1, the protein phosphatase 1G, and the E3 ubiquitin-protein ligase Praja2.

Proteomic approaches identified a novel transcription elongation complex with which Tat associates (He et al. 2010; Sobhian et al. 2010). This so-called super elongation complex (SEC) combines active P-TEFb with another elongation factor PAF1c and several proteins that are known as mixed lineage leukemia fusion partners, including ELL2, AFF4, ENL, and AF9. ELL2 enhances transcriptional elongation by increasing the catalytic rate and preventing the pause by pol II. Several studies reproducibly showed that Tat interacts with the SEC to deliver the elongation complex to the site of viral transcription. In this way, Tat allows for the cooperative activities of P-TEFb, PAF1c, and ELL2 to enhance HIV transcriptional elongation at multiple levels, including both the recruitment and activation of P-TEFb and the assembly and stability of the SEC.

Interestingly, Tat and P-TEFb both have functions beyond elongation and may regulate distinct phases of HIV transcription, such as transcript splicing. The HIV Tat-specific factor 1, necessary for efficient Tat-dependent transcription, was implicated in mRNA splicing through its

interaction with components of the spliceosomal machinery. Similarly, a distinct Tat interaction partner, CA150, affects transcriptional elongation and splicing. P-TEFb itself localizes to nuclear speckles where splicing occurs and interacts with the splicing factor c-Ski-interacting protein, an interaction important for Tat's transcriptional function. These findings and others suggest that Tat regulates HIV transcription at multiple levels.

Posttranslational Modifications Fine-Tune Tat Function

Tat may accommodate interactions with different cofactors in a timely manner through its repertoire of posttranslational modifications. The list of these modifications – clustered mainly in the Tat ARM – is growing (Table 1). Posttranslational modifications add new protein-protein interfaces to Tat and allow for tunable, highly regulated interactions with host cell factors. Certain modifications provide recognition surfaces for specific protein domains, such as bromodomains, which recognize and bind acetyl-lysine residues. Indeed, acetylation of Tat at Lys50 enables interaction of the Tat ARM with the bromodomain of the acetyltransferase PCAF (► [Histone Acetyltransferase \(HAT\): CBP/p300, p/CAF, GCN5](#)), thereby

Tat Expression and Function, Table 1 Tat posttranslational modifications

Modification	Tat residues modified	Enzyme(s) adding	Enzyme(s) removing	Function
Acetylation	K28	PCAF	HDAC6	Enhances interaction among Tat, P-TEFb, and TAR
Acetylation	K50, K51	GCN5, p300	SIRT1	Dissociates TAR from Tat and P-TEFb Generates interaction interface for PCAF bromodomain
Methylation	R52, R53	PRMT6	Unknown	Inhibits formation of Tat:TAR:P-TEFb complex Increases Tat half-life
Methylation	K50, K51	SETDB1	Unknown	Knockdown of SETDB1 enhances Tat-mediated transcription
Methylation	K51	Set7/9	LSD1	Enhances interaction among Tat, P-TEFb, and TAR
Phosphorylation	S62, S68 and T64	PKR	Unknown	Phosphorylation-deficient Tat triple-mutant reduces Tat-mediated transcription
Phosphorylation	S16, S46	Cdk2	Unknown	Phosphorylation-deficient Tat mutant reduces Tat-mediated transcription
Ubiquitination	K71	Hdm2	Unknown	Unclear; does not impact Tat half-life

recruiting the acetyltransferase activity to the site of viral transcription (Mujtaba et al. 2002). In contrast, certain Tat modifications negatively regulate protein-protein and protein-RNA interactions. For example, methylation of Arg52 and Arg53 within the Tat ARM by the protein methyltransferase 6 (PRMT6) interferes with Tat binding to both TAR and P-TEFb.

The functional relevance of some known Tat modifications is unclear. For example, Tat mutants deficient in phosphorylation sites display reduced transactivation capacity, although the mechanism through which phosphorylation enhances transactivation is unknown. Similarly, while Tat is ubiquitinated, and this modification has little effect on the half-life of Tat, the molecular mechanism of how this modification enhances Tat transcriptional activity is not understood.

Is Tat Involved in HIV-1 Latency?

A key role in understanding Tat's involvement in the establishment or maintenance of latency (► [Immunology of Latent HIV Infection](#)) is rooted within the feed-forward mechanism regulating Tat expression (Fig. 1). Viral latency is defined as a state of reversibly nonproductive infection often associated with a transcriptionally silenced integrated provirus in subsets of resting memory CD4⁺ T cells (► [Central Memory CD4⁺ T Cells](#)). These latent reservoirs are found in every infected individual and are widely recognized as premier barriers to eradicating HIV from patients. The persistence of infection requires lifelong treatment with antiretroviral therapy and is associated with shortened life span and numerous HIV-associated comorbidities. While the role of Tat in the establishment, maintenance, and reversal of HIV-1 latency remains under investigation, reports point to levels of Tat expression and activity as central contributors to latency.

In latent cells, HIV LTR activity is expected to be silenced – resulting in a lack of Tat production. Key transcription factors, such as NF-κB and NFAT, are sequestered in the cytoplasm in resting central memory T cells, a critical reservoir for latent HIV infection in patients, and are therefore

unavailable within the nucleus to activate viral transcription. In addition, levels of cyclin T1 are low in resting CD4⁺ T cells, preventing low levels of Tat from effectively transactivating HIV transcription.

Evidence that lack of functional Tat contributes to latency comes from in vitro experiments in which increased intracellular expression of Tat is sufficient to inhibit the establishment of latency and even reactivate most of the latent cell populations tested. Moreover, viruses that were recovered from nonproductively infected CD4⁺ T cells in patients are enriched for Tat variants with attenuated transactivation activity, suggesting that impaired Tat activity contributes to the establishment and/or maintenance of latency. Interestingly, small stochastic fluctuations in Tat gene expression are sufficient to drastically affect the Tat feedback loop and determine whether viral latency or productive infection ensues (Weinberger et al. 2005). Thus, similar to actively infected cells, Tat activity in latent cells auto-regulates its own production or, in this case, the lack of its production; this lack of production contributes critically to the robust silencing of HIV transcription in latency. The precise mechanisms of how Tat function is inactivated in latently infected cells and full-length transcript production is kept low are currently the subject of intense research.

Tat Is an Important Effector of HIV Pathogenesis

In addition to its role in regulating HIV-1 transcription and latency, numerous intracellular and extracellular activities have been attributed to Tat. They include modulating host immune responses, mediating survival and apoptotic processes (► [Lymphocyte Apoptosis](#)), and stimulating cellular growth/proliferation, ultimately ensuring viral persistence and effective viral spread (Table 2). Some of these effects are linked to Tat interaction partners, such as the (NAD⁺)-dependent deacetylase SIRT1. Tat binds avidly to SIRT1 and inhibits SIRT1's catalytic activity, thus preventing the timely deacetylation of

Tat Expression and Function, Table 2 Extra-transcriptional effects of intracellular and extracellular forms of Tat

Functions	Cell-type affected	Mechanisms	Contributions to pathogenesis
Immune activator	T-cells Dendritic cells Monocytes Microglia	Induces expression of pro-inflammatory cytokines: IL-1, IL-6, and TNF- α Increase CCR5, CXCR4, and CD40 expression Binds chemokine receptors: CXCR4, CCR2, and CCR3	Recruits and activates immune cells for propagation of infection
Immune suppressor	T-cells Monocytes	Reduces expression of MHC Class I molecules, IL-2, and NO Induces expression of IL-4 and IL-10 Degrades RON receptor tyrosine kinase	Promotes evasion of immune surveillance
Pro-apoptotic	T-cells Monocytes	Perturbs microtubule dynamics and promotes mitochondria-dependent apoptosis (i.e., Bim) Increases TRAIL, caspase-3, and caspase-8 activity Modulates Fas/FasL system Associates with PTEN and PP2A promoters	Increases susceptibility to infections by weakening immune system
Anti-apoptotic	T-cells Monocytes	Increases Bcl-2 expression Reduces expression of p53	Allows for viral replication Oncogenic
Neurotoxic	Neurons Microglia Astrocytes Endothelial	Alters neuronal calcium flux Activates excitatory amino acid receptors Effects inflammatory and oxidative processes Interferes with Nerve Growth Factor (NGF)	Promotes development of dementia
Growth factor	Kaposi's Sarcoma Endothelial	Stimulates growth of Kaposi's sarcoma cells Activates VEGFR-1, VEGFR-2, and integrins	Oncogenic Angiogenic
Gene regulator	T-cells Monocytes	Activates NF- κ B activity through ROS production, degradation of I κ B- α , interaction with p50-p65 heterodimers, and suppression of SIRT1 deacetylase activity Regulates mRNA capping and splicing Binds and inhibits Dicer function to suppress production of host siRNA	Promotes HIV-1 transcription Enhances pleiotropic cellular effects Subverts cellular defense

downstream SIRT1 targets, such as NF- κ B. As acetylated NF- κ B is transcriptionally more active, this effect of Tat causes increased NF- κ B transcriptional activity and elevated expression of immune activating cytokines that contribute to a state of generalized immune activation (► [Chronic Immune Activation](#)) observed in HIV infection. Increased immune activation is a hallmark of HIV infection directly linked to the progression to AIDS and the unrestricted spread of HIV infection.

Tat also associates with host gene promoters *in vivo*. A genome-wide chromatin immunoprecipitation approach identified ~450 cellular promoters that were occupied by Tat, including the promoters of the phosphatase PTEN and two subunits of the phosphatase PP2A (Kim et al. 2010). This study links Tat with the activation of

phosphoinositide 3-kinase-mediated apoptotic pathways in HIV-infected CD4⁺ T cells, a possible explanation of how Tat contributes to the depletion of CD4⁺ helper T cells (► [CD4+ T Cell Depletion](#)) in patients. Other research has demonstrated the ability of Tat to bind and inhibit the function of the host protein Dicer, an RNase III-like enzyme that processes microRNA (miRNA) and short interfering RNA (siRNA). Because RNA silencing may also serve as a defense mechanism against viral infection, Tat suppression of Dicer's ribonuclease-directed processing activity may allow HIV-1 to more effectively evade cell-based immunity.

The ability of Tat to translocate to neighboring cells theoretically amplifies its pathogenic effect and may be specifically relevant in distinct sanctuaries, such as the central nervous system (CNS).

Before crossing the plasma membrane and accessing the extracellular space, Tat is recruited and inserted into the membrane via its basic ARM in a phosphatidylinositol (4,5) biphosphate-dependent manner. Outside the cell, Tat functions as a promiscuous ligand that binds a number of chemokine and integrin surface receptors on a variety of cell types. The majority of these proposed Tat “receptors” (e.g., lipoprotein receptor-related protein, CXC chemokine receptor type 4 (► [CXCR4, Co-Receptors](#)), and heparin sulfate proteoglycans) are endocytic receptors that may internalize Tat upon binding. In addition, the Tat ARM functions as a protein transduction domain (PTD), a stretch of basic amino acids also found in antennapedia, a *Drosophila* homeodomain transcription factor, and VP22, a herpes simplex virus 1 structural protein, that may allow for spontaneous translocation of Tat across plasma membranes through direct electrostatic interactions with negatively charged phospholipids or through the formation of inverted micelles.

Therapeutic Strategies Surrounding Tat

Because Tat plays essential roles in HIV replication and pathogenesis as well as in protein transduction, it is not surprising that many therapeutic approaches (► [Therapeutic Clinical Trials, Introduction to Evidence and Research](#)) have focused on Tat. These include therapeutic attempts to disrupt Tat transcriptional activity and binding to TAR RNA, a Tat-based vaccine, and the use of Tat peptides as vehicles for cellular delivery of various macromolecules into cells.

A main focus of Tat-based therapy research is small molecules that target the crucial interaction between Tat and TAR RNA, with the promise to halt HIV replication at an early stage. One example is 7-chloro-5-(2-pyrryl)-3H-1,4-benzodiazepine-2(H)-one, which inhibits Tat-dependent transcription in a manner relying on TAR. This compound inhibits replication of HIV-1 and HIV-2. Despite the demonstrated promise for this molecule *in vitro*, a limited therapeutic window and observed toxicity of this drug were issues that preempted its further development. Other examples of

compounds that inhibit the Tat:TAR interface include quinoline derivatives, diphenylfuran derivatives, beta-carboline derivatives, and aminoglycoside-arginine conjugates.

Tat is also considered a promising candidate target for an anti-AIDS vaccine (► [Vaccine Induced Immune Responses; Preventing HIV-1 Transmission Through](#)). Tat is the most conserved HIV protein, thus limiting the risk for the development of resistance. In both primates and humans, cellular and humoral immune responses against Tat are rapid and positively correlated with slower progression to AIDS. Results from recent cross-sectional and longitudinal studies have demonstrated that a Tat vaccine based on the biologically active Tat protein alone is safe and may reduce HIV-1 replication and disease progression.

Since the discovery that the Tat ARM serves as a PTD, research into the use of short peptides mimicking Tat in the cellular uptake of various molecular cargo has dramatically expanded. A key property of these cell-penetrating peptides is that they are cationic and typically enriched in positively charged residues, such as lysines or arginines, as found in the Tat ARM. Peptide fragments encoding tyrosine 47 through arginine 57 of the Tat ARM have been used to transport DNA, proteins, and other materials through the cell membrane and into the cell nucleus. Prominent examples of Tat fusion peptides include a Tat-Bcl-xL fusion to inhibit apoptosis in ischemia, a Tat-p53 fusion to increase the tumor suppressive activity of p53 in tumor cells, and gp91dsTat, which blocks the assembly of the NAD(P)H oxidase complex to alleviate the increase in systolic blood pressure caused by angiotensin II injections. Although the use of Tat peptides holds great promise for gene transfer and drug delivery, current use is limited by a lack of tissue specificity and an incomplete understanding of uptake modes.

Conclusion

Research into Tat biology has been successful in providing an increasing molecular understanding of HIV transcription and latency. Studies of Tat

have fueled important biological discoveries, such as the central role of P-TEFb in transcriptional regulation and the design and usage of Tat-adapted PTDs for the transport of molecular cargo into cells. The “pathogenic” functions of Tat (i.e., in modulating immune activation and cell survival of infected and neighboring cells) have gained molecular shape in part by identifying novel Tat interaction partners. The urgency in better understanding and eliminating viral latency has refocused the field on possible therapeutic interventions targeting HIV transcription. Future pursuits will inevitably need to acknowledge the prominent role of Tat as a central regulator of HIV transcription and address the powerful auto-regulatory feedback loop through which Tat regulates its expression.

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T-Cell Homeostasis

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Definition

During healthy aging, CD4⁺ and CD8⁺ T-cell numbers remain relatively stable, despite a significant decline in thymus output. This T-cell homeostasis is dramatically disturbed during HIV infection, during which naive and memory CD4⁺ and naive CD8⁺ T-cell numbers gradually decline, eventually leading to increased susceptibility to opportunistic infections. In contrast, memory CD8⁺ T-cell numbers are significantly increased in HIV infection. Although the causes of disturbed lymphocyte homeostasis in HIV infection have been subject of much debate, there is a current consensus that the deleterious effects of chronic immune activation play a central role.

During effective combination antiretroviral therapy (cART), CD4⁺ T-cell numbers tend to come back to healthy control levels, albeit very slowly, while memory CD8⁺ T-cell numbers tend to remain elevated for long periods of time. It remains unclear whether active homeostatic mechanisms kick in to reestablish lymphocyte homeostasis or whether the slow reconstitution of the T-cell pools reflects the rate at which lymphocytes are being generated under healthy circumstances.

Pathogenesis

CD4⁺ T-Cell Loss

Acute HIV-1 infection is characterized by a peak of viral load and high levels of immune activation. During this phase, the major part of the memory CD4⁺ T-cell pool in the gut is irreversibly lost (Clayton and Clayton 1997). During the following, chronic stage of infection, CD4⁺ T-cell numbers in the blood gradually decline; both naive and memory CD4⁺ T cells are lost (McCune 2001), while the viral load remains relatively stable. There has been much debate about the exact mechanisms underlying this depletion; factors that have been suggested to be involved vary from direct HIV-induced cytopathicity to immune senescence, thymic impairment, and the deleterious effects of chronic immune activation.

Loss of Virally Infected Cells

In vitro studies in which CD4⁺ T cells were infected in culture with HIV have shown that HIV can directly kill infected cells by inducing apoptosis (Meyaard et al. 1992). As the fraction of productively infected CD4⁺ T cells at any moment in time is low [in the order of 10⁸ out of a total pool of 10¹² CD4⁺ T cells (Clark et al. 1999)], it has been questioned whether HIV-induced cytopathicity by itself could explain the CD4⁺ T-cell loss occurring during HIV infection. In line with this, several studies have shown that the level of in vitro cytopathicity of HIV does not correlate with CD4⁺ T-cell depletion: no difference in CD4⁺ T-cell loss was found between humanized SCID mice infected with non-cytopathic or cytopathic HIV isolates (Mosier et al. 1993), and Simian Immunodeficiency Virus (SIV) isolates that are equally cytopathic to rhesus macaque and sooty mangabey cells in vitro cause CD4⁺ T-cell loss and progression to AIDS in rhesus macaques, while CD4⁺ T-cell numbers in sooty mangabeys are not affected (Silvestri et al. 2003). The CD4⁺ T-cell loss that is characteristic for HIV infection can thus not be explained by the cytopathic effects of productive HIV infection.

Indeed, the number of infected cells in HIV-infected individuals is much smaller than

the number of apoptotic cells, and in fact only a small proportion of productively HIV-infected cells undergo apoptosis (Finkel et al. 1995). T-cell apoptosis in HIV infection is not even restricted to CD4⁺ T cells as the majority of apoptotic T cells express CD8 (Meyaard et al. 1992). The majority of T cells that are lost in HIV infection are thus “bystander” cells, which are not productively infected with HIV themselves. Instead these cells are thought to die as a result of chronic immune activation (see below), or because their refractoriness to HIV infection, leading to the accumulation of incomplete DNA transcripts, triggers an inflammatory form of programmed cell death called pyroptosis (Cooper et al. 2013; Doitsh et al. 2014).

Thymic Involvement: Is There a Lack of CD4⁺ T-Cell Production?

It has been proposed that the gradual loss of CD4⁺ T cells in HIV-infected individuals may not only be due to increased cell death but also to decreased production of new CD4⁺ T cells by the thymus. Experiments in humanized SCID mice (Bonyhadi et al. 1993) and thymic tissue from HIV-1 infected neonates (Rosenzweig et al. 1993) have shown that HIV can infect thymocytes, leading to decreased CD4:CD8 thymocyte ratios, and thymic biopsies from individuals who died of AIDS show signs of severe thymocyte loss (Haynes et al. 1999). It remains unclear, however, to what extent HIV infection of the thymus contributes to the gradual loss of CD4⁺ T cells during asymptomatic infection, especially in adults.

In HIV-infected human adults, T-cell receptor excision circles (TRECs) have been measured to study the in vivo effects of HIV on thymic output (Douek et al. 1998). TRECs are formed through the deletion of the TCR-D locus during gene rearrangement of the TCR α -chain and are not copied during cell division. Therefore, they are produced only upon de novo T-cell formation in the thymus. In HIV-1 infected individuals, the average number of TRECs per T cell (referred to as the average “TREC content”) of naive CD4⁺ and CD8⁺ T cells is significantly lower than in age-matched healthy controls, suggestive of reduced thymic output in HIV-1 infection

(Douek et al. 1998). However, mathematical modeling pointed out that the decreased average TREC content of the naive T-cell population in HIV-infected individuals is much more likely due to the increased rates of T-cell division that are typically observed in HIV-infected individuals than to changes in thymic output (Hazenberg et al. 2000a). In asymptomatic HIV-infected adults, there is thus no evidence that thymic output is reduced.

In the light of the well-known process of thymic involution during aging, one may even wonder how much effect thymic impairment could have on lymphocyte homeostasis in human adults. The contribution of thymic output to the maintenance of the peripheral T-cell pool is a topic of much debate. Recent studies in healthy adults have pointed out that the contribution of thymic output to peripheral naive T-cell maintenance is small (Braber et al. 2012): while an estimated 10% of newly generated naive T cells in adults is generated by the thymus, the other 90% are produced by peripheral naive T-cell division. This suggests that even total abrogation of thymic function by HIV would not lead to significant changes in peripheral T-cell numbers in adults. In fact even in children, in whom thymic function is optimal, peripheral T-cell division contributes significantly to peripheral T-cell homeostasis (Hazenberg et al. 2004). Despite the numerous analogies between the mouse and human immune system, the central contribution of the thymus to the maintenance of the peripheral naive T-cell pool is thus quite specific for mice, and its role in human lymphocyte homeostasis tends to be exaggerated (Braber et al. 2012).

Increased T-Cell Turnover

Quantification of T-cell turnover using deuterium or BrdU labeling has revealed that the rate at which T cells are produced in HIV infection and in SIV-infected macaques by far exceeds that in healthy controls (Mohri et al. 2001; de Boer et al. 2003). The observed negative correlation between CD4⁺ T-cell numbers and their rate of production has raised the idea that the increased CD4⁺ T-cell turnover represents a homeostatic response to the gradual loss of CD4⁺ T cells, leading to

accelerated aging and exhaustion of the regenerative capacity of the immune system, eventually resulting in severe CD4⁺ T-cell depletion (Ho et al. 1995). A proposed mechanism of immune senescence would be telomere erosion through extensive T-cell proliferation. Telomeres – the unique structures at the end of chromosomes consisting of tandem DNA repeats – are known to shorten upon cell division and thereby provide a record of the replicative history of cells. Rather remarkably, in HIV infection CD8⁺ but not CD4⁺ T-cell telomere lengths are reduced, suggesting that – if anything – the CD8⁺ and not the CD4⁺ T-cell pool becomes exhausted (Wolthers et al. 1996).

Increased rates of T-cell production have long been thought to contribute to T-cell homeostasis and to help sustain clinical latency in HIV-infected individuals. Studies in humans and monkeys strongly suggest, however, that increased T-cell proliferation in HIV infection does not reflect a homeostatic response to the loss of CD4⁺ T cells. It turned out that division of CD4⁺ T cells in HIV-infected patients declines dramatically and rapidly after the start of combination antiretroviral therapy (cART), long before CD4⁺ T-cell counts in the blood have normalized (Hazenberg et al. 2000b). The majority of T cells proliferating in untreated HIV infection thus seem to respond to the virus, which decreases rapidly upon the start of cART, rather than to the lack of CD4⁺ T cells (Lempicki et al. 2000).

These findings have put the correlation between low CD4⁺ T-cell numbers and high levels of T-cell proliferation in a different light and have suggested that high levels of immune activation and the resulting T-cell proliferation may even contribute to CD4⁺ T-cell depletion. Indeed, immune activation turned out to be one of the strongest prognostic markers for HIV disease progression, even stronger than, and independent of, HIV viral load (Giorgi et al. 1999; Hazenberg et al. 2003). Interestingly, even levels of immune activation *before* seroconversion are predictive for disease progression. In a study among homosexual men, low CD4⁺ T-cell counts and high CD70 expression by CD4⁺ T cells before HIV seroconversion were associated with fast disease

progression upon HIV infection (Hazenberg et al. 2003). Taken together, these studies have emphasized the deleterious effects of chronic immune activation and the resulting high levels of T-cell proliferation in HIV infection.

Lessons from Nonhuman Primates

Studies in nonhuman primates have further substantiated the central role of immune activation in HIV pathogenesis and have shown that in the absence of chronic immune activation, irrespective of viral load, HIV infection does not lead to AIDS. In SIV-infected sooty mangabeys, who remain healthy despite high SIV load, T-cell turnover rates are normal (Chakrabarti et al. 2000), while in pathogenic SIV infection in rhesus macaques, high levels of immune activation are associated with progression to AIDS. Absence of disease progression in sooty mangabeys cannot be explained by low viral loads, strong cytotoxic T-cell or neutralizing antibody responses, or reduced cytopathicity of SIV for sooty mangabey CD4⁺ T cells, while in rhesus macaques, progressive CD4⁺ T-cell decline and AIDS develop despite strong and ongoing immune responses to SIV (Silvestri et al. 2003). Since the dynamics of virus and virus-infected CD4⁺ T cells in these animal models of SIV infection are also comparable, excessive indirect activation-induced bystander killing of T cells in rhesus macaques seems to be the major pathological difference (Rey-Cuille et al. 1998; Chakrabarti et al. 2000; Silvestri et al. 2003). Viral replication is thus required in HIV pathogenesis but is insufficient to cause disease. It is the immune activation that is induced by the virus that is driving the eventual CD4⁺ T-cell loss and disease progression.

The Cycle Between T-Cell Activation and Death

The major cause of chronic immune activation in HIV-1 infection is currently topic of intense investigation (Miedema et al. 2013). Levels of bacterial products such as LPS in the circulation appear to be increased in HIV-infected individuals, as a consequence of the rapid and drastic loss of CD4⁺ T cells from the gut, leading to translocation of bacterial products and thereby to chronic

activation of the immune system (Brenchley et al. 2006). HIV has also been shown to trigger immune cells more directly, through activation of the innate immune system via Toll-like receptors (TLR) 7 and 8, which recognize single-stranded HIV RNA (Meier et al. 2007). The consequence of this chronic immune activation may in part be the natural process of apoptosis of activated T cells. Indeed, some situations of chronic immune activation independent of HIV infection show that chronic immune activation by itself can induce T-cell loss. In transgenic mice that constitutively express CD70 on the cell surface of B lymphocytes, antigen-activated T cells are continuously co-stimulated via interaction with CD27 on the T-cell surface. Such mice develop a phenotype reminiscent of HIV infection, in that their peripheral T-cell pool becomes progressively depleted, eventually leading to death by opportunistic infections (Tesselaar et al. 2003). Similarly, in healthy Ethiopians increased levels of immune activation, probably caused by common parasitic infections, are related to low CD4⁺ T-cell counts, low percentages of CD4⁺ and CD8⁺ naive T cells, and decreased TREC contents, all characteristics reminiscent of HIV infection. More recently, it has been shown that abortive HIV infection of CD4⁺ T cells can lead to caspase-1 mediated pyroptosis, an inflammatory form of programmed cell death (Doitsh et al. 2014). Since pyroptotic cell death leads to the release of cytoplasmic contents and pro-inflammatory cytokines, this may drive a vicious circle between cell death and immune activation.

CD8⁺ T-Cell Changes

The effects of HIV on immune homeostasis are not confined to CD4⁺ T cells. In fact also the CD8⁺ T-cell pool undergoes major changes in HIV infection. CD8⁺ T cells typically show increased levels of activation, proliferation, and cell death, just like CD4⁺ T cells, but their numbers are typically markedly increased in HIV-infected individuals. Moreover, telomere shortening and TREC dilution are more pronounced in CD8⁺ T cells than in CD4⁺ T cells. In response to HIV infection, there is a massive peripheral expansion of the effector/memory CD8⁺ T-cell pool, while

naive CD8⁺ T cells start to decline shortly after infection (Roederer et al. 1995). The effector/memory CD8 subset only begins to decline at the AIDS stage (Margolick et al. 1995). The fact that telomeres of CD8⁺ T cells are shortened in HIV infection, while those of CD4⁺ T cells are not, suggests that in contrast to CD4⁺ T cells, CD8⁺ T cells that have divided tend to survive and thereby cause the increased CD8⁺ T-cell numbers that are typical for HIV-infected individuals.

Immune Reconstitution During cART

Although there is little evidence that active homeostatic mechanisms are called into action during untreated HIV infection, cell numbers tend to normalize on effective treatment, albeit very slowly. The reconstitution of the CD4⁺ T-cell population can be divided into two phases. During the first 2–3 months following therapy initiation, the number of CD4⁺ T cells in the blood increases rapidly due to redistribution of memory T cells from the tissues and the lymph nodes to the blood (Pakker et al. 1998). The second phase of reconstitution takes much longer and involves the renewal of the naive T-cell pool. CD4⁺ T-cell numbers typically steadily increase during cART (Hunt et al. 2003a), and under sustained viral suppression and if CD4⁺ T-cell numbers at the start of therapy are not too low, the immune system has the potential to fully recover the CD4⁺ T-cell pool without any signs of accelerated immunological aging within 7 years of cART (Vrisekoop et al. 2008).

On the basis of experiments in mice, it is generally assumed that homeostatic mechanisms are called into action when the lymphocyte pool becomes severely depleted, thereby helping to reestablish lymphocyte homeostasis. Although T-cell proliferation levels clearly decline after initiation of cART, they generally remain higher than in healthy age-matched controls and could in principle reflect homeostatically increased levels of T-cell proliferation due to a long-term lack of CD4⁺ T cells. Increased T-cell proliferation levels during cART are, however, generally related to residual immune activation, which itself is

associated with poor immune reconstitution on cART (Hunt et al. 2003b), again confirming the central deleterious role of immune activation in HIV infection. Alternatively, recovery of thymic output during cART may contribute to the recovery of the CD4⁺ T-cell pool, especially in children. Indeed, thymic tissue in HIV-infected children has been shown to increase with time on cART, eventually reaching levels comparable to age-matched healthy controls (Sandgaard et al. 2014). In HIV-infected adults, thymic size, as measured by chest computed tomography (CT) scans, was found to correlate with CD4⁺ T-cell reconstitution on cART (Teixeira et al. 2001). There is discussion whether thymic output could even reach supranormal levels in lymphopenic circumstances to compensate for the lack of peripheral T cells, a phenomenon known as thymic rebound. Although average naive TREC contents in lymphopenic patients can indeed reach supranormal levels (Douek et al. 2000), it is important to realize that a handful of TREC⁺ T cells that migrate from the thymus into a virtually empty peripheral T-cell pool are expected to cause a rapid increase in the average TREC content of the peripheral naive T-cell pool which may reach supranormal levels even in the absence of thymic rebound (Hazenberg et al. 2002). Although T-cell homeostasis is thus generally reestablished after years of effective cART, up till now there is no evidence that this is due to active homeostatic mechanisms that are called into action to increase the generation of naive or memory T cells.

Conclusion

During HIV infection, T-cell homeostasis of both CD4⁺ and CD8⁺ T cells is dramatically disturbed. Although the cause of the CD4⁺ T-cell loss is not fully understood, it is clear that chronic immune activation plays a central role. Both direct activation of the innate immune system by HIV and bacterial translocation due to the severe loss of CD4⁺ T cells from the gut are thought to be important drivers of immune activation in HIV infection. Death of abortively HIV-infected cells is thought to add to the increased levels of

immune activation, through release of cytoplasmic products and inflammatory signals thereby creating a vicious cycle between chronic activation and cell death. During effective long-term cART, T-cell homeostasis is reestablished, albeit very slowly. It remains unclear whether this normalization reflects the normal slow production of T cells under healthy circumstances or whether the body manages to compensate for the loss of CD4⁺ T cells through increased T-cell production.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [Immunological Responses to Antiretroviral Therapy](#)
- ▶ [Lymphocyte Apoptosis](#)
- ▶ [Overview: Immunopathogenesis](#)
- ▶ [Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense](#)
- ▶ [SIVmac Infection of Macaques, Immunopathogenesis of](#)
- ▶ [Thymic Function](#)

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Th17 Cells

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Definition

Th17 cells are a distinct functional CD4+ T cell subset, characterized by their expression of the master transcription factor ROR γ t and secretion of interleukin-17 (IL-17). These cells are found predominantly in the gastrointestinal tract (GI tract) and act there to protect the host against extracellular bacteria and fungi. Th17 cells further contribute to mucosal immunity by promoting the regeneration of gut epithelial cells, the maintenance of tight junctions, and the production of antimicrobial peptides. Th17 cells have also been implicated in driving inflammation during certain autoimmune conditions.

Introduction of CD4+ T Cell Subsets

Naïve CD4+ T cells, upon TCR stimulation and costimulation by antigen-presenting cells (APCs), differentiate into distinct CD4+ helper T cell subsets, characterized by their unique cytokine profiles, transcription factors, and different effector functions. The original paradigm for CD4+ T cell differentiation identified two polarized subsets in both mice and humans: Type 1(Th1) and Type 2(Th2) cells. Th1 cells express the transcription factor T-bet, produce IFN- γ , and are primarily devoted to protection against intracellular pathogens. Th2 cells, which express the transcription factor GATA-3, secrete IL-4, -5, -9, and -13,

and are involved in host protection against parasitic helminthes, but can also be involved in allergic disorders (Abbas et al. 1996; Romagnani 1997). However, this paradigm shifted upon the discovery of additional Th subsets that were able to produce other cytokines and possessed functions distinct from Th1 and Th2 cells.

Th17 cells, a third subset of effector Th cells, were first identified through mouse models of autoimmunity. Originally, self-reactive Th1 responses were proposed to drive disease development in two autoimmune mouse models, experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA), based on the absence of disease development upon neutralizing IL-12p40, a cytokine critical for driving Th1 polarization (Gately et al. 1998). However, following the later discovery that the cytokine IL-23 shares the p40 subunit with IL-12, it was revealed that the development of EAE and CIA was dependent on IL-23, a cytokine that expands IL-17-producing T cells (Cua et al. 2003). Since then, the potent inflammatory properties of Th17 cells have been shown to play a critical role not only in autoimmune pathology but also in antibacterial immunity.

Th17 Phenotype and Function

Th17 cells are a subset of memory CD4⁺ T cells that are enriched in mucosal tissues and preferentially produce IL-17, in addition to IL-21, IL-22, TNF- α , and IL-2. The differentiation of Th17 cells, in humans, requires a combination of cytokines, including IL-6, transforming growth factor- β (TGF- β), IL-21 and IL-1 β , and later IL-23 for their expansion and survival (Paiardini 2010). Their differentiation is further regulated by the expression of the master transcription factor retinoic acid-related orphan receptor (ROR) γ t. In fact, overexpression of RORc, the gene which encodes ROR γ t, in human naïve T cells induces IL-17A and IL-17F production, while concurrently decreasing production of IFN- γ . Phenotypically, Th17 cells are characterized by a myriad of surface markers, none of which exclusively define this effector Th subset alone. Human Th17 cells

express the IL-23 receptor (IL-23R); the chemokine receptors CCR6, CCR4, and CCR2; and the C-type lectin receptor CD161. The enrichment of these chemokine receptors directs the preferential migration of Th17 cells to mucosal tissues, which express chemokines including CCL20 (the ligand for CCR6) during periods of chronic inflammation. Additionally, human Th17 cells have been found to originate from a small population of naïve CD4⁺ CD161⁺ T cells found in the umbilical cord blood and thymus and retain the expression of CD161 upon Th17 lineage commitment (Annunziato et al. 2012).

Th17 cells secrete IL-17, which plays a critical role in the clearance of extracellular bacteria and fungi. IL-17 induces the expression of cytokines (IL-6, GM-CSF, TNF- α), chemokines (CXCL1, CXCL8, CXCL10), and metalloproteinases from epithelial and endothelial cells, which coordinate the recruitment, activation, and migration of neutrophils (Korn et al. 2009). In addition to IL-17, Th17 cells produce several other proinflammatory cytokines, including IL-21, IL-22, TNF- α , and IL-2 (Klatt and Brenchley 2010). IL-21 acts on Th17 cells in an autocrine fashion to amplify Th17 responses, regulates long-term maintenance of functional CD8⁺ T cells, and stimulates the generation of memory B cells and antibody-producing plasma cells. IL-22 is able to drive the production of antimicrobial peptides, such as β -defensins, further supporting the role of this effector cell subset in host defense (Liang et al. 2006). To complement their role in host protection against infections, Th17 cells are also critical in the maintenance of mucosal structural integrity. IL-17 and IL-22 contribute to the integrity of the intestinal barrier by promoting enterocyte proliferation (Kim et al. 2012), inducing the expression of claudins (critical in the formation of intestinal tight junctions) (Kinugasa et al. 2000), and stimulating mucin expression (Sugimoto et al. 2008). These protective roles of Th17 cells have been demonstrated in vivo in several infection models, wherein the absence of IL-17 and other Th17 family cytokines is associated with increased susceptibility to disease and bacterial/fungal burden (Korn et al. 2009). Many of these bacterial species represent those responsible for opportunistic

infections in the setting of HIV/AIDS. However, Th17 cells are not specific for viruses, including HIV, CMV, or influenza, as evidenced by a lack of IL-17 production following stimulation of CD4⁺ T cells with viral antigens (Brenchley et al. 2008).

Role of Th17 Cells in Pathogenic SIV/HIV Infection

HIV and SIV infections are characterized by massive dysregulation of the host immune system. During the acute phase of infection, there is massive depletion of mucosal CD4⁺ T cells. The majority of CD4⁺ T cells infiltrating the mucosa are memory CD4⁺ T cells that express the primary coreceptor for HIV/SIV, CCR5, thus making them preferential targets for viral replication and depletion. It is well established that this robust depletion of mucosal CD4⁺ T cells results in structural and immunological damages of the mucosa. As a result, microbial translocation from the gut into the peripheral blood occurs, which is associated not only with HIV disease progression but also with chronic immune activation (Brenchley et al. 2006). Yet, the loss of mucosal CD4⁺ T cells alone cannot explain these pathogenic phenomena, since SIV natural hosts, such as sooty mangabeys (SMs) and African green monkeys (AGMs), do not progress to AIDS despite a significant depletion of CD4⁺ T cells from mucosal sites (Gordon et al. 2007; Pandrea et al. 2007). Consequently, the regulation of specific CD4⁺ T cell subsets was compared between pathogenic and nonpathogenic SIV/HIV infections to best understand the events responsible for disease progression and chronic immune activation. One critical manifestation of the mucosal disruption that occurred was the preferential loss of Th17 cells during pathogenic SIV and HIV infections.

While an initial description of Th17 cell dynamics during HIV infection found a significant increase in the blood of HIV-infected individuals compared to uninfected, additional studies have generated conflicting results, demonstrating both a maintenance as well as a depletion of Th17 cells from the peripheral blood after infection (Maek-A-Nantawat et al. 2007; Macal et al. 2008; Favre

et al. 2010). However, Th17 cells are enriched in the gastrointestinal tract (GI) compared to the blood, and therefore, subsequent efforts focused on evaluating their maintenance in the mucosa following infection. Th17 cells were found to be depleted throughout the entire GI tract of HIV-infected patients. This Th17 cell loss is specific to mucosal tissues and is not seen in the blood or bronchoalveolar lavage (BAL) of HIV-infected individuals (Brenchley et al. 2008). Consistent with these findings, additional studies have demonstrated that both IL-21-producing and IL-22-producing CD4⁺ T cells are lost from the mucosa during pathogenic SIV infection of RMs (Klatt et al. 2012; Micci et al. 2012). The loss of IL-17-producing lymphocytes is associated with epithelial damage of the colon, suggesting that a preferential loss of Th17 cells likely contributes to microbial translocation in the setting of HIV infection. Moreover, in addition to a loss in Th17 cell number during HIV infection, recent work has demonstrated that mucosal Th17 cells are dramatically less functional in their ability to co-secrete IL-22, IFN- γ , and TNF- α from the earliest stages of HIV infection (Kim et al. 2013).

The mechanisms responsible for the loss of Th17 cells during pathogenic SIV and HIV infections have been extensively explored. Th17 cells express the HIV/SIV coreceptor, CCR5, although its expression appears to be tissue-specific. Blood Th17 cells express low levels of CCR5, while Th17 cells in the mucosa are predominantly CCR5-positive, thus making them highly susceptible targets of HIV/SIV infection (Brenchley et al. 2008). However, despite low CCR5 levels, *in vitro* studies have found that Th17 cells from peripheral blood are highly permissive to HIV infection, compared to Th1 cells, although these findings have been difficult to recapitulate *in vivo* (El Hed et al. 2010; Gosselin et al. 2010; Alvarez et al. 2013). Nevertheless, it is less certain whether this increased susceptibility *in vitro* reflects the mechanism by which mucosal Th17 cells are depleted. For example, no correlation was found between levels of plasma viral load and the frequencies of IL-17-producing lymphocytes in the mucosa of SIV-infected RMs, which suggests that viral replication alone is unlikely to be responsible

for the preferential depletion of Th17 cells from the GI tract (Klatt et al. 2012). Investigations of a potential impact of HIV infection on the proliferative capacity of Th17 cells similarly revealed no differences from Th1 cells, illustrating that Th17 cells are not more susceptible to activation-induced cell death (Brenchley et al. 2008). Th17 cells were also found to express comparable levels of Bcl-2, an antiapoptotic molecule, to Th1 cells. Apart from a direct impact of HIV on Th17 cells, one mechanism that may contribute to the preferential loss of Th17 cells is the loss of CD103⁺ dendritic cells (DCs) from the colon and mesenteric lymph nodes following infection, as was demonstrated in SIV-infected RMs. CD103⁺ DCs have been shown to promote Th17 differentiation of naïve CD4⁺ T cells in vitro, and their levels are positively correlated with levels of Th17 cells in vivo (Klatt et al. 2012). In addition, intestinal IL-21-producing lymphocytes are significantly depleted during chronic SIV infection of RMs, which is associated with the loss of Th17 cells (Micci et al. 2012). This finding suggests that the loss of cytokines that are critical for the generation and maintenance of Th17 cells during SIV/HIV infection may also contribute to the preferential depletion of mucosal Th17 cells. Consistent with this model, the administration of recombinant IL-21 to SIV-infected macaques results in a transient increase in mucosal Th17 cell frequencies (Pallikkuth et al. 2013). Therefore, the depletion of Th17 cells is likely influenced by a dysregulation in the priming environment upon HIV/SIV infection, although additional mechanisms that may contribute to this preferential depletion are continually being explored.

The extent of Th17 cell depletion has been shown to be predictive of chronic immune activation and disease progression in HIV-infected individuals (Favre et al. 2010). Interestingly, this loss of Th17 cells during pathogenic SIV/HIV infection is accompanied by an increase in the frequencies of regulatory T cells (Tregs) (Favre et al. 2009). Th17 and Tregs, two distinct effector CD4⁺ T cell subsets, derive from a common progenitor and share reciprocal maturation pathways based on the cytokine milieu. In contrast to Th17

cells, Tregs generally function to suppress T cell responses. However, the expansion of Tregs during HIV infection is insufficient to dampen chronic immune activation and may instead be accelerating disease progression through the suppression of antiviral T cell responses. In fact, the loss of balance between Th17 cells and Tregs is predictive of generalized T cell activation in acute HIV infection, as measured by the frequency of activated CD8⁺ T cells (Chevalier et al. 2013). The importance of this delicate balance in resisting disease progression is further illustrated by the fact that elite controllers, a group of HIV-infected individuals who spontaneously (without antiretroviral therapy) control plasma viral load at undetectable levels, maintain a ratio of Th17 cells to Tregs similar to that seen in uninfected individuals (Favre et al. 2010). Pre-existing levels of Th17 cells may also predict the severity of HIV/SIV pathogenesis, since it was shown that RMs with larger blood and intestinal Th17 compartments prior to SIV infection had lower peak and set-point viral loads following infection than those starting with smaller compartments (Hartigan-O'Connor et al. 2012).

Despite an unclear understanding of the mechanisms responsible for their loss, it is clear that Th17 cells are preferentially depleted from the mucosal tract during pathogenic HIV/SIV infections of humans and nonhuman primates. The loss of this critical cell subset disrupts mucosal immunity, impacting the integrity of the intestinal epithelium that can then lead to microbial translocation and systemic immune activation. Thus, maintaining Th17 cells represents a key feature for preserving mucosal immunity during HIV/SIV infection.

Th17 Cells in Nonprogressive SIV/HIV Infection

In direct contrast to HIV-infected humans, natural SIV hosts, such as sooty mangabeys (SMs) and African green monkeys (AGMs), generally preserve healthy CD4⁺ T cell levels and avoid generalized immune activation, thus remaining AIDS-free. Studying features which distinguish

the host response to SIV in these natural hosts from those in pathogenic SIV infection of rhesus (RMs) and pigtail (PTMs) macaques has helped elucidate mechanisms of AIDS pathogenesis in humans. As was stated previously, HIV pathogenesis has long been associated with gastrointestinal pathology, characterized by mucosal CD4⁺ T cell loss, structural damage, and microbial translocation. Similarly, SIV infections in natural host SMs and AGMs result in a depletion of mucosal CD4⁺ T cells. However, the lack of microbial translocation in these natural hosts following SIV infection suggested that a loss of CD4⁺ T cells, alone, was not sufficient to trigger the loss of mucosal integrity, microbial translocation, and systemic immune activation present in progressive disease models (Paiardini et al. 2009). Because of the proposed role of Th17 cells at mucosal sites in antibacterial immunity, several groups investigated the frequencies of Th17 cells during SIV infection in natural hosts.

Th17 cells in SMs and AGMs were first found to have a similar phenotype as those found in both RMs and humans, with the majority being memory CD4⁺ T cells expressing IL-23R and CCR6 (Brenchley et al. 2008; Favre et al. 2009). Functionally, these Th17 cells were of a distinct lineage, expressing IL-17, TNF- α , and IL-2, but with minimal production of IFN- γ , IL-4, IL-10, or TGF- β , which validated that these cells were the same as those lost during pathogenic SIV infection. However, examination of the frequencies of Th17 cells in the GI tract of SIV-infected SMs and AGMs revealed that this cell subset was maintained in the mucosa of natural hosts, despite an overall loss of mucosal CD4⁺ T cells. The sampling of additional anatomical sites in AGMs demonstrated that Th17 cells were preserved in the spleen, axillary and iliac lymph nodes, and colon following SIV infection, which was unseen upon pathogenic SIV infection in PTMs. These findings illustrate that the maintenance of Th17 cells in the GI tract of natural hosts for SIV is a feature critical for resisting disease progression; in fact, these findings are consistent with data obtained through investigations of Th17 levels in nonprogressive HIV infections in humans (Brenchley et al. 2008; Favre et al. 2009).

Two groups of HIV-infected individuals have been the center of numerous investigations into the mechanisms of AIDS pathogenesis due to their lack of disease progression and improved clinical outcomes – elite controllers (ECs) and long-term nonprogressors (LTNPs). ECs are able to spontaneously control HIV-1 replication below the clinical level of detection (<50 HIV-1 RNA copies/mL of plasma) without antiretroviral therapy (ART). LTNPs, on the other hand, represent a cohort of individuals who are able to preserve CD4⁺ T cell levels in the absence of ART. While most have low viremia, a rare subpopulation of LTNPs also exhibit high viral loads, similar to the manifestation of SIV in natural host SMs and AGMs. Investigations into the frequencies of Th17 cells after HIV-1 infection in these two cohorts have reached similar conclusions as those found in comparative SIV studies. Elite controllers are able to preserve a ratio of Th17 cells to Tregs at levels seen in uninfected individuals, both in the peripheral blood and the mucosal tract, which is lost in HIV-infected non-controllers (Favre et al. 2010). Furthermore, LTNPs have higher levels of Th17 cells than normal progressors in the blood and are able to preserve levels of Th17 cells in the mucosa that are comparable to those found in uninfected individuals (Cicccone et al. 2011; Salgado et al. 2011).

By maintaining their frequencies of Th17 cells in the mucosal tract, SIV natural hosts and HIV-infected nonprogressors are able to preserve mucosal immunity and avoid pathogenic events, such as microbial translocation and chronic immune activation, even when viral replication is high. Continuing investigations into the mechanisms which allow for the preservation of this critical cell subset in natural hosts, but their loss in pathogenic infection, will be invaluable for better understanding AIDS pathogenesis and designing therapeutic interventions that can promote sustained mucosal immunity.

Th17 Cells During Antiretroviral Therapy

Highly active antiretroviral therapy (HAART) successfully suppresses viral replication in the

majority of HIV-infected individuals and restores circulating CD4⁺ T cell levels to near-normal levels, thus representing a major advancement in the treatment of HIV disease. While HAART initiation has dramatic effects on CD4⁺ T cell restoration in the blood, HAART has been less efficacious in the reconstitution of mucosal CD4⁺ T cells that are lost early in primary HIV infection. Instead, HAART results in a delayed and often incomplete restoration of gut mucosal CD4⁺ T cells (Guadalupe et al. 2006). Nevertheless, numerous groups have demonstrated that restoration of mucosal CD4⁺ T cells is possible with HAART, albeit with varying degrees of reconstitution. This variability in the levels of mucosal CD4⁺ T cell reconstitution has been hypothesized to be an effect of the timing of HAART initiation, with earlier initiation supporting a more complete restoration of mucosal CD4⁺ T cells, although this has not been formally proven.

Even less is known about the ability of HAART to reconstitute levels of Th17 cells in the GI tract of HIV-infected individuals. In a cross-sectional study in which HAART-treated patients had modest to high levels of overall CD4⁺ T cell restoration in the mucosal tract, Th17 cells were found at frequencies similar to, or even greater than, those found in uninfected individuals (Macal et al. 2008). This reconstitution of mucosal CD4⁺ T cells and concomitant accumulation of Th17 cells was found to be associated with increased polyfunctional anti-HIV CD4⁺ and CD8⁺ T cell responses. In another study, HAART was found to not only increase the frequency of mucosal Th17 cells in HIV-infected patients but also to restore the polyfunctionality of this critical cell subset (Kim et al. 2013). However, the recovery of Th17 functionality was only seen after prolonged periods of HAART, suggesting that the length of HAART impacts the degree to which mucosal immunity is restored. Nevertheless, additional studies have found variable and incomplete reconstitution of mucosal Th17 cells despite viral suppression by HAART (Chege et al. 2011). This variability may be related to the degree of overall CD4⁺ T cell restoration. Despite this proposed reconstitution

of Th17 cells, though, the majority of studies have been unable to demonstrate the ability of HAART to fully decrease plasma LPS levels, a common indicator of microbial translocation, to baseline levels reported in healthy individuals.

Collectively, these studies indicate that HAART positively impacts CD4⁺ T cell numbers in the mucosa. Furthermore, HAART is able to specifically restore mucosal Th17 cells in HIV-infected patients following their preferential depletion during acute infection. However, the degree of reconstitution of both total CD4⁺ and Th17 cells varies per individual. Longitudinal studies that examine the kinetics of Th17 levels in the mucosa will be critical to validate these findings, as well as identify the key factors which contribute to the level of mucosal Th17 cell, and overall CD4⁺ T cell, reconstitution.

Conclusion

Th17 cells, a CD4⁺ T cell subset enriched in the GI tract and chiefly involved in antibacterial immunity, are preferentially depleted from the mucosal tract of SIV-infected macaques and HIV-infected humans. The depletion of this critical effector CD4⁺ T cell subset is associated with the loss of intestinal integrity, which may ultimately facilitate microbial translocation and systemic immune activation. The impact of this Th17 cell loss on disease pathogenesis is further underlined by the fact that mucosal Th17 cells are preserved during SIV infection of natural host sooty mangabeys and African green monkeys, two species which are resistant to AIDS pathogenesis. Therefore, therapeutic interventions that aim to maintain and restore Th17 cells, and thus restore mucosal immunity, will be critical in slowing HIV disease progression. HAART has been able to partially restore CD4⁺ T cell levels, including Th17 cells, following long-term viral suppression; however, Th17 cell reconstitution is highly variable and further investigations will be necessary to better understand what factors are needed for full mucosal restoration. While gaps remain regarding the mechanisms of their loss and reconstitution, it is undeniable that Th17 cells are

crucial for the maintenance of mucosal immunity and resisting HIV disease progression.

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Thymic Function

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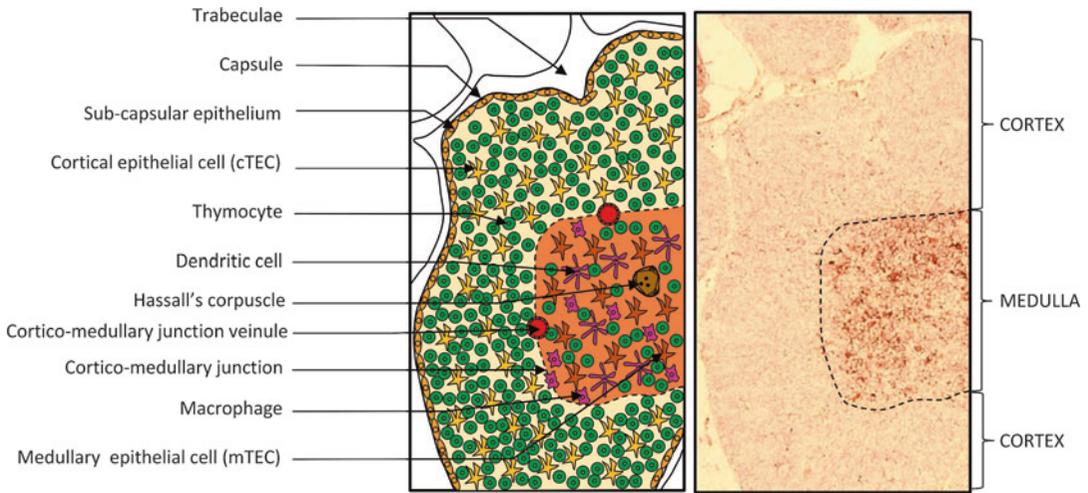
Definition

T-cell maturation within the thymus is key for naïve T-cell homeostasis as it allows maintaining naïve T-cell pool and diversity, essential parameters for preserved capacity to develop efficient immune responses against invading pathogens. HIV infection deeply alters T-cell homeostasis through diverse mechanisms and especially premature thymic aging and exhaustion. Nevertheless, thymic function is implicated in immune reconstitution in HIV-infected patients under highly active antiretroviral therapy (HAART).

Normal Thymopoiesis

Thymus Architecture

The thymus is a specialized poly-lobular organ of the immune system, localized in the anterior superior mediastinum, on top of the heart and aortic trunks, and beneath the breastbone. It was for a long time considered as an endocrine gland or



Thymic Function, Fig. 1 Anatomic structure and organization of the thymus. Schematic representation of the organization of a thymic lobule (*left panel*) compared to a thymic slice stained for HLA-DR molecule in *brown* (*right panel*). Thymic lobules are separated by trabeculae and can

be divided in two distinct compartments, the cortex, which mostly contains thymocytes and cortical epithelial cells (*cTEC*), and the medulla, which contains thymocytes, dendritic cells, macrophages, and medullary epithelial cells (*mTEC*). The corticomedullary junction is highly irrigated

even as a gland without any function. Its immunologic importance was only discovered in 1961 (Miller 1961). The thymus is very active during fetal life and childhood, producing large amounts of naïve T cell that built the highly diversified T-cell repertoire. Following puberty the thymus starts to involute, thymic epithelium being progressively replaced by adipose tissue while perivascular space is augmented. However, despite this involution, adult thymus still maintains the capacity to sustain T-cell maturation and contains thymocytes at different stage of differentiation, even after 70 years old (Douek et al. 1998).

The thymus is divided in lobules separated by trabeculae. Each lobule comprises two histologically and functionally different regions: (i) a cell-dense outer region that constitutes the cortex and (ii) a lighter inner part that constitutes the medulla. The cortex is mostly composed of thymocytes and cortical epithelial cells (cTECs), whereas the medulla contains thymocytes, medullary epithelial cells (mTECs), macrophages, and dendritic cells (DCs). In between these regions, the corticomedullary junction is a highly irrigated zone. Within the medulla, Hassall's corpuscles are concentric structures composed of reticular epithelial cells. They are involved in the

regulation of medullary DCs through thymic stromal lymphopoietin (TSLP) secretion and in the maturation of regulatory T cells (Treg) (Fig. 1).

T-Cell Maturation

The primary role of the thymus is to sustain thymocyte maturation, leading to the production of fully functional mature naïve T cells. Bone marrow-derived $CD34^+$ thymocyte progenitors enter the perimedullary cortex of the thymus through extravasation from the corticomedullary junction postcapillary venules. This entry is dependent upon P-selectin expression by endothelial cells and is highly regulated by the availability of intrathymic niches (Rossi et al. 2005). These precursors are called triple negative (TN, $CD3^-CD4^-CD8^-$) cells. TN thymocytes mature in the cortex and sequentially acquire CD2, CD5, and CD1a expression. At the TN stage of differentiation, thymocytes extensively proliferate under Notch1 and IL-7 signaling, allowing large amounts of cells to reach subsequent maturation stages. At the end of the TN differentiation stage ($CD34^+CD1a^+CD3^-CD4^-CD8^-$), future $\alpha\beta$ T cells start to acquire T-cell receptor β -chain (TCRB) expression. The TCRB locus, composed of 52 TCRBV segments and 2 TCRBDJ loci,

undergoes rearrangement through V-D-J recombination. This rearrangement is a stochastic mechanism by which non-contiguous V (variable), D (diversity), and J (junction) segments are associated to form a complete and variable TCR sequence. This recombination is driven by RAG enzymes (recombination-activating gene), which recognize specific recombination signal sequences flanking the different segments. Following TCRB rearrangement, cells that express a TCRB chain able to associate with p-T α chain receive survival signals through the CD3 ϵ and CD3 γ chains (β -selection). Unselected cells die by apoptosis.

During this entire step, thymocytes migrate through the thymic cortex to the subcapsular region. This migration is driven by both CCL25, expressed by cortical epithelial cells and macrophages, and CXCL12, expressed by subcapsular fibroblasts (Dutrieux et al. 2014). Thymocytes that survive β -selection express CXCR4 and, by interacting with CXCL12, are retained in the subcapsular region where they become intermediate simple positive cells (ISP, CD3^{low}CD4⁺CD8⁻) and extensively proliferate. This proliferation increases the number of cells expressing an in frame and correctly folded TCRB chain that will further differentiate. It also permits enhancing TCR diversity through α/β pairing (Arstila et al. 1999). Indeed, at early DP stage (CD3⁻CD4⁺CD8⁺), a second wave of RAG expression triggers TCRA chain rearrangement. Thus, ISP cell proliferation allows each selected TCRB chain to pair with a large number of TCRA chains, leading to further increased TCR diversity.

TCRA locus is composed of 70 V α and 61 J α segments which are rearranged using a process similar to TCRB chain. Preceding TCRA chain rearrangement, TCRD locus, which is localized between V α and J α segments on chromosome 14, must be excised. This deletion, as well as all other rearrangements at both TCRA and TCRB loci, leads to the circularization of the excised DNA. Such circular DNA molecules or T-cell receptor excision circles (TRECs) persist in the nucleus as extrachromosomal DNA but are lost upon cell cycling. Of note, several types of TRECs are generated during the entire T-cell

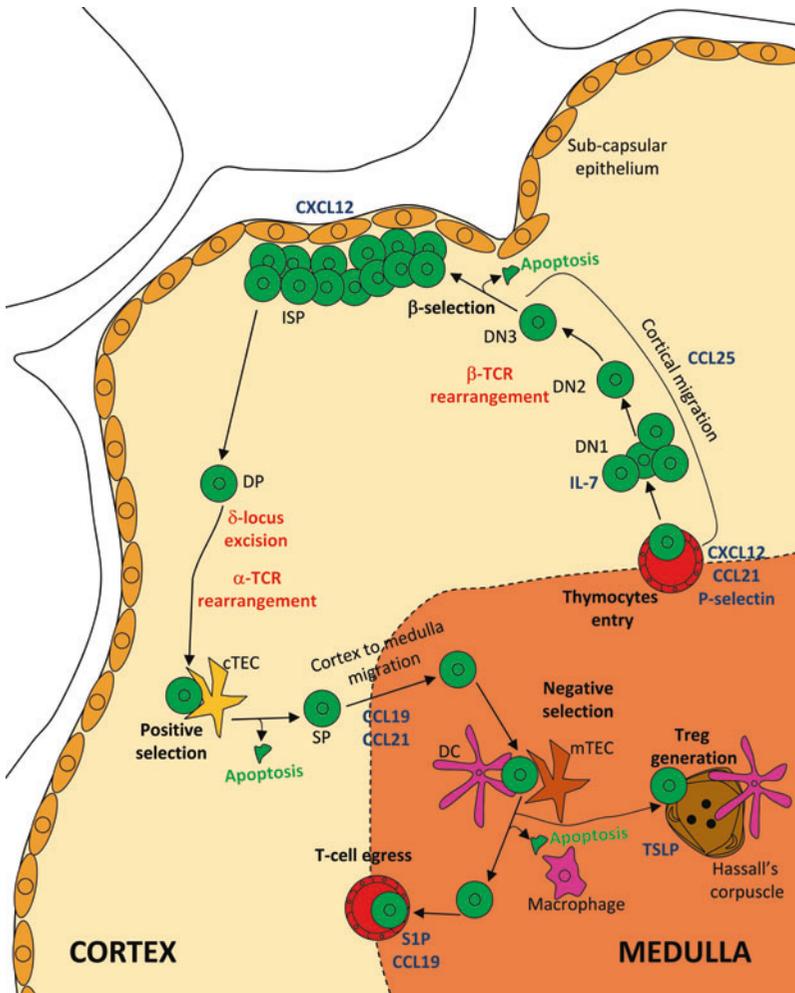
maturation process. The sjTREC (signal joint TREC), byproduct of TCRD locus deletion, is present in the vast majority of cells that exit the thymus (70%); it is thus used as a marker for recent thymic emigrants. Similarly, TCRB and TCRA rearrangements lead to the generation of different TREC molecules (DJ β TRECs, VD β TRECs, and VJ α TRECs). During the TCRA rearrangement process, thymocytes depend upon IL-7 to survive until the positive selection (Guillemard et al. 2001). DP cells expressing a full TCR (CD3⁺CD4⁺CD8⁺TCR⁺) able to interact with class I or class II major histocompatibility complexes (MHC-I and MHC-II) expressed by cTECs downregulate RAG expression and further differentiate into simple positive (SP) cells. Downregulation of CD8 expression allows MHC-II-reacting cells to differentiate into SP4 (CD3⁺CD4⁺CD8⁻). Contrariwise, DP cells that lose signal downmodulate CD4 molecule, reexpress CD8 molecule, and become SP8 cells (CD3⁺CD4⁻CD8⁺). Furthermore, thymocytes recognizing the nonclassical MHC-I CD1d differentiate into NKT cells.

Positively selected SP thymocytes migrate back through the cortex following gradients of CCR7-dependent chemokines (CCL19 and CCL21) (Ueno et al. 2004). In the medulla, they will be subjected to mTECs-driven negative selection. These cells present a variety of self-peptides on their MHC (spermine, prostaglandin, preproinsulin II, etc.) as a consequence of their expression of the transcription factor AIRE (autoimmune regulator) (Anderson and Su 2011). mTEC-reactive SP cells will be eliminated by apoptosis.

Finally, selected CD4 and CD8 mature T-cells expressing CCR7, sphingosine-1-phosphate receptor 1 (S1P1), and CD31 follow CCL19 and sphingosine-1-phosphate (S1P) gradients and exit the thymus through CD31-expressing corticomedullary microvessels. Newly exported cells (recent thymic emigrants – RTE) enter the blood stream and contribute to the diversification of circulating naïve T-cell pool (Fig. 2).

Studying Thymic Function

Thymic function can be measured through measuring the volume and metabolic activity of the



Thymic Function, Fig. 2 The maturation of $\alpha\beta$ T cells within the thymus. Thymocytes (in green) enter in the thymus through corticomedullary junction led by P-selectin. They migrate through the cortex following CCL25 gradient during double negative (DN, $CD3^-CD4^-CD8^-$) maturation. They undergo TCRB rearrangement and β -selection and become intermediate single positive (ISP, $CD3^{low}CD4^+CD8^-$) which are retained in the subcapsular region by CXCL12. ISPs extensively proliferate in this area then become double positive (DP, $CD3^+CD4^+CD8^+$) before acquiring the TCR α -chain. DPs interact with cortical epithelial cells (cTEC) which drive the positive selection. Thymocytes that are able to recognize MHC acquire the simple positive phenotype (SP, $CD3^+CD4^+CD8^-$ or $CD3^+CD4^+CD8^+$). SPs migrate in the medulla through CCR7-dependent chemokines and undergo the negative selection through interaction with medullary epithelial cells (mTEC) and dendritic cells (DC). Autoreactive cells died through apoptosis or are oriented to Hassall's corpuscle to become regulatory T cells (Treg). SPs that survive to negative selection are exported through S1P and CCL19 gradient

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gland, quantifying RTEs in the blood, and evaluating thymocyte proliferation.

Computed tomography (CT-scan), associated to positron-emission tomography (PET-scan), allows qualitative in vivo evaluation of the volume and the metabolism of the thymus through measurement of thymic glucose uptake using

a radioactive tracker (^{18}F -fluorodeoxyglucose) (Lelievre et al. 2012).

Since the demonstration that the thymus remains a functional organ in adults, its role in various pathologies has been extensively assessed. Different molecular markers of RTEs have been proposed in the literature. Quantification of the

sjTREC content in blood T-cells is considered as a good surrogate to measurement of thymic function. Indeed, TREC molecules are devoid of replication origin; they are thus not duplicated during mitosis and are diluted down when T-cells proliferate. Accordingly, sjTREC content in blood T-cells marks the presence of cells recently emigrated from the thymus and is informative for evaluating thymic activity (Douek et al. 1998). However, this parameter is also impacted by modifications of naïve T-cell half-life and proliferation. Indeed, in thymectomized patient, sjTREC levels only very slowly decrease as a consequence of increased naïve T-cell survival. In contrast, enhanced RTE proliferation that is often observed in lymphopenic patients leads to the reduction of sjTREC frequencies, leading to erroneous estimate of thymic function.

To circumvent the inaccuracy of sjTREC measurement in estimating thymic function, Dion and colleagues set up an assay aimed at evaluating intrathymic precursor T-cell proliferation. Indeed, as specified above, thymocytes extensively proliferate between TCRB and TCRA rearrangements. This proliferation increases the number of TCRB-expressing cells that reach late DP stage and rearrange TCRA chain, enhancing thymic output. As DJ β TRECs are diluted down during this proliferation, the relative concentration of sjTRECs and DJ β TRECs is dependent upon the extent of thymocyte proliferation. Consequently, their ratio (sj/ β TREC), measured in peripheral blood cells, directly reflects the intrathymic proliferation history of the studied T-cell population and consequently thymic activity (Dion et al. 2007). Thereby, any change in the sj/ β TREC ratio signs modification of precursor T-cell proliferation thus of thymic output.

Finally, several cell surface markers have been proposed to identify RTEs within naïve T-cells. In the CD4 T-cell compartment, naïve T-cells expressing either PTK7 (tyrosine-protein kinase-like 7) or CD31 (platelet endothelial cell adhesion molecule implicated in thymic egress and homing to lymph nodes) present a higher sjTREC content than their negative counterparts (Kimmig et al. 2002; Haines et al. 2009). In contrast, these markers cannot be used in the CD8 compartment.

HIV Infection Affects Thymic Function

With the demonstration of persistent thymic function in adults, the question of its alteration during HIV infection arose. Indeed, naïve T-cell subsets are deeply reduced following infection (Silvestri and Feinberg 2003). However, probably due to increased plasma IL-7 levels that generally characterize HIV-infected patients – even without marked lymphopenia – naïve T-cell homeostatic proliferation does not seem to be significantly impaired following HIV infection. Consequently, naïve T-cell decline in patients could be a consequence of impaired thymic activity and reduced naïve T-cell neo-production.

From the late 1990s, several reports demonstrated that sjTREC content of circulating T-cells (sjTREC/ 10^6 cells) was strongly reduced in chronically HIV-infected patients, suggesting thymic defect (Douek et al. 1998; Poulin and Sekaly 1999). These papers opened a long-lasting debate concerning the reliability of measuring sjTREC frequencies in lymphopenic individuals as their decline in patients' blood could also be a consequence of global increase of T-cell cycling leading to dilution of TREC molecules and/or impaired survival of TREC-containing cells (Hazenberg et al. 2003). However, the observed decline in global sjTREC concentration in the blood (sjTREC/mL) argued against the implication of modified naïve T-cell turnover in the evolution of sjTREC frequencies in HIV-infected patients. This debate was finally solved by the identification of the direct impact that HIV infection has on thymic physiology. Indeed, quantifying sjTREC and DJ β TRECs, Dion and colleagues demonstrated that reduced thymocyte proliferation characterizes HIV-infected patients. Diminished thymocyte proliferation, leading to reduced thymic output, certainly participates to naïve T-cell lymphopenia observed early on following HIV infection (Dion et al. 2004, 2007). Indeed, thymic dysfunction began early on during the first months following infection, suggesting its importance in the establishment of HIV-1 physiopathology, and persists over the chronic phase of the infection. The mechanisms leading to diminished thymocyte proliferation in HIV-infected patients remain

barely studied, but several hypotheses can be envisaged.

On the one hand, even though *in vivo* evidences of thymocyte infection in human remain sparse, it can be supposed that thymus cells constitute a target for HIV due to their expression of CD4, CCR5, and CXCR4. Indeed, ISP, DP, and SP4 thymocytes as well as thymic DCs express both CD4 molecule and HIV coreceptors and are susceptible to HIV infection *in vitro* (Schmitt et al. 2006). Of note, only a small fraction of CD4⁺ thymocytes express CCR5 leading to preferential infection by CXCR4-tropic viruses and suggesting low infection rates during primary infection. However, cytokines expressed in the thymus (IL-2, IL-4, and IL-7) were shown to up regulate coreceptor expression on thymocytes (Pedroza-Martins et al. 1998). Furthermore, thymic epithelial cells (mTECs and cTECs) can also be productively infected *in vitro* suggesting that they could participate to the propagation of the infection within the thymus (Braun et al. 1996). Indeed cellular contacts between thymocytes and TECs are necessary for an intense viral replication in thymocytes (Rothe et al. 1998). In thymocyte, as in circulating T-cells, HIV replication depends upon NF κ B activation, suggesting that proliferating cells are more susceptible to express HIV proteins when infected. Such an expression may lead to specific elimination of proliferating cells, leading to the export of cells presenting with low proliferation history (low sj/ β TREC ratio). However, *in vivo*, infection of the thymus remains strongly limited. Sparse infected cells can be identified in the thymus of acutely SIV-infected rhesus macaques (Dutrieux et al. 2014) as well as *postmortem* on histological thymic tissues from AIDS patients (Pekovic et al. 1987). It is thus unlikely that killing of proliferating infected cells alone can strongly impact on thymic output. Nonetheless, infection of immature T-cells in the thymus may result in the export of infected RTEs that significantly contribute to HIV reservoir (Cameron et al. 2010; Fabre-Mersseman et al. 2011).

On the other hand, HIV infection also triggers modification of thymic physiology leading to the observed reduction of thymocyte proliferation

and the initiation of antiviral innate immune responses. Indeed, infection of fetal thymus in culture stimulates the expression of IFN- α in the supernatant and leads to reduced SP4 production (Sivaraman et al. 2011). Thymic DCs and macrophages (which express CD4) are permissive to CCR5-tropic HIV-1 strains, suggesting that these cells can be infected during the early phases of the infection (Schmitt et al. 2006) and produce IFN- α within the thymus (Bendriiss-Vermare et al. 2001). Lastly, Dutrieux and colleagues recently demonstrated that, in Chinese rhesus macaques, SIV infection stimulates the production of various IFN- α subtypes within the thymus, some of these molecules harboring a strong antiproliferative activity on developing thymocytes (Dutrieux et al. 2014). At the same time, thymic chemokine networks are also altered. While CCL19 and CCL25 expressions are deeply enhanced by day 3 of infection, CXCL12 expression is reduced. Considering the role of these chemokines in triggering either intrathymic cell migrations (CCL19 and CCL25) or cell retaining in the subcapsular area (CXCL12), these modifications are compatible with faster cell trafficking within the thymus (Dutrieux et al. 2014). Interestingly, IFN- α production and modified chemokine networks in the thymus probably have the paradoxical consequence of transiently enhancing thymic output through acceleration of thymocyte differentiation (Dutrieux et al. 2014). Such an increased thymic output was observed following measles infection-induced lymphopenia in both children and rhesus macaques. However, in the long term, IFN- α -dependent inhibition of thymocyte proliferation, leading to reduced numbers of DN cells in the thymus, could affect thymic epithelial cells/thymocytes cross talk and stromal cell survival, leading to thymic exhaustion.

Finally, besides its quantitative consequences on thymopoiesis, HIV infection in the thymus and HIV-induced modifications of thymic physiology could also impact on the quality of thymic production. Indeed, the death of HIV-infected cells within the thymus and expression of HIV proteins by mTECs should lead to the presentation of HIV peptides on MHC molecules, mimicking

self-antigen presentation and triggering the deletion of HIV-specific T-cell clones. Such an immune dysfunction was observed following Gross virus infection of thymic epithelium in mice. Moreover, IFN- α expression in the thymus, known to increase HLA class I expression in the thymus, could also impact on the global affinity of T-cell receptor repertoire, leading to reduced activation following antigen exposure.

Immune Regeneration Under Treatment

Patients achieving sustained virus control under HAART usually reconstitute, at least partly, their CD4 T-cell compartment. During the first months of therapy, most of the increase in circulating T-cell counts occurs in the naïve compartment, suggesting a role of thymopoiesis. Indeed, PET scan analysis of the thymus of HAART-treated patients showed that thymic function is boosted following treatment initiation (Lelievre et al. 2012). Similarly, both sjTREC frequency and sj/ β TREC ratio were significantly enhanced during the first year of therapy, demonstrating the implication of the thymus in the early phase of immune recovery under treatment (Markert et al. 2001; Dion et al. 2004). In contrast, some patients with poor immunological response to antiretroviral therapy barely regain naïve T-cell counts and did not recover normal thymic output (Dion et al. 2007). In these patients, naïve T-cell frequencies strongly correlated with sjTREC frequency and sj/ β TREC ratio, suggesting that increased thymic output plays a major role in immune reconstitution under HAART (Dion et al. 2007). Interestingly, naïve T-cells produced under therapy also present a longer half-life and a diminished sensitivity to apoptosis (Aladdin et al. 2003). However, efficient thymopoiesis is not sustained following T-cell reconstitution. In long-term-treated good immunological responder patients, the sj/ β TREC ratio was similar to that of patients who failed to reconstitute T-cell compartment (Delobel et al. 2006). This suggests that, similarly to FOXN1-deficient patients receiving a thymic graft (Albuquerque et al. 2012), as well as following allogeneic hematopoietic cells transplant

(Castermans et al. 2011), the thymus was able to sustain T-cell production in lymphopenic patients but could not remain active in reconstituted hosts presenting close to normal T-cell homeostasis. Following the initial phase of thymus-dependent immune reconstitution, naïve T-cell counts are mostly maintained through IL-7-driven survival and proliferation.

Finally, even in HAART-treated poor immunological responder patients, thymic function can be boosted through immunotherapeutic intervention. Indeed, in the first IL-7-based clinical trials, both thymic output and circulating naïve CD4⁺ T-cell counts were significantly enhanced in virally controlled poor immunological responders (Sereti et al. 2009; Levy et al. 2012). Similarly, increased thymic function and diversification of T-cell repertoire were observed in IL-7-treated SIV-infected rhesus macaques (Parker et al. 2010).

Conclusion

The thymus is an essential primary lymphoid organ providing the immune system with new naïve T cells in childhood but also in adults. Through continuous production of new T-cells, thymopoiesis ensures maintaining naïve T-cell diversity allowing the development of adequate immune responses to invading pathogens. Viral replication within the thymus can be observed in chronically infected patients as well as from the first days of experimental SIV infection of rhesus macaques. In this model, viral infection in the thymus coincides with major modifications of thymic physiology. While changes in thymic physiology may transiently increase thymic output during acute infection as a consequence of accelerated thymopoiesis, thymic impairment is observed during the chronic phase of the infection. However, the thymus keeps a renewal capacity and, upon antiretroviral or immunostimulating therapy, can functionally regenerate and participate to immune restoration. Indeed, an increased thymic activity is undeniably mandatory for efficient immune reconstitution leading to extended TCR repertoire during antiretroviral treatment.

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Tim-3

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Definition

T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) is a member of the T cell/transmembrane, immunoglobulin, and mucin (TIM) gene family of phosphatidylserine-binding proteins (Freeman et al. 2010). It is a type I transmembrane glycoprotein consisting of an N-terminal IgV-like domain and a heavily glycosylated mucin domain, a transmembrane region, and a cytoplasmic tail. Tim-3 plays a role in the regulation of both innate and adaptive immune responses during autoimmunity, cancer, and chronic infections (Han et al. 2013; Zhu et al. 2011). While it has been shown to both negatively and positively regulate innate immune responses, Tim-3 plays a distinct negative regulatory role in regards to T cell responses. During human immunodeficiency virus type 1 (HIV) infection, Tim-3 marks dysfunctional T cells during chronic stages of infection and acts to dampen responses to viral antigen, contributing to the “exhausted” state of the immune system and lack of viral control (Jones et al. 2008).

Introduction

Virus infections are typically controlled by potent T cell responses, particularly a potent CD8+

cytolytic T cell (CTL) response. Viruses, like HIV, that are chronic and persistent are able to evade these potent responses via multiple mechanisms, including epitope mutation and dysregulation of T cell “help” in the case of HIV infection. Virus persistence leads to ongoing CD4⁺ and CD8⁺ T cell activation. Persistent T cell activation in the presence of chronic virus infections, such as observed with HIV, has led to unique phenotype termed “T cell exhaustion.” T cell exhaustion is characterized by the hierarchical loss of effector function, initially with defective proliferation and an enhanced tendency to apoptosis, followed by sequential loss of effector function such as IL-2 and TNF- α production, and then followed by loss of IFN- γ production and cytotoxic ability (Wherry et al. 2003). Efforts to characterize exhausted T cells led to the observation that expression of multiple co-inhibitory molecules, including Programmed-Death 1 (PD-1), Lymphocyte Activation Gene-3 (LAG-3), and Tim-3 (Khaitan and Unutmaz 2011), was a feature of these cells. It is unclear whether T cell exhaustion is a manifestation of a virus evasion strategy or a way for the immune system to limit excessive T cell responses and avoid autoimmunity to cross-reactive self-antigens. This review will focus mainly on the role of Tim-3 in T cell exhaustion in HIV infection.

Tim-3 was originally identified as a marker of IFN- γ secreting CD4⁺ Th1 cells but is also expressed on naïve T cells, CD4⁺ T regulatory cells (Tregs), CD4⁺ Th17 cells, activated CD8⁺ T cells, constitutively on monocytes, activated macrophages, dendritic cells (DCs), and natural killer (NK) cells (Han et al. 2013; Khaitan and Unutmaz 2011). Tim-3 binds to multiple ligands to regulate signaling in cells of both the innate and adaptive immune system, resulting in a complex regulation between the two arms of the immune system. Indeed, multiple studies have described Tim-3 as a negative regulator of immune responses, while others have described Tim-3 as a positive regulator of immune responses (Khaitan and Unutmaz 2011; Ferris et al. 2014). However, these effects appear to be context specific, as Tim-3 appears to play various roles in

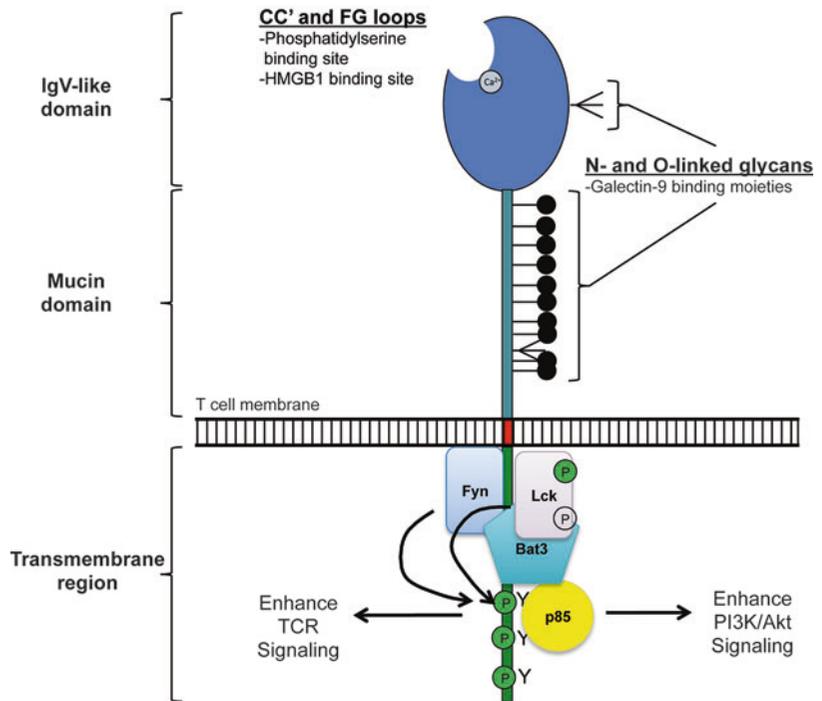
regulating autoimmunity and in immune responses to cancer and acute and chronic infections.

Tim-3 Ligands

Tim-3 binds to multiple ligands, including galectin-9, phosphatidylserine (PS), and high mobility group box 1 (HMGB1) protein (Freeman et al. 2010; Zhu et al. 2005; Chiba et al. 2012). Galectin-9 is a soluble tandem-repeat S-type lectin containing two carbohydrate recognition domains (CRD) joined by a flexible peptide linker. Binding to Tim-3 is mediated via interactions with carbohydrates on the IgV-like and mucin domain of Tim-3 and the galectin-9 CRDs (Fig. 1; Zhu et al. 2005). Galectin-9 does not exclusively bind to Tim-3. Its lectin-binding properties and bivalent nature allow for heterogeneous cross-linking of multiple glycoproteins such as CD45 and CD148 (Clayton et al. 2014). Galectin-9's interaction with Tim-3 results in apoptosis of Th1/Tc1 cells supporting its role as a co-inhibitory molecule (Zhu et al. 2005). However, the signaling cascade responsible for this death induction is still unclear.

PS is a phospholipid, which normally localizes to the inner leaflet of the plasma membrane, but gets “flipped” to the outer leaflet during the early stages of apoptosis, thus acting as an “eat me” signal to mediate clearance of the apoptotic cell via phagocytosis. Like other members of the TIM family, Tim-1 and Tim-4, Tim-3 binds to PS via the unique pocket formed by the calcium-coordinating CC' and FC loops of the IgV-like domain (Freeman et al. 2010) with a rather high affinity (nM range). TIM family molecules insert their IgV-like domains into the opposing membrane to stabilize the interaction with PS. However, molecular modeling suggests that PS binding does not inhibit potential galectin-9 binding, as binding sites for each ligand are on opposite sides of the IgV-like domain (Fig. 1; Freeman et al. 2010). Binding to PS mediates differential function of Tim-3 depending on cell type expressing Tim-3. For example,

Tim-3, Fig. 1 Tim-3 is a type 1 membrane protein of 301 amino acids, belonging to the immunoglobulin superfamily, and contains an IgV-like domain, a mucin-like domain, a transmembrane region, and an intracellular tail. Glycans on surface Tim-3 can bind to galectin-9, and PS and HMGB1 can bind to the region bound by CC' and FG loops of Tim-3. The cytoplasmic tail does not contain recognizable ITIM/ITAM motifs but can be phosphorylated and can bind to a number of upstream signaling proteins



macrophages, DCs, and T cells that express Tim-3 can bind to PS. However, unlike Tim-3 on DCs, which mediates phagocytosis and cross-presentation of apoptotic cells, the effect of PS binding to Tim-3 on T cells is unclear (Nakayama et al. 2009).

Recently, Tim-3 was described as a suppressor of DC-mediated antitumor responses via binding to HMGB1 (Chiba et al. 2012), a nuclear, cytosolic, and extracellular protein with varying functions depending on the cellular or extracellular compartment. This interaction was mapped to the PS-binding calcium-coordinating CC' and FC loops of the IgV-like domain (Fig. 1). When released from necrotic or inflammatory cells, HMGB1 acts as a danger-associated molecular pattern (DAMP) molecule, or alarmin, mediating activation of nucleic acid-sensing systems via pattern recognition receptors (PRRs) and ultimately an inflammatory innate immune response. Tim-3 on DCs binds to extracellular HMGB1 and targets it for degradation, thus suppressing PRR-mediated responses in tumors (Chiba et al. 2012). No work has yet described the whether this

HMGB1-Tim-3 interaction also plays a role in T cell responses.

Tim-3 Signaling

Tim-3 is an important regulator of both innate and adaptive immune responses. Interestingly, despite its role in negatively regulating T cell immune responses, the Tim-3 cytoplasmic tail lacks known signaling motifs, such as the immunoreceptor tyrosine-based inhibition motif (ITIM), for the recruitment of inhibitory factors, such as the phosphatase SHP-1. Common Tim-3 characteristics observed in innate and adaptive immune cell signaling are tyrosine phosphorylation and subsequent binding to Src-family kinases (SFKs). In the innate immune system, engaging Tim-3 on DCs results in tyrosine phosphorylation of its cytoplasmic tail and subsequent activation of the nonreceptor tyrosine kinases Bruton’s tyrosine kinase (Btk) and c-Src. This leads to the release of a soluble inhibitory factor that dampens NF-κB signaling (Maurya et al. 2014). However,

studies have also shown that engagement of Tim-3 can lead to NF- κ B activation (Ferris et al. 2014), whose mechanism is unclear. Likewise, there exists conflicting evidence for Tim-3 signaling in adaptive immune cells. In T cells, initial work showed that Tim-3 plays a role in enhancing T cell receptor (TCR) signaling via recruitment of Fyn, Lck, and p85 (the PI3K adaptor), resulting in enhanced TCR-induced NFAT/AP-1 and NF- κ B signaling as well as Akt/mTOR signaling (Fig. 1; Ferris et al. 2014). Indeed, subsequent studies showed that Tim-3 was recruited to the immunological synapse, further suggesting a role in TCR signaling (Clayton et al. 2014; Rangachari et al. 2012). However, these studies also showed recruitment of receptor phosphatase CD45/CD148 to Tim-3 via galectin-9 to dampen Lck signaling (Clayton et al. 2014) and recruitment of the chaperone Bat3 to dampen Tim-3-induced negative signaling (Rangachari et al. 2012), suggesting that Tim-3 acts to inhibit TCR signaling. However, the exact pathways and downstream targets have still yet to be determined. In addition, it is still unclear which Tim-3 ligand mediates these diverse effects.

Tim-3 and the Innate Immune System

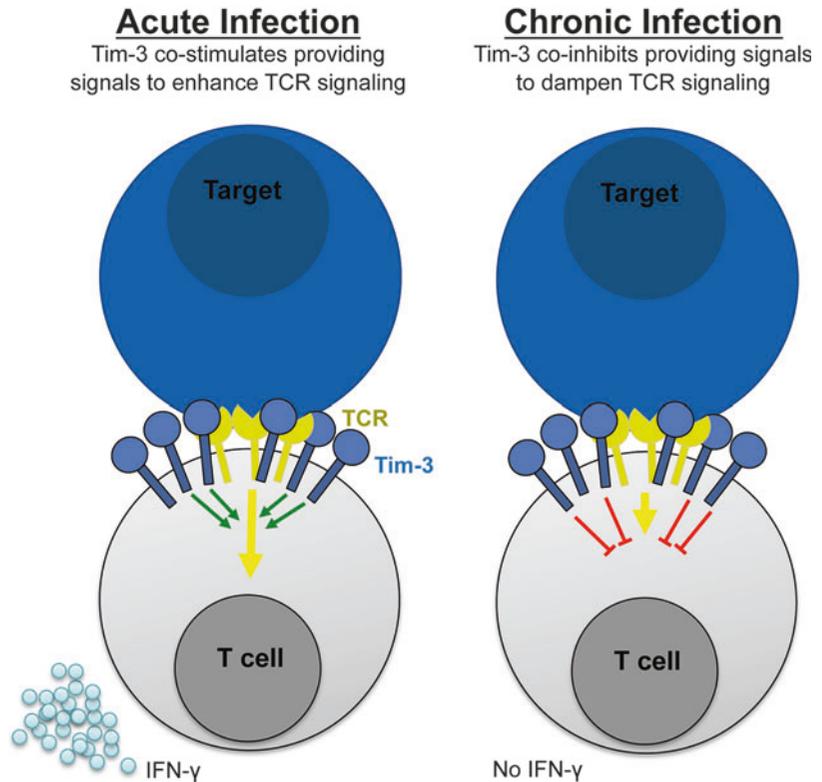
Tim-3 plays an important role in regulating innate immune responses. As mentioned, it is expressed on monocytes, activated macrophages, DCs, and NK cells (Han et al. 2013). On monocytes, it acts to dampen IL-12 on resting monocytes, but after monocyte activation, Tim-3 is downregulated, resulting in expression of pro-inflammatory cytokines. Tim-3 is also upregulated on stimulated macrophages, which suppresses macrophage activation. In addition, it also mediates phagocytosis and cross-presentation of cell debris via its interaction with PS (Nakayama et al. 2009). Depression of the Tim-3 pathway in these cells contributes to the development of autoimmune disease (Nakayama et al. 2009). Expression of Tim-3 on DCs also negatively regulates innate responses. This was shown in the case of tumor-associated DCs with high levels of Tim-3, which bound to the HMGB1 and dampened cellular responses to nucleic acid

(Chiba et al. 2012). In addition, Tim-3 engagement results in release of a soluble negative factor, which dampens NF- κ B signaling (Maurya et al. 2014). The situation is more complicated for NK cells. Activation of NK cells with certain cytokines upregulates the expression of Tim-3, which then marks highly functional, cytotoxic NK cells. However, engagement of Tim-3 suppresses NK cell function. Overall, it appears that Tim-3 expression on innate cells acts a central negative regulator of the innate immune system in terms of cytokine production but favors antigen clearance and cross-presentation.

Tim-3 in the Adaptive Immune System

Tim-3 has been extensively studied in the context of autoimmunity and T cell exhaustion in both cancer and chronic infection. Early work characterizing Tim-3 suggested it acts to dampen Th1 and Th17 responses to self-antigen, as blocking this pathway resulted in development of autoimmune disease (Freeman et al. 2010). Tumor infiltrating lymphocytes (TILs) and virus-specific T cells show characteristics of exhaustion, which include loss of effector function and increased levels of multiple co-inhibitory molecules, including Tim-3 (Ferris et al. 2014). Indeed, expression of Tim-3 correlates with lack of IFN- γ production, and blocking Tim-3 using antagonistic antibodies or soluble Tim-3 has shown to promote rejection of solid tumors in murine mice (Ferris et al. 2014). In some studies, this effect was mediated in part by suppressing Tim-3⁺ Treg responses, which produced high levels of IL-10. In addition, Tim-3 blockade rescued ex vivo function of Tim-3⁺ TILs from patients (Ferris et al. 2014). Thus, Tim-3 can inhibit T cell effector responses via direct and indirect mechanisms. Interestingly, in active tuberculosis (TB) infection, Tim-3 is highly upregulated on T cells, which possess more potent anti-TB responses, and engaging Tim-3 provides a co-stimulatory signal and enhances anti-TB T cell effector responses. Further, Tim-3 is required for effective responses to clear *Listeria monocytogenes* (LM) (Ferris et al. 2014). Thus, Tim-3 plays divergent roles in terms

Tim-3, Fig. 2 Tim-3 appears to show divergent effects on T cells depending on the stage of infection and the balance of co-stimulatory or inhibitory molecules at the surface. During acute infection, Tim-3 may act as a co-stimulator, but during chronic infection, Tim-3 has the opposite effect, in part by bringing in more negative regulators



of controlling T cell responses during acute versus chronic antigen stimulation (Ferris et al. 2014; Fig. 2).

Tim-3 in HIV

During chronic HIV infection, Tim-3 is found highly upregulated on both CD4⁺ and CD8⁺ T cells (Jones et al. 2008). These levels are high in acute/early infection (less than 6 months) and untreated chronic infection (more than 1 year), but not in long-term non-progressors (LTNPs), when compared to healthy controls. These levels positively correlate with viral load, T cell activation (marked by CD38 expression), and negatively with CD4⁺ T cell counts, suggesting Tim-3 expression correlates with HIV disease progression. Recognition of HIV-infected DCs and stimulation via TCR or common γ -chain cytokines mediates this induction of Tim-3 expression (Mujib et al. 2012; Larsson et al. 2013). Highly active antiretroviral therapy (HAART) has

varying effects on Tim-3 expression; however, changes in Tim-3 expression levels correlate with ongoing T cell activation, measured by CD38 expression, further suggesting that chronic immune activation controls Tim-3 expression during HIV infection. Tim-3 expression however is not consistently decreased after HAART when antigen levels drop. As seen with cancer and other chronic infections, Tim-3⁺ T cells fail to respond to stimuli. However, these responses can, in part, be rescued via blockade with either a soluble Tim-3 decoy or antagonistic antibody, suggesting that Tim-3 plays a functional role in T cell exhaustion during HIV infection (Jones et al. 2008). Interestingly, HLA-B*27- and HLA-B*57-restricted HIV-specific CD8⁺ T cells fail to upregulate Tim-3 to levels seen with other MHC-restricted CD8⁺ T cells. In addition, these cells are more functional and correlate with control of HIV infection as is seen in LTNPs (Larsson et al. 2013). Thus, Tim-3 is upregulated on HIV-specific CD8⁺ T cells, which are exhausted. The exact signaling pathways downstream of

Tim-3 that mediate this phenotype are still poorly defined, and much work needs to be accomplished in this regard.

Conclusion

Tim-3 is a receptor found on various cells of the immune system, which acts to dampen excessive inflammatory responses. While this is essential for autoimmune diseases such as multiple sclerosis, it contributes to lack of T cell control of cancer and chronic viral infections, like HIV. This inhibition is mediated, in part, via Tim-3 binding to its ligands galectin-9, PS, or HMGB1, resulting in signaling pathways, which mediate apoptosis, phagocytosis, or inhibition of PRR sensing pathways, respectively. Tim-3 signaling in innate and adaptive immune cells share common characteristics, such as Tim-3 tyrosine phosphorylation and recruitment of SFKs; however, how this results in modulation of cellular responses is still unclear. In addition to Tim-3's inhibitory role, new data suggests that Tim-3 plays a positive role in enhancing T cell responses during acute infections (Fig. 2). It remains to be determined if this is the case for acute HIV infection, which would contrast with its role during chronic HIV infection. Indeed, blocking this pathway using antagonistic antibodies suggests a method to enhance T cell responses during HIV infection, but disease state (acute versus chronic infection) and cell types affected (innate versus adaptive immune cells) are important factors to consider when designing therapeutics to manipulate the Tim-3 pathway. We still lack a complete understanding of downstream signaling pathways of Tim-3 on T cells during acute and chronic infections or in cancer. A fuller understanding of these pathways will guide antibody-based therapies in these diseases so that the correct effect (immune suppression or co-stimulation) can be achieved without unforeseen consequences.

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Toxoplasmosis in HIV-Infected Patients

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Definition

Toxoplasmosis is the infection caused by *Toxoplasma gondii*, an obligate intracellular protozoan that belongs to the phylum Apicomplexa. In immunocompromised patients, such as AIDS patients with low CD4 cell count, reactivation of latent disease can cause life-threatening encephalitis. The disease usually presents with a focal neurological deficit associated with the formation of multiple *Toxoplasma* abscesses. Treatment requires combination of anti-toxoplasmic drugs and its continuation as suppressive therapy until immune reconstitution occurs.

Biology, Epidemiology, and Transmission

Biology

Toxoplasmosis is an endemic infection of worldwide distribution caused by *Toxoplasma gondii*, an intracellular protozoan of the order Coccidia,

phylum Apicomplexa. *Toxoplasma* can present in three forms in its life cycle: the oocyst, tissue cyst, and tachyzoite.

The infection is acquired orally by ingestion of contaminated food or water oocysts or by ingestion of undercooked meat containing tissue cysts. The infection can also be acquired through the vertical route (mother-to-child transmission). Once inside the body, the cysts and oocysts release tachyzoites, which have the ability to affect intestinal epithelial cells, to invade the mesenteric lymph nodes, and then via the bloodstream to spread to different tissues. Tachyzoites are responsible for the clinical presentation of acute toxoplasmosis; they replicate intracellularly, infesting and destroying cells until the immune system develops the appropriate response. Subsequently, the parasites that survive this immune response encyst in various tissues, including the brain, the retina, skeletal muscle, the myocardium, and occasionally the lungs, where they can stay in a quiescent form for life. Years later, if T cell-mediated immunity is impaired, the cysts in tissues may break releasing tachyzoites, which produce a reactivation of the infection.

Oocysts develop in the cells of the intestinal mucosa of cats, domestic cats, and other wild felines, like the leopard and lions being the definitive host. Cats become infected by ingesting cysts in the raw meat of infected animals or oocysts present in feces eliminated by other felines. After 1–2 weeks, oocyst shedding ceases and rarely restarts. Oocysts must sporulate to become infectious, a process that is favored by heat and humidity, conditions typically found in litter boxes used to deposit cat feces in homes. Sporulated oocysts can remain infectious for more than 1 year.

Tissue cysts can be found in the cells of almost any organ of the host. Maintaining the cysts in a quiescent state and controlling any breakage to avoid the periodical release of tachyzoites is a process mediated by T cells and cytokines. Thus, in individuals previously infected and with T cell impaired immunity, residual cysts are an important source of tachyzoites that may escape immune control. However, cases of retinal involvement can occur in immunocompetent and

immunocompromised individuals, which are presumed to be mediated by additional mechanisms to those described.

Epidemiology and Transmission

The most common route of transmission of *T. gondii* to humans is the eating of raw or undercooked pork or lamb meat. In theory, any animal that ingests infected oocysts or tissue cysts can become infected with toxoplasmosis. If the meat is not cooked above 60 °C or frozen below –20 °C, cysts remain viable (Montoya and Liesenfeld 2004).

The three main forms of transmission to humans of *T. gondii* are:

- Ingestion of oocysts eliminated by cats or eating undercooked meat containing cysts
- Transplacental transmission
- Infection through blood transfusion or organ transplant

Cats living in a strictly domestic environment have little chance of spreading infection. Congenital infection occurs if the mother acquires a toxoplasmic infection during pregnancy (primary infection). However, there have been cases of vertical transmission in HIV-positive women with latent toxoplasmosis after reactivation of the parasite.

Clinical Presentation

Primary infection with *T. gondii* is asymptomatic in most immunocompetent individuals. Up to a third of cases, and more frequently in children, may present an adenitis, a flu-like or a mononucleosis-like syndrome.

Most cases of toxoplasmosis with central nervous system (CNS) involvement associated to AIDS or to other states of severe cellular immunosuppression are the consequences of the reactivation of a previous infection in seropositive individuals. Clinical manifestations depend on where in the CNS the infection is reactivated and the intensity of the local inflammatory response, which can vary according to the CD4⁺ T cell count of the patient.

The clinical description given below is observed in patients with AIDS, who represent the vast majority of cases of cerebral toxoplasmosis. Other patients with cellular immunodeficiency (such as stem cell transplant recipients) have extra-neurological forms, such as lung disease (pneumonitis) or disseminated disease, more often than patients with AIDS, but in the case of brain involvement, the clinical presentation is very similar.

Neurological Disease

About 50% of patients with intracerebral infection present with headaches, confusion, and altered consciousness. The beginning of the disease can be insidious or abrupt. Up to 30% of patients suffer seizures as the initial manifestation, and 50–60% show signs of focal neurological deficiency. High fever with chills, neck stiffness, and other meningeal signs are unusual and should suggest other diagnoses (Miro et al. 2008).

Since cerebral toxoplasmosis is often multifocal, intracerebral masses are very destructive and inflammatory and can develop in any part of the brain, although the cortical-subcortical union of the cerebral hemispheres is the most frequent site. Virtually, any neurological syndrome can develop and produce motor or sensory deficits, among other focal deficits. The brainstem, the basal ganglia, and the cerebellum may also be affected, and patients can present with movement disorders, neuropsychiatric findings, and varying levels of depression in their level of consciousness, including coma.

Hemiparesis is the most common focal deficit, but there may be cranial nerve injuries, focal seizures, aphasia, visual loss, ataxia, dysmetria, tremors, hemiballismus, and other extrapyramidal signs. The involvement of the spinal cord can produce transverse myelitis or conus syndrome. Hydrocephalus, brain hemorrhage, and choroiditis may also occur less frequently (Antinori et al. 2004).

Extra-neurological Disease (Isolated or Concomitant to Cerebral Involvement)

In addition to the CNS, reactivation foci can also be observed in other organs, most frequently the

lungs in form of pneumonitis and the eyes as chorioretinitis, concomitantly or not with brain involvement. Since parasites may form cysts in any organ, and since recurrent parasitemia can occur in association with reactivations, new parasitic implants may occur in other organs, and thus the clinical manifestations of extracerebral toxoplasmosis can be quite diverse. In fact, autopsy studies often show unrecognized multi-organic involvement; up to 50% of patients with no concomitant CNS disease have extracerebral lesions, but these are frequently subclinical (Rabaud et al. 1994).

Ocular Disease

Ocular disease is probably the most common clinical manifestation of extracerebral HIV-associated toxoplasmosis. In 30–60% of chorioretinitis cases, encephalitis is also present. Conversely, relatively few patients with encephalitis present concomitant chorioretinitis. Visual symptoms due to *Toxoplasma* retinitis include loss of visual acuity and myodesopsia. Funduscopic examination reveals areas of yellow-white-colored necrotizing retinitis, sometimes with hemorrhage and vasculitis. Lesions are predominantly unilateral. Fluorescein angiography shows hyperfluorescence from the periphery and progressing toward the center of the lesions. *Toxoplasma* retinitis must be differentiated from retinitis caused by cytomegalovirus, varicella zoster virus, syphilis, and fungi, including *Pneumocystis jirovecii* retinitis.

Pulmonary Disease

Pulmonary manifestations of toxoplasmosis represent up to 35% of extrapulmonary forms. Fever and dyspnea are the most common symptoms, while cough and sputum may be missing. Chest x-rays usually show bilateral diffuse infiltrates in the lungs (similar to *Pneumocystis jirovecii* RX pattern). Multiple nodular infiltrates have been also reported. Typically, there is a massive increase in lactate dehydrogenase (LDH). The diagnosis can be established by examining the bronchoalveolar lavage, which reveals *T. gondii* trophozoites detected by special staining or by molecular biology, i.e., polymerase chain reaction (PCR).

Other Organ Involvement

Along with pneumonitis and chorioretinitis, extracerebral manifestations have been reported in virtually every organ and even sepsis-like clinical presentations in severely immunocompromised patients (generally non-AIDS immunosuppressed patients) with elevated massive LDH increases, to which molecular biology techniques, such as PCR performed in blood, are highly sensitive and specific in establishing a diagnosis.

Central Nervous System Involvement During Acute Toxoplasmic Infection

Although it is much less common, the CNS can be affected during the *Toxoplasma* primary infection. Patients with primary infection and with severe forms of cellular immunosuppression can produce severe visceral manifestations and disseminated infection, including pneumonitis, myositis, myocarditis, orchitis, and encephalitis, manifested in the form of diffuse involvement or intracerebral masses.

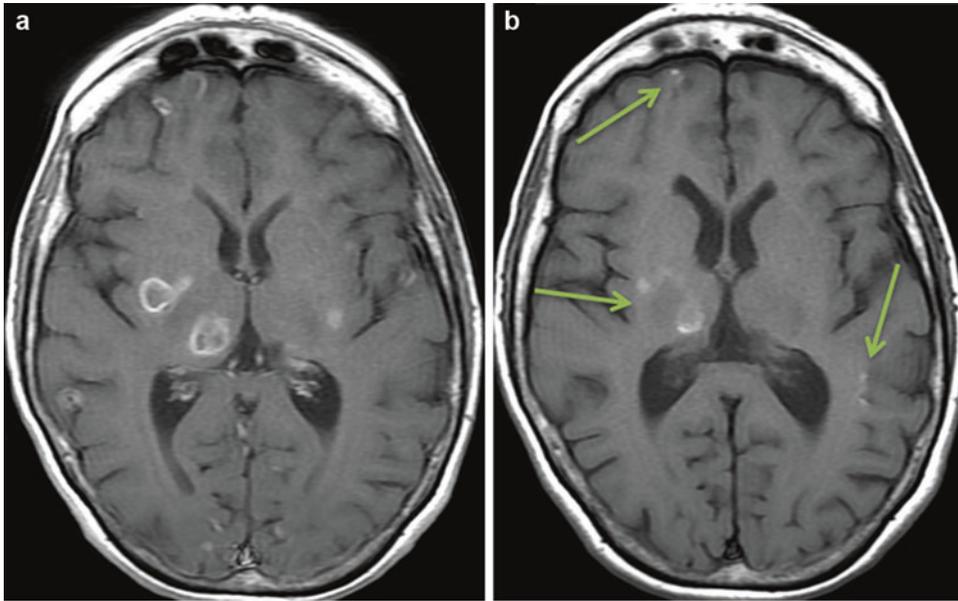
Diagnosis

Clinical Suspicion

Anatomical Neuroimaging (CT/MRI)

When cerebral toxoplasmosis is suspected, neuroimaging must be performed urgently. In most clinical series, 10–43% of patients with toxoplasmic encephalitis have a solitary parenchymal injury, demonstrated by computed tomography (CT); the remaining patients have more than one injury. It is unusual to have a negative CT scan in symptomatic patients (3–10%).

The most typical presentation of cerebral toxoplasmosis is the presence of multiple focal intracerebral lesions (Fig. 1), which are detected more often with magnetic resonance imaging (MRI), as it is more sensitive than CT. With MRI, over 80% of patients are found to have multiple lesions. Therefore, if there is a single lesion on CT, and this is confirmed by MRI, a lymphoma or other causes of focal injury associated with AIDS (as tuberculoma or histoplasmosis) should be ruled out. In cerebral toxoplasmosis, injuries are more often bilateral and



Toxoplasmosis in HIV-Infected Patients, Fig. 1 Toxoplasmosis in a 55-year-old female. Panel (a) Post-contrast axial T1-weighted MRI demonstrating multiple hemorrhagic enhancing lesions (nodular and ring

enhancing) with edema. Panel (b) Non contrast axial T1-weighted MRI taken 4 months later showing marked decrease in the number, size, and edema of lesions with residual calcified images (*green arrows*)

present contrast ring enhancement (80–90% cases) and produce a mass effect and edema. They often develop in the basal ganglia, the thalamus, or the corticomedullary junction of the cerebral hemispheres.

However, no presentation using CT or MRI is considered absolutely diagnostic of cerebral toxoplasmosis. Lymphoma and toxoplasmosis can produce very similar imaging, for example, multifocal disease and contrast reinforcing ring lesions can be seen in up to 40–50% of AIDS patients with primary CNS lymphoma (PCNSL). Hemorrhage before treatment may sometimes be seen in toxoplasmosis, and this finding can help differentiate toxoplasmosis from lymphoma. Furthermore, a “target sign” (an eccentric nodule inside an enhancing ring) is highly suggestive of toxoplasmosis (Smith et al. 2008). Advanced MRI techniques can also help differentiate diagnoses. Lymphoma lesions show reduced diffusion in diffusion-weighted imaging, and MR perfusion shows an elevated relative cerebral blood volume. HMR spectroscopy in lymphoma indicates elevated peaks of choline.

Functional Neuroimaging (SPECT/PET)

In an effort to clarify the noninvasive diagnosis of cerebral toxoplasmosis (which is always presumptive), other imaging techniques have been evaluated, particularly to help to differentiate it from PCNSL. Single-photon emission CT (SPECT) using thallium 201 and positron emission tomography (PET) using marked 2-fluorodeoxyglucose are useful techniques. In both techniques, infections (such as cerebral toxoplasmosis) are observed as cold or hypometabolic lesions, while lymphoma behaves like a hypermetabolic lesion. Both techniques are insensitive to small lesions, less than 8 mm, and, although rare, false positives and false negatives may occur. Although these techniques are useful to diagnose a PCNSL, they are not necessary to confirm toxoplasmosis. If cerebrospinal fluid (CSF) is obtained, these functional imaging techniques can be combined with molecular techniques, such as PCR for toxoplasmosis or the Epstein-Barr virus (EBV), in order to make reliable differential diagnoses between cerebral toxoplasmosis and primary CNS lymphoma (Antinori et al. 1999).

Therapeutic Trial as a Diagnostic Method and Indication to Brain Biopsy

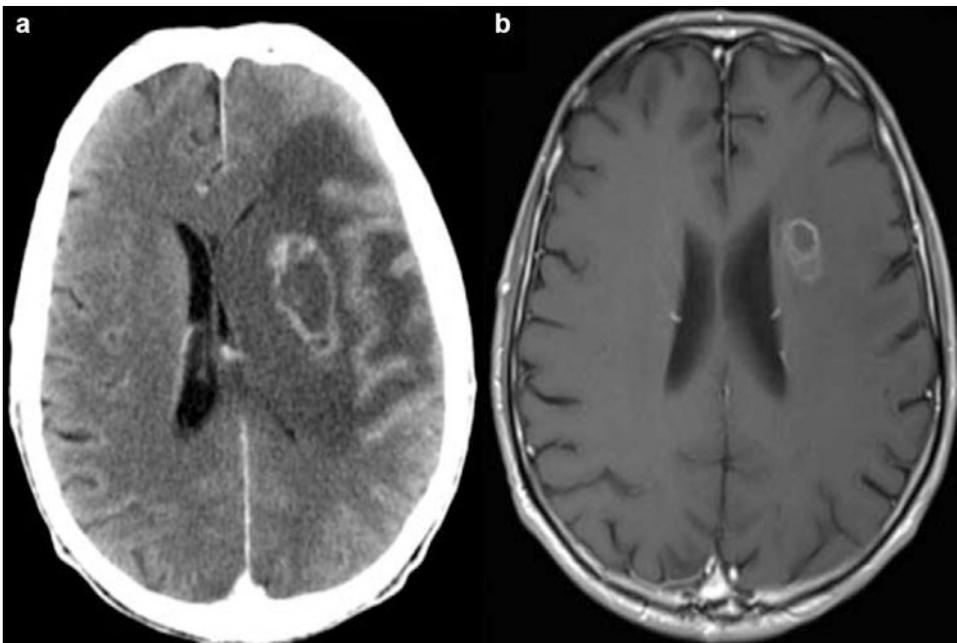
From a practical perspective, however, most of these patients undergo an empirical trial of anti-*Toxoplasma* therapy for 10–14 days. If after this period there is no clinical or radiological improvement, a brain biopsy should be performed. Most patients with cerebral toxoplasmosis (65–90%) respond readily to a treatment of two drugs: pyrimethamine, with either sulfadiazine, or clindamycin, with response rates of over 90% within 2–3 weeks (Fig. 2). Brain biopsy has been transformed over the years into a procedure now only reserved for a limited number of clinical situations, particularly for those patients who do not respond to empirical treatment or who have an alternative diagnosis suggested by imaging or microbiological CSF findings.

Microbiological Diagnosis

The definitive diagnosis of the cause of toxoplasmosis encephalitis is performed by the

demonstration of tachyzoites in the brain tissue (Fig. 3). However, as already mentioned, because of the invasiveness of brain biopsies and the lack of experience in many centers, this option is limited to rare cases in which diagnosis cannot be confirmed by other techniques or in which there has been no response to therapeutic treatment.

Until the advent of molecular biology techniques, the etiologic diagnosis of toxoplasmosis was made almost exclusively by serology. However, if the patient presents severe immunodeficiency, serology may fail or give misleading results, and there have been reports of a lack of *Toxoplasma* IgG response in serum in HIV-positive patients. However, in Western Europe and the United States, undetectable levels of IgG are very rare in determinations performed in reference laboratories. The presence of IgM anti-*Toxoplasma*, however, is difficult to detect in HIV-positive patients; and, since in most cases cerebral toxoplasmosis is caused by reactivations, it has limited value (Marcos et al. 2008).

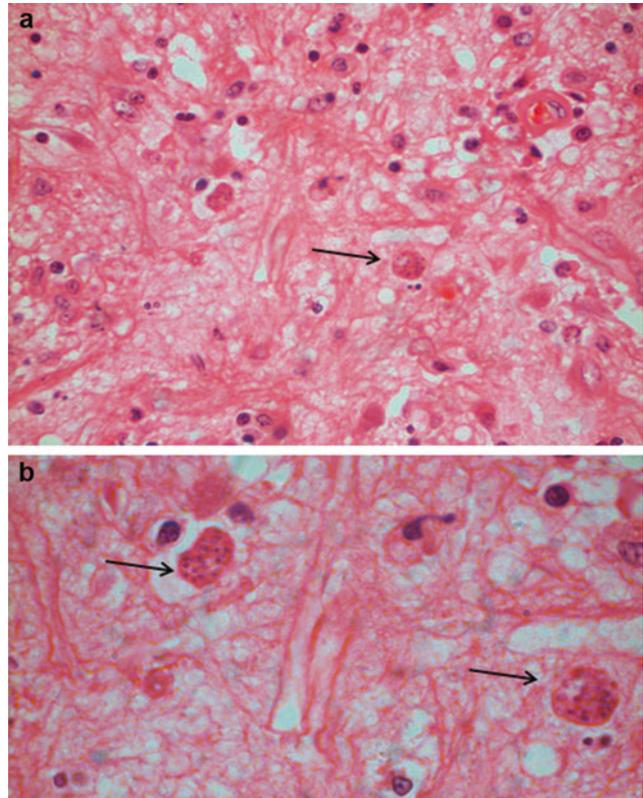


Toxoplasmosis in HIV-Infected Patients, Fig. 2 Cerebral toxoplasmosis. Panel (a) Post-contrast axial CT showing a large deep lesion in the left hemisphere with significant edema, mass effect, and contrast ring enhancement. A second smaller lesion seems to be present

behind the bigger one. Panel (b) Post-contrast axial T1-weighted MRI taken 3 weeks after anti-*Toxoplasma* treatment showing a marked reduction in lesion size, mass effect, and edema

Toxoplasmosis in HIV-Infected Patients,

Fig. 3 *Toxoplasma* encephalitis in humans (AIDS related) – ©LSHTM. Panel (a) Tachycyst of *T. gondii* in brain 100 × (arrows) – hematoxylin and eosin stain. Panel (b) Tachycyst of *T. gondii* (arrows) at bigger magnification – hematoxylin and eosin stain



Anti-*Toxoplasma* IgG detection in CSF is positive in 30–70% of patients with toxoplasmic encephalitis, although this alone does not constitute a diagnosis of brain disease. It does, however, have a high specificity when clinical presentation is also suggestive. Moreover, despite the detection of intrathecal IgG production being very useful, obtaining CSF is not always possible. In many patients with suspected toxoplasmosis, CSF extraction is avoided due to the risk of herniation, and in many centers it is not part of the routine diagnostic procedures for cerebral toxoplasmosis. If it is obtained, CSF frequently does not show any significant alteration, since these lesions are frequently not in contact with the subarachnoid space (Miro and Alvarez-Martinez 2013).

Molecular diagnostic techniques are not exempt from difficulties either. The main problem for the amplification of the toxoplasmic genetic material by PCR techniques is the absence of standardized protocols. Various types of PCR

have been used: conventional PCR, final-time PCR (semi-nested and nested formats), real-time PCR, and PCR followed by oligochromatography. Likewise, several genes have been used as *T. gondii* target amplification. The B1 is the most widely used gene, having 35 copies in the genome, and also the most stable. Other genes used as targets are the P30 gene, a uni-copy gene encoding the major surface antigen; rRNA, encoding the small subunit ribosomal RNA, with 110 copies in the genome; the fragment 529 bp with 300 copies per genome, the genes encoding α -tubulin and β -tubulin; and the DNA repetitive noncoding fragment TGR1E.

In addition to CSF, PCR can be also performed in the bronchoalveolar lavage, the vitreous and aqueous humor, pleural and peritoneal fluid, bone marrow aspirates, peripheral blood, and affected tissues, such as the brain.

Until the development of PCR-based techniques, brain biopsy was the gold standard for

diagnosis confirmation. Currently, the diagnosis is confirmed by PCR from blood or preferably from CSF, preventing the morbidity of intraoperative biopsy. The sensitivity of PCR blood is 16–86%, depending on the type of test used. With CSF, sensitivity is also very variable, from 17% to 100%. For both samples, the sensitivity decreases dramatically if the patient has previously received anti-*Toxoplasma* treatment. Specificity of CSF PCR is higher than 90%. Despite the variable sensitivity, the high specificity and the high positive predictive value of CSF PCR make it a very useful technique in the diagnosis of focal brain lesions in immunosuppressed patients with clinical and radiological suspicion. It must be remembered, however, that a positive PCR for *T. gondii* in the blood does not mean that the intracerebral process has the same etiology. Indeed, multiple opportunistic infections in extremely immunosuppressed AIDS patients are not unusual.

The most common form of extracerebral and extraocular toxoplasmosis in immunocompromised including HIV-infected patients is lung infection. Detection of *T. gondii* by PCR in lung tissue samples or bronchoalveolar lavage has a sensitivity and specificity of 100% in these cases. In the diagnosis of disseminated forms, detection by PCR in blood has a sensitivity and specificity of close to 100%. In ocular toxoplasmosis, DNA detection of the parasite in the aqueous humor is less sensitive than local antibody detection; indeed, PCR has a sensitivity of 18–37%. However, when the diagnosis is made in the vitreous humor, the sensitivity approaches 100%.

Therefore, despite the lack of standardization for molecular diagnostic methods for toxoplasmosis, they should still be considered to supplement or confirm a clinical, radiological, and serological diagnosis in immunocompromised patients as well as to be useful in monitoring the progress of patients with, or at risk of, toxoplasmosis.

Antimicrobial Treatment

Cerebral Toxoplasmosis

Treatment of cerebral toxoplasmosis includes treating the clinically active infection, followed

by maintenance therapy to suppress recurrent disease in patients with CD4⁺ T cell count below 200 cells/mm³. The recommendations for treatment almost always involve drug combination (Table 1).

The standard treatment for toxoplasmosis includes an association of pyrimethamine (loading dose of 100 or 200 mg followed by 75 or 50 mg/day) and sulfadiazine 1,000 mg (<60 kg) or 1,500 mg (>60 kg) every 6 h. In the outpatient setting, the sulfadiazine daily dose can also be given twice daily taking into account the serum half-life of 7–12 h. Intravenous trimethoprim-sulfamethoxazole is a recommended therapy for patients unable to receive oral therapy.

For patients with intolerance to sulfonamides, pyrimethamine plus clindamycin (600 mg four times daily, intravenously or orally) has shown comparable or slightly lower effectiveness in most studies (Katlama et al. 1996). Clindamycin has the advantage of an IV formulation. Clindamycin/pyrimethamine does not prevent *Pneumocystis jirovecii* pneumonia; therefore, a pneumocystis prophylactic regimen must be added. Patients with pneumonitis, chorioretinitis, the involvement of other organs, or a disseminated infection should receive the same treatment. Folinic acid (leucovorin calcium, 10–20 mg/day orally, although this dose can be increased to 50 mg/day or more) must be associated with a regimen containing pyrimethamine to reduce toxicity to bone marrow.

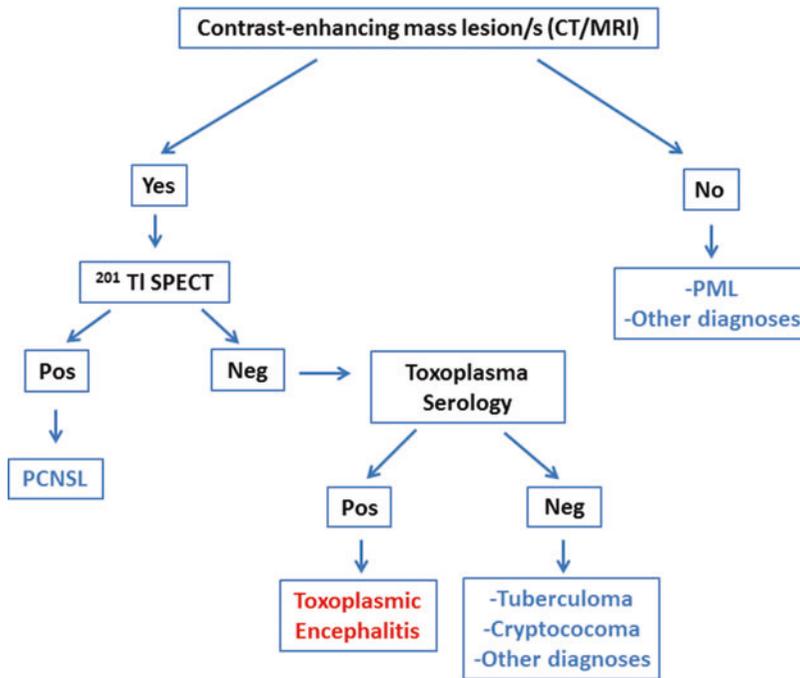
The clinical and radiological response of patients depends on the level of neuronal destruction and the timing of the initiation of therapy and of the immune response, but it should be evident during the first 3 weeks, at least clinically. Resistance of *T. gondii* to drugs used for therapy has not been documented. Most patients with chorioretinitis also respond and show an improvement in visual acuity within 6 weeks. Mortality rates in the acute phase of *Toxoplasma* encephalitis ranged between 5% and 20% in published series. Death usually occurs due to very advanced illness at the time therapy was initiated and is often due to other simultaneous opportunistic diseases. The prognosis for medium and long-term recovery depends on the state of

Toxoplasmosis in HIV-Infected Patients, Table 1 Prophylaxis and treatment of cerebral toxoplasmosis in patients with AIDS^a

Primary prophylaxis			
Indication	Preferred	Alternatives	Suspension/reinitiation
Positive IgG anti- <i>Toxoplasma</i> Ab and CD4 < 100/mcl (US) (AIII) or <200/mcl (Europe) All the regimens recommended for 1ry prophylaxis are effective against <i>P. jirovecii</i> <i>Prevention of exposure</i> Eat cooked enough (or previously frozen at -20 °C) meat, wash fruits and vegetables, and wear gloves if close contact with raw meat or gardening activities (BIII) Wear gloves and apply hygiene measures if close contact with domestic animals (cats) and their droppings Ensure that cats stay at home and avoid eating raw or undercooked meat (BIII)	Co-trimoxazole 1 comp DD (trimethoprim/sulfamethoxazole 160/800 mg) PO/d (AII)	Co-trimoxazole 1 comp DD ×3/sem PO (BIII) Co-trimoxazole 1 comp (trimethoprim/sulfamethoxazole 80/400 mg)/d PO (BIII) Dapsone 50 mg/d VO + pyrimethamine 25 mg + folic acid 25 mg ×2/sem PO (BI) Dapsone 200 mg + pyrimethamine 75 mg + folic acid 25 mg/sem PO (BI) Atovaquone 1,500 mg with/without pyrimethamine 25 mg + folic acid 15 mg/d PO (CIII)	Stop prophylaxis after ≥ 6 months of ART, if CD4 > 200 cel/mcl and VL undetectable during ≥ 3 months (AI) Resume prophylaxis if CD4 < 100–200 cells/mcl (AIII)
Treatment of acute episode			
Disease	Preferred	Alternatives	Comments
Focal abscesses in CNS or retinochoroiditis	Pyrimethamine 200 mg PO (loading dose), followed by: Pyrimethamine 50 mg/d PO + sulfadiazine 1,000 mg/6 h PO (<60 kg) Pyrimethamine 75 mg/d PO + sulfadiazine 1,500 mg/6 h PO (≥60 kg) + folic acid 15 mg/d VO (AI)	Pyrimethamine 50–75 mg/d PO + clindamycin 600 mg/6 h IV o PO + folic acid 15 mg/d PO (AI) Co-trimoxazole (trimethoprim/sulfamethoxazole, -trimethoprim 10 mg/kg/d-) IV o VO (BI) Atovaquone 1,500 mg/12 h PO + pyrimethamine 50–75 mg/d PO (and folic acid 15 mg/d PO) or + sulfadiazine 1,000–1,500 mg/6 h (BII) Pyrimethamine 50–75 mg/d VO + azithromycin 900–1,200 mg/d PO + folic acid 15 mg/d PO (CII)	Minimum duration: 6 weeks (prolong it if growing lesions of incomplete response) (BII) Clindamycin regimens do not protect against <i>Pneumocystis jirovecii</i> ; add specific prophylaxis (AII) If intracranial hypertension, add dexamethasone (BIII) If seizures, add antiepileptic drugs (not as prophylaxis) (AIII)
Suppressive therapy (secondary prophylaxis)			
Indication	Preferred	Alternatives	Suspension/reinitiation
All the patients who have completed the acute phase of therapy	Pyrimethamine 25–50 mg/d PO + sulfadiazine 1,000 mg/6–12 h VO + folic acid 15 mg/d VO (AI)	Pyrimethamine 25–50 mg/d VO + clindamycin 600 mg/8 h VO + folic acid 15 mg/d VO (BI) Co-trimoxazole 1 comp DD/12 h VO (BII) Atovaquone 750–1,500 mg/12 h PO + pyrimethamine 25 mg/d PO (+ folic acid 15 mg/d VO) or + sulfadiazine 1,000 mg/6–12 h (BII) Pyrimethamine 25–50 mg/d PO + azithromycin 500–1,000 mg/d PO + folic acid 15 mg/d PO	Stop if CD4 > 200 cells/mcl and ART > 6 months and viral load undetectable (BI) Resume prophylaxis if CD4 < 200 cel/mcl (AIII)

^aThe level of recommendation (A, B, C and I, II, III) corresponds to the Infectious Diseases Society of America (IDSA) Adapted from Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents, GESIDA and National AIDS Plan Expert Panel (2008), Guidelines of the AIDS Study Group (GESIDA), and National AIDS Plan (2008)





Toxoplasmosis in HIV-Infected Patients, Fig. 4 Neuroimaging work-up plan in AIDS patients with CNS lesions. A cerebrospinal fluid sample should be obtained when possible for viral (*CMV*, *EBV*, *JCV*), parasitic (*T. gondii*), and mycobacterial molecular diagnosis. *CT* computed tomography, *MRI* magnetic resonance image, *201TI SPECT* 201 thallium single-photon emission

computed tomography, *PML* progressive multifocal leukoencephalopathy, *PCNSL* primary central nervous system lymphoma, *CMV* cytomegalovirus, *EBV* Epstein-Barr virus, *JCV* JC virus. 201TI SPECT: useful to differentiate from PCNSL, but not necessary to confirm toxoplasmosis

immunosuppression. Patients with pneumonia or disseminated infection have a poor prognosis, often associated to the base state of immunosuppression.

Treatment of cerebral toxoplasmosis should continue for at least 6 weeks. Most clinicians continue therapy longer if the clinical manifestations do not improve or if lesions on MRI remain stable or do not significantly decrease in size for many weeks. Imaging studies should be repeated within 2 weeks to ensure that toxoplasmosis was the correct and only cause of the injury (Fig. 3). If the lesions have not improved, a biopsy (or a repeat biopsy) should be considered to determine whether more than one process was present. If toxoplasmosis is the only proven diagnostic and neurological symptoms have not improved after 2 weeks, an alternative regimen could be used, but there is no evidence that this strategy may improve the prognosis. Although serial scans

during the following months can demonstrate the complete or nearly complete resolution of the localized and multifocal disease, no repeat studies are required in a patient with clinical improvement receiving standard treatment. The initial diagnosis algorithm in a patient with suspected cerebral toxoplasmosis can be seen in Fig. 4.

Adverse Effects of Treatment and Alternative Regimens

Rashes and other adverse inflammatory reactions to sulfonamides are well recognized in HIV-infected patients. Additionally, patients treated with sulfadiazine can develop crystalluria, hematuria, renal colic, and occasionally some degree of kidney failure. Adequate fluid intake should be guaranteed to minimize tubular crystal formation.

Blood count should be monitored since pyrimethamine is associated with pancytopenia, especially with neutropenia. This effect may be more

pronounced if pyrimethamine is combined with sulfa drugs than with clindamycin.

The main adverse effect of clindamycin is diarrhea. In patients to whom no orally drugs can be given, trimethoprim-sulfamethoxazole has shown comparable activity in some small clinical trials and can be administered intravenously. Some authors consider trimethoprim-sulfamethoxazole as one of the preferred regimens.

Atovaquone is also an antimicrobial with antitoxoplasmic activity and may be associated with both pyrimethamine and sulfadiazine. It can also be used as monotherapy in patients unable to tolerate either of these drugs, but its effectiveness is reduced compared to the previously discussed regimens. Macrolides (azithromycin and clarithromycin) are also active molecules against *Toxoplasma*, but should be used in combination with pyrimethamine.

Adjuvant Use of Corticosteroids and Anticonvulsants

Adjunctive corticosteroids (e.g., dexamethasone) should not be administered routinely but only if a clinical evaluation indicates the need to reduce intracerebral pressure where there is considerable mass effect from focal lesions or when seizures are intractable. Patients receiving corticosteroids must be closely monitored to prevent the development of other opportunistic infections such as cytomegalovirus retinitis, disseminated cryptococcosis, and tuberculosis.

Anticonvulsants should be given to patients with proven seizures but not routinely to all patients with *Toxoplasma* encephalitis. Phenobarbital or phenytoin sodium is not recommended due to potential drug interactions with many antiretroviral drugs. Sodium valproate is preferred. There is no clear evidence of how long they should be continued after an initial convulsive attack. Anticonvulsants should probably be continued for at least the duration of the acute treatment.

Suppressive Therapy (Secondary Prophylaxis) After the Acute Episode

After at least 6 weeks of treatment, with clinical and radiographical response, patients should

maintain treatment for life (secondary prophylaxis or suppressive therapy), unless there is effective immune reconstitution in response to antiretroviral therapy with an increase in CD4⁺ T cells to above 200 cells/ μ l. Maintenance therapy should only be discontinued once CNS lesions show no contrast enhancement on CT/MRI. Without prophylaxis, rates of relapse for *Toxoplasma* encephalitis are 50–80% at 6–12 months in patients without effective immune reconstitution.

For maintenance therapy, most clinicians maintain the oral dosing regimen at a half dosage. Recurrences (10–40%) may occur and, as already mentioned, are due to nonadherence either to drug treatment or to serious immunodeficiency. Drug resistance is thought to be a rare event. In patients who were treated with trimethoprim-sulfamethoxazole, a reduction of the dose to a daily double-dose tablet seems a reasonable alternative to secondary prophylaxis.

Suppressive treatment can be stopped with immune reconstitution and is generally accepted after measurements of CD4 > 200 cells/ μ L in patients whose viral loads of HIV-1 remain undetectable for 3–6 months and who are adherent to treatment. In this situation, discontinuation is considered without risk of recurrence (Miro et al. 2006). The summary of the recommendations of treatment of acute episodes and primary and secondary prophylaxis are shown in the table.

Differential Diagnoses

As previously explained, PCNSL represents the most important diagnosis to exclude. PCNSL presents more frequently as a single lesion, hypermetabolic in SPECT or PET. If a lumbar puncture is not contraindicated, PCR for EBV is frequently positive. Indeed, the association of a single lesion in CT/MRI, a positive PET/SPECT, and a positive PCR for EBV has extremely high specificity and positive predictive value for PCNSL (Fig. 4).

In single lesions, with negative SPECT/PET and negative *T. gondii* IgG serology and no anti-*Toxoplasma* response, alternative diagnoses such as cryptococcoma or tuberculoma should be considered (Fig. 4). Both *Cryptococcus* and *M. tuberculosis* present more frequently as chronic meningitis, but they can produce single or multiple

intracerebral masses, which are normally hypometabolic in functional imaging techniques. PCR for *M. tuberculosis* or capsular Ag for *Cryptococcus* is usually positive in CSF. However, in some cases, confirmation may require the aspiration of material or a biopsy (Skiest 2002).

Other causes of multiple cerebral abscesses, such as a *Nocardia* infection or other bacterial and fungal diseases, may also be considered.

ART Initiation

In AIDS patients with opportunistic diseases, the early initiation of antiretroviral therapy (ART) within 2 weeks of the diagnosis of an opportunistic infection improves prognosis (reduces the risk of clinical progression to new AIDS events and death), with the exception of cryptococcal meningitis and probably of tuberculous meningitis. Therefore, the initiation of ART should not be delayed, although this entails an additional burden given the number of tablets and the risk of interactions. For toxoplasmosis, drug interactions do not represent a major problem, and there is no preferred antiretroviral regimen. Some clinicians prefer an antiretroviral combination with good penetration into the CNS, although it has not been shown that this is associated with a better prognosis. As with most opportunistic infections in patients with AIDS, there is a risk of worsening clinical manifestations when patients improve their immune status, a phenomenon known as immune reconstitution inflammatory syndrome (IRIS). However, it is rarely seen in cerebral toxoplasmosis, even if steroids are not used. Nevertheless, there should be very close monitoring of neurologic outcomes in patients during the first weeks of ART treatment. If IRIS is suspected, other concomitant neurological processes should be excluded. Treating IRIS involves the use of anti-inflammatory drugs such as corticosteroids.

Antimicrobial Treatment of Extracerebral Toxoplasmosis

Data on the outcome of treatment of AIDS patients with toxoplasmosis outside the CNS are limited; available information on the therapy of

ocular and pulmonary involvement indicates that these forms of toxoplasmosis are also responsive to treatment. Therapeutic combinations are the same used for toxoplasmic encephalitis, although duration of therapy is less well established.

Prevention

Primary Prophylaxis

Primary prophylaxis is indicated in patients with a positive serology for *T. gondii* with CD4 < 100 cells/ μ L according to the US guidelines and in patients with CD4 < 200 according to some European experts. In practice, the patients should receive trimethoprim-sulfamethoxazole where CD4 < 200 cells/ μ L to prevent *P. jirovecii* pneumonia, typically a double-dose tablet (160/800 mg) three times a week. This regimen protects very effectively against toxoplasmosis. Pyrimethamine alternative schemes are associated with dapsone, sulfadoxine, or atovaquone or also atovaquone alone (Table 1).

HIV-Infected Patients Seronegative for *T. gondii*

In severely immunocompromised patients who are seronegative for *T. gondii*, primary infection with *T. gondii* should be avoided (by not eating raw meat or poorly washed vegetables).

Conclusions

Cerebral toxoplasmosis continues to be a major problem with high morbidity and mortality in severely immunocompromised HIV-positive patients and, less frequently, in other patients with depressed cellular immunity. The diagnostic approach, treatment, and prognosis have remained relatively unchanged in recent years. Neurological *Toxoplasma* involvement is still prevalent in patients with late diagnosis of HIV infection, so detailed knowledge is essential for specialists in neurology, infectious diseases, clinical microbiology, and internal medicine.

Cross-References

- ▶ [AIDS-Related Primary Central Nervous System Lymphoma](#)
- ▶ [Antiretroviral Medications, Adult Care, and Treatment](#)
- ▶ [Cryptococcosis and HIV](#)
- ▶ [Tuberculosis and HIV](#)

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Transcription (Initiation, Regulation, Elongation)

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Definition of HIV Transcription

After integration, the HIV provirus serves as a template for the transcription of both viral messengers and genomic RNA by the cellular RNA polymerase II (RNAPII). Proviral transcription is initiated by the viral promoter and is determined by cellular factors and environment. In contrast, HIV-1 transcription elongation is dependent on the viral trans-activator protein Tat in addition to cellular elongation factors. Indeed, HIV-1 gene expression is characterized by an early Tat-independent phase, where the elongation is inefficient, and a late Tat-dependent phase, where the elongation is processive. The regulation of HIV-1 transcription also depends on a number of other elements discussed in this entry.

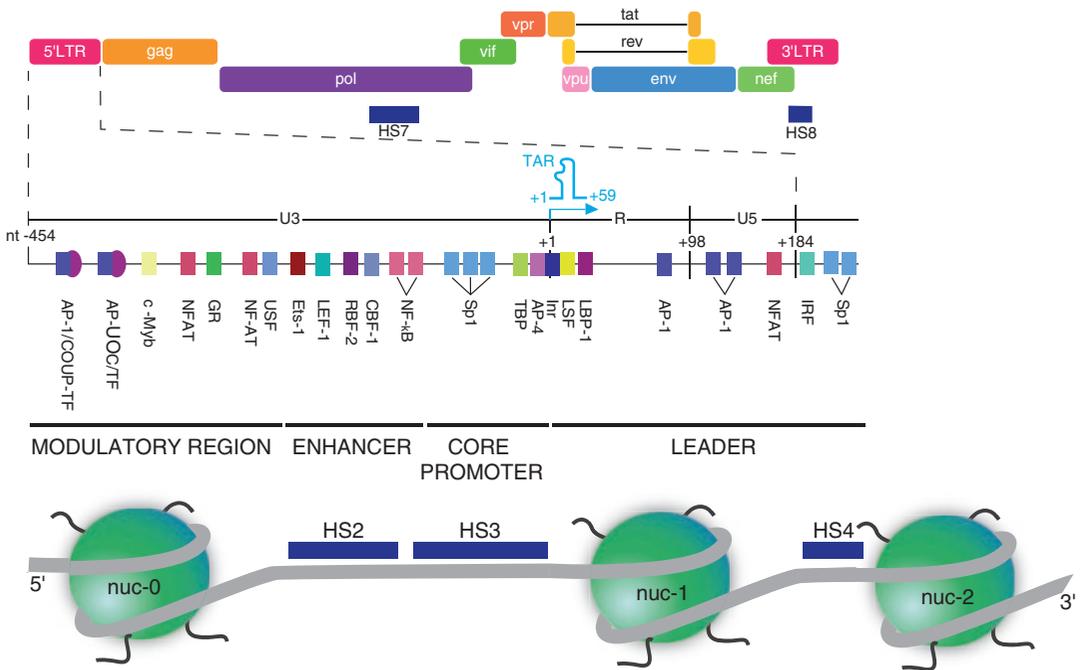
Mechanisms of HIV-1 Transcription

Organization of the HIV-1 Promoter

The main HIV-1 transcriptional *cis*-regulatory elements are located in the viral long terminal repeats (LTRs). These are present at both extremities of the integrated DNA and have been divided in three regions, named U3 (unique in 3'), R (repeated), and U5 (unique in 5') in reference to their respective origin in the viral RNA genome (Fig. 1). Transcription initiates at the U3/R junction in the 5' LTR and transcripts are polyadenylated at the R/U5 junction in the 3' LTR.

The HIV promoter has been extensively characterized *in vitro* and is divided in four functional domains that control basal and activated transcription. Binding sites for several transcription factors (TFs) have been identified in each of these domains using *in vitro* footprinting and gel retardation assays (Fig. 1). From the 5' end to the 3' end, the

four domains are as follows. Firstly, the modulatory region contains two binding sites for the AP-1 and/or the COUP cellular TFs, as well as binding sites for the glucocorticoid receptor (GR), NFAT, and USF/TFE-3. Secondly, the enhancer is composed of a distal region and a proximal region. The distal enhancer region contains DNA-binding sites for the cellular TFs TCF-1/LEF-1 and Ets-1, and the proximal enhancer region contains two binding sites for NF-κB. Thirdly, the core promoter contains three Sp1 binding sites, a TATA box, and an initiator (Inr) element close to the transcription start site. Finally, the leader region contains the inducer of short transcript element and binding sites, for example, UBP-1/LBP-1, AP-1, NFAT, and Sp1. Furthermore, the latter region encodes the transactivating response (TAR) element (nucleotide (nt) 1–59, where nt +1 represent the transcription start site) whose RNA forms a stable stem-loop structure (Fig. 1).



Transcription (Initiation, Regulation, Elongation), Fig. 1 Schematic representation of the HIV-1 5' LTR. The HIV-1 genome is represented at the top of the figure. Major hypersensitive sites (HS, blue boxes) located through the genome and nucleosomal organization of the HIV-1 genome 5' region are indicated. The U3, R, U5, and leader regions are indicated. Nucleotide 1 (nt +1) is the

transcription start site of HIV-1. The four functional regions involved in transcriptional regulation as well as the transcription factor binding sites identified in these regions are shown. See text for details (This figure is adapted from Colin and Van Lint, *Retrovirology* 2009, with permission from BioMed Central)

The HIV-1 pandemic is caused by at least nine subtypes (A, B, C, D, F, G, H, J, and K) from the major (M) group and an increasing number of recombinant forms. The LTR of the HIV-1 genome is one of the most conserved regions among virus isolates and subtypes. This relative conservation indicates that the LTR is subject to strict constraints because it is important for viral gene expression and replication. Although, most HIV-1 isolates have three Sp1 sites and at least two NF- κ B sites, the subtype C promoter contains three canonical NF- κ B motifs. There is considerable variation in the presence of other binding sites, such as AP-1, NFAT, and USF, and some of these differences are subtype specific.

Under basal conditions, two major DNase I-hypersensitive sites (short region of chromatin detected by its super sensitivity to cleavage by DNase I and other various nucleases) are present in the 5' LTR: HS2 (nt -234- > -132) and HS3 (nt -67- > -7), which map to the HIV-1 promoter in the U3 region (Fig. 1). Site HS4 (nt 201- > 265) is located immediately downstream of the 5' LTR in a region overlapping the primer binding site (Fig. 1).

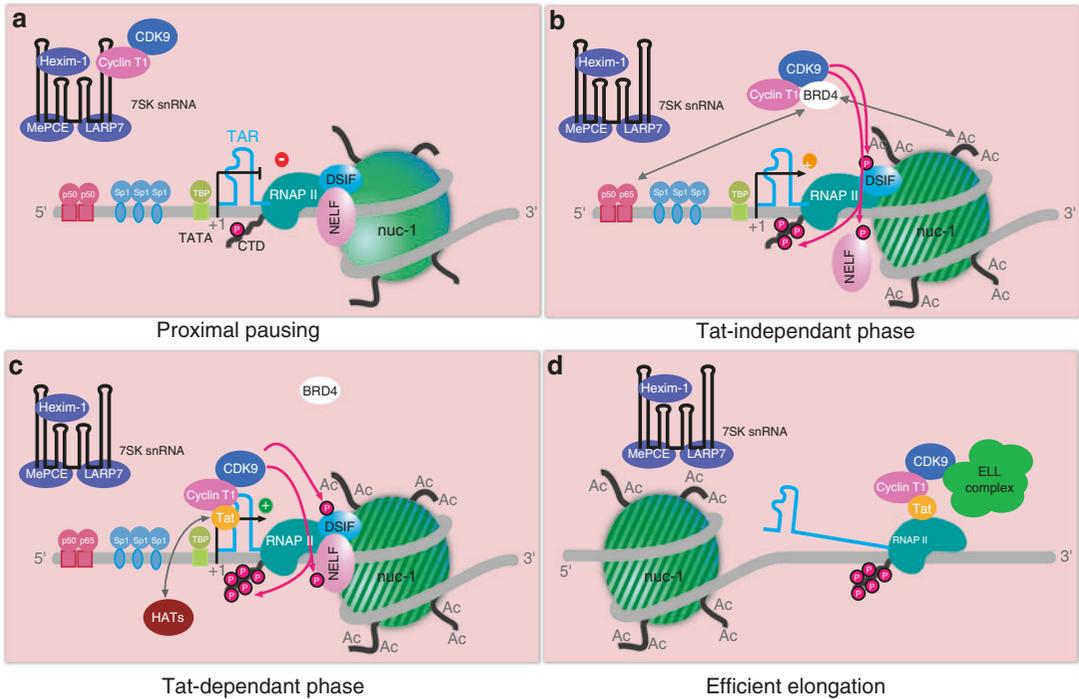
The 3' LTR, containing the polyadenylation signal for viral transcripts, exhibits a pattern of DNase I hypersensitivity that is different from the 5' LTR, with only a single major hypersensitive site (HS8) mapping to nt 8,867- > 9,034 (Fig. 1).

Besides the presence of DNase I-hypersensitive sites in the LTRs, a single major hypersensitive site (named HS7) has been identified in the part of the *pol* gene coding for the integrase (centered on nt 4,035- > 4,311, Fig. 1). Several ubiquitous and cell-specific TFs have been shown to be recruited to the HS7 region (including Oct-1, Sp1/Sp3, PU.1, and AP-1) and to be important for viral infectivity (Colin et al. 2011; Goffin et al. 2005).

Chromatin analyses with micrococcal nuclease (which cuts DNA in nucleosome-free regions and in linker regions separating nucleosomes) and with restriction enzymes have allowed establishing the nucleosomal organization of the 5' LTR. Two nucleosomes (called nuc-0 and nuc-1) are positioned at the viral promoter DNA at precise locations with respect to regulatory

elements (Fig. 1). Nuc-0 is positioned immediately upstream of the modulatory region and nuc-1 immediately downstream of the viral transcription start site. These nucleosomes define two open regions of chromatin corresponding to the modulatory region plus the enhancer/core promoter region (HS2 + 3) and a regulatory domain in the leader region downstream of the transcription start site (HS4) respectively. Interestingly, nucleosomes within the LTR are not deposited according to their thermodynamically most favorable positions (Mahmoudi 2012). Indeed, the comparison between the predicted nucleosome affinity of the HIV LTR sequence (using NuPoP software tool) and the known *in vivo* nucleosome distribution demonstrated a striking reverse correlation. The DNA sequence encompassing the HS2 and HS3 region displayed the highest affinity for nucleosome formation, while the strictly positioned nuc-0 and nuc-1 sequences displayed lower nucleosome propensity. The concerted effect of various TF binding sites in the HS2 and HS3 regions and their relative orientation mediate combinatorial interactions between multiple DNA TFs and cofactor complexes, resulting in a structure thought to be inconsistent with the coexistence of stable nucleosomes. Indeed, the BAF subcomplex of ATP-dependent SWI/SNF chromatin-remodeling complex specially functions to counteract intrinsic histone-DNA sequence preferences at the LTR and to move a preferred nucleosome from HS2 and HS3 regions to position nuc-1 over suboptimal sequences immediately downstream of the transcription start site, explaining that this region is devoid of nucleosome. However, this region still contains a loosely positioned nucleosome, which is evicted upon activation. A genome-wide study revealed that hypersensitive regions to nucleases, which are often assumed to be devoid of nucleosomes, are in fact occupied by histone variants (Mahmoudi 2012).

However, nucleosome positioning is not static and in a growing number of cases, changes in nuclease sensitivity secondary to the disruption of specific nucleosomes have been observed during transcriptional activation. During HIV transcriptional activation, only one change occurs in



Transcription (Initiation, Regulation, Elongation), Fig. 2 Mechanisms of transcriptional activation by the viral protein Tat and the cellular elongation factor P-TEFb. In the absence of Tat (a), transcriptional initiation at the HIV-1 promoter is driven by the recruitment of RNAPII via TBP. However, transcription elongation is impeded as the result of the presence of the nuc-1 nucleosome, the activity of DSIF and NELF, and the sequestration of P-TEFb (composed of cyclin-dependent kinase Cdk9 and Cyclin T1) in an inactive snRNP complex containing 7SK RNA, HEXIM-1, and the RNA-binding proteins MePCE and LARP7. Upon activation (b), P-TEFb is released from its inactive form and is recruited to the HIV-1 promoter through interactions with Brd4 and NF-κB. Once recruited to the HIV-1 promoter, Cdk9

phosphorylates the CTD of RNAPII, DSIF, and NELF, allowing occasional full-length transcripts. Following the synthesis of the first molecules of Tat (c), Tat binds to the TAR RNA and enhances RNAPII processivity by recruiting P-TEFb from its inactive form and by competing with Brd4. This recruitment allows enhanced transcription of the full HIV-1 genome. Transcriptional elongation of the HIV-1 genome (d) is further stimulated because Tat can also recruit, in addition to P-TEFb, other elongation factors (such as ELL2, AFF4, ENL, and AF9), thereby forming the super elongation complex. This larger complex contains notably members of the mixed-lineage leukemia (MLL) family, including ELL2 that stimulate the rate of RNAPII transcriptional elongation. See text for details

this organization: nuc-1 is specifically disrupted (Verdin et al. 1993). In conclusion, nucleosome positioning in the 5' LTR appears to be an intrinsic property of the LTR, as the same positions were observed independently of the different integration sites in cell lines.

Initiation and Elongation of HIV-1 Transcription

As previously described, the HIV-1 5' LTR region contains numerous sites for cellular TFs. These factors help control the rate of RNAPII transcription initiation from the provirus, and their

abundance in different cell types or at different times likely determines whether a provirus is actively transcribed or not. Despite the importance of these factors, HIV-1 transcription is strongly dependent on the viral trans-activator Tat. Therefore, transcription of the HIV-1 provirus is characterized by an early Tat-independent phase and a late Tat-dependent phase (Fig. 2).

During the Tat-independent phase, the HIV-1 promoter is strictly under the control of the local chromatin environment and cellular TFs binding to *cis*-acting elements in the viral promoter region. However, transcription is initiated normally but

elongation is inefficient and results in short abortive transcripts that cannot support viral replication. Indeed, at initiation, the TATA-Binding Protein (TBP) recognizes the TATA box and initiates the recruitment of the TBP-associated factors (TAFs), which assemble into the TFIID complex. Next, additional general TFs associate with RNAPII to form the pre-initiation complex (PIC). The C-terminal domain (CTD) of the largest subunit of the RNAPII is not phosphorylated and binds the Mediator complex. In the absence of Tat, critical TFs, such as NF- κ B and Sp1, are required for the formation of the PIC and the recruitment of TBP and TAFs. Then, TFIIF binds to the promoter and phosphorylates serine 5 of the CTD. This event stimulates promoter clearance and synthesis of the first nucleotides of RNA. However, after synthesis of a short RNA that includes TAR, NELF (negative elongation factor) and DSIF (DRB sensitivity-inducing factor) are directly recruited and induce RNAPII pausing (Fig. 2a). The pausing induced by DSIF and NELF could allow the cleavage/polyadenylation factor Pcf11 to dismantle the elongation complex. In addition to the presence of negative transcription elongation factors (N-TEF), RNA also could contribute to pausing by an RNA interference-independent mechanism. Nuc-1, by its position immediately after the transcription start site, could also impede the progression of RNAPII by accentuating a natural pausing site. In the early phase, cellular TFs, especially NF- κ B, and acetylated histones both via interaction with the bromodomain-containing protein 4 (BRD4) recruit the positive transcription elongation factor b (P-TEFb), composed of cyclin-dependent kinase 9 (Cdk9) and Cyclin T1 (Fig. 2b). P-TEFb is required to overcome early blocks after promoter clearance, thus allowing for elongation of transcription. Once recruited to the transcriptional complex, Cdk9, the catalytic subunit of P-TEFb, phosphorylates serine at position 2 of the CTD and marks the transition from initiation to elongation of transcription (Fig. 2b). Moreover, P-TEFb also phosphorylates NELF and DSIF (Fig. 2b). The phosphorylation of NELF causes its dissociation from TAR and releases paused transcription elongation complexes. Although the unmodified DSIF

inhibits elongation, phosphorylation of Spt5 separates it from the rest of the complex and converts it into a positive elongation factor that stabilizes transcription complexes at terminator sequences.

Despite elongation defect, occasional full-length genomic transcripts are generated, leading to the synthesis of a few Tat molecules. The synthesis of a few molecules of Tat is sufficient to stimulate HIV-1 transcription and causes an increase of the RNAPII processivity that allows the transcription of the entire provirus. The viral trans-activator protein Tat is an atypical transcriptional activator that functions through binding RNA TAR, present at the 5' end of all nascent viral transcripts (Fig. 2c). The binding of Tat to TAR enhances the processivity of transcribing RNAPII complex by recruiting cellular proteins. Indeed, Tat recruits Cyclin T1/Cdk9 either from the inactive complex containing the 7SK snRNA, HEXIM1, the La-related protein 7 (LARP7), and the 7SK-specific methyl-phosphate-capping enzyme (MePCE), by competing with HEXIM for Cyclin T1 binding, or from the active Cyclin T1/Cdk9 complex containing BRD4 present on cellular genes (Fig. 2c; Karn and Stoltzfus 2012; Taube and Peterlin 2013). Cyclin T1 interacts cooperatively with both the N-terminal transactivation domain of Tat (amino acid 1–48) and the loop sequence at the top of the TAR's stem-loop structure. As a consequence, P-TEFb and Tat bind TAR with higher affinity and specificity than Tat alone. Cdk9 then phosphorylates the CTD of the previously bound RNAPII complex. In the presence of Tat, the substrate specificity of Cdk9 is altered, such that the kinase phosphorylates both serine 2 and serine 5 of the CTD instead of serine 2 alone. Moreover, Tat can recruit PCAF, a histone acetyltransferase (HAT), which could play a role in chromatin-remodeling following transcriptional activation. Tat itself is also post-translationally modified (► Tat) by acetylation, for example, in order to promote the recruitment of P-TEFb to the HIV-1 promoter or dissociation of Tat from TAR leading to its subsequent transfer to the elongating RNAPII complex (Fig. 2d). After, the Tat/P-TEFb complex dissociates from the RNAPII complex following transcription initiation and undergoes subsequent cycles of

association/dissociation. Tat deacetylation by Sirtuin 1 (SIRT1), a class III protein deacetylase, allows its dissociation from the RNAPII and PCAF complex and its recycling to initiate a new cycle of transcriptional activation. This function might be important when the amount of Tat is a limiting factor, especially at the early phase of infection. The binding of Tat to TAR also promotes the recruitment of various cellular cofactors to the HIV-1 5' LTR including histone-modifying enzymes, such as the HATs p300 and CBP and chromatin-remodeling complexes, likely reinforcing an acetylated and open chromatin environment. Tat first recruits the PBAF subcomplex of ATP-dependent remodeling complex SWI/SNF via its interaction with BRG-1 and In1 (integrase interactor-1) subunits, allowing the initiation of nuc-1 remodeling (Mahmoudi 2012). Consequently, the optimal activity of Tat is furthermore dictated by its association with different cellular factors while being subject to other post-translational modifications (► [Tat](#)). In this way, Tat increases the production of viral mRNA by ~100-fold and allows the production of a large amount of mRNA in a short time. Tat is also an effective elongation factor because it is able to recruit the “super elongation complex” (SEC) in addition to P-TEFb (Sobhian et al. 2010; Fig. 2d). SEC components of the Tat/P-TEFb complex include ELL1 and its homologue ELL2, AFF1 and its homologue AFF4, ENL, AF9, and the components of the polymerase-associated factor complex. ELL1 and ELL2 are well-characterized transcription elongation factors that stimulate the processivity of RNAPII and act in concert with P-TEFb. In addition to its classically recognized role in the induction of transcriptional elongation and chromatin remodeling, Tat may also influence transcriptional initiation by facilitating the assembly of the pre-initiation complex requiring the Sp1 and NF- κ B binding sites. Increasing evidence suggests that Tat also plays a role in splicing, capping, and polyadenylation processes. Consequently, Tat acts at several levels in HIV-1 transcription (► [Tat](#)).

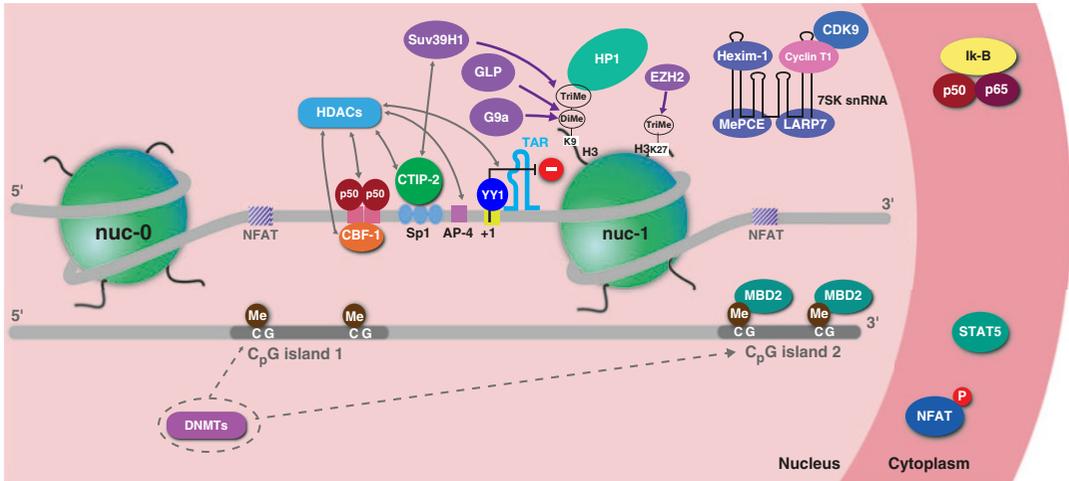
Splicing, capping, and polyadenylation processes occur mostly cotranscriptionally (Karn and Stoltzfus 2012). First, pre-mRNA is capped

at its 5' end by capping enzymes: RNA triphosphatase, RNA guanylyltransferase, and RNA (guanine-N7) methyltransferase. This step is facilitated by RNAPII pausing. The capping reaction is also stimulated by Tat via the TAR-dependent phosphorylation of the CTD by P-TEFb. Further processing of viral pre-miRNA by the host splicing machinery gives rise to a variety of transcripts and consequently, many proteins, due to the alternative splicing. Indeed, nascent transcripts include several 5' splice donor (five), 3' splice acceptor (nine), and branch point sites in order to result in over 40 differently spliced mRNAs. Generated mRNAs are divided into three size classes: (i) unspliced RNA (9 kb) serving as genomic RNA or to produce the Gag and Gag-Pol precursors; (ii) singly spliced (4 kb) RNA encoding Tat exon1, Vif, Vpu-Env, and Vpr; or (iii) fully spliced (2 kb) RNA expressing Tat exon1 + 2, Rev, and Nef (Fig. 1; ► [HIV Life Cycle](#)). Transport of unspliced and partially spliced mRNAs from the nucleus to the cytoplasm is mediated by the viral Rev protein which interacts with the rev responsive element (RRE) in the viral RNA and the cellular export factor Crm1 to connect these viral RNAs to the export machinery (Rev). Before the transport to the cytoplasm, the final step in the processing of the nascent transcript is polyadenylation. The 3' processing and polyadenylation of pre-mRNAs involves recognition of the upstream AAUAAA and downstream GU-rich motifs surrounding the cleavage and poly (A) addition site. For this, host cellular proteins such as the cleavage/polyadenylation specificity factor (CPSF), CstF, CF1m, CF2m, and poly (A) polymerase are required for endonucleolytic cleavage and polyadenylation of viral pre-mRNA. HIV provirus contains two identical LTR and each of them has a poly(A) site. However, the poly (A) site in the 5' LTR has to be ignored for HIV transcription.

HIV-1 Transcriptional Regulation

Transcriptional Regulation

At the level of transcription, the major contributors to transcriptional downregulation are



Transcription (Initiation, Regulation, Elongation), Fig. 3 Mechanisms of HIV-1 transcriptional repression. The main transcription factor binding sites and the nucleosomal organization of the HIV-1 genome 5' region are schematically represented. The transcription start site (nucleotide +1, nt +1) and the nuc-1 nucleosome are indicated. During latency, nuc-1 blocks transcriptional initiation and/or elongation and is maintained hypoacetylated by HDACs recruited to the HIV-1 promoter via several transcription factors or the CTIP2 cofactor. Histone methyltransferases (Suv39H1, G9a, GLP, and EZH2) are also recruited and methylate histone 3 tails on lysines 9 and 27. In addition, the HIV-1 proviral promoter is

hypermethylated by DNMTs in two CpG islands surrounding the transcription start site. Moreover, transcription factors essential for HIV-1 transcription are maintained in an inactive form (in the cytoplasm: sequestration of the active form of the NF-κB (p65/P50 heterodimers) by IκBα, phosphorylated NFAT, and unphosphorylated STAT5; in the nucleus: sequestration of inactive P-TEFb in the nuclear snRNP complex). In this context, several compounds have been proposed for HIV-1 transcriptional reactivation from latency. See text for details (This figure is adapted from Van Lint et al., *Retrovirology* 2013, with permission from BioMed Central)

transcriptional interference, the chromatin environment, lack of key host TFs, and lack of Tat and associated cofactors (Fig. 3).

Transcriptional Interference

HIV-1 integrates into the host genome in a non-random manner (► **Integration**). The finding that latent HIV-1 proviruses integrate in actively transcribed regions may seem paradoxical, considering the establishment of a transcriptionally latent state. Transcriptional interference (TI) has been proposed to explain HIV-1 promoter repression when integrated into introns of highly expressed genes. TI refers to the direct negative impact of one gene on another in *cis* by the following: (i) **Promoter occlusion**: when the provirus integrates in the same transcriptional orientation as the host gene, “read-through” transcription from an upstream promoter displaces key TFs from the HIV-1 promoter and prevents the assembly of the pre-initiation complex on the viral promoter,

thereby hindering HIV-1 transcription. (ii) **Steric hindrance**: provirus integration in the opposite orientation to the host gene may lead to the collision of elongating polymerases from each promoter, resulting in a premature termination of transcription from either the weaker or both promoters. Convergent transcription may also allow for the elongation of both viral DNA strands. The subsequent formation of double-stranded RNAs might lead to RNAi, RNA-directed DNA methylation, or generation of antisense RNAs. (iii) **Enhancer trapping**: this phenomenon can occur when an enhancer of provirus or host gene is placed out of context near the promoter of another gene.

Role of Transcription Factors

Molecular regulation of HIV-1 transcription is a multifaceted process dictated in part by the abundance of cellular TFs that induce or repress HIV-1 promoter activity (Van Lint et al. 2013). HIV-1



promoter activity is also tightly linked to the level of activation of its host cell. It is widely accepted that the lack of inducible key cellular TFs (NF- κ B, NFAT, and STAT5) is one element involved in the initiation and elongation repression of viral transcription in resting CD4⁺ T cells. The presence of host transcription repressors (i.e., DSIF, YY1, CTIP2, c-myc, CBF-1, p50 homodimers) also contributes to HIV-1 silencing.

Firstly, HIV-1 transcription is tightly coupled to the cellular activation status because transcriptional repressors are present under basal conditions, while transcriptional activators, such as NF- κ B and NFAT, are sequestered in the cytoplasm of quiescent T cells but recruited to the nucleus following T-cell activation. Recruitment of HATs by NF- κ B and NFAT proteins promotes chromatin-remodeling of the HIV-1 5' LTR. In contrast, YY1 in cooperation with LSF inhibits transcription.

Secondly, host TFs allow recruitment of chromatin-remodeling enzymes and cooperate in transcriptional regulation (Fig. 3). Indeed, several of these factors, including AP-1, c-Myb, GR, C/EBP, NFAT, E-box binding proteins, Ets-1, TCF-1 α /LEF-1, NF- κ B, Sp1, IRF, and the HIV-1 trans-activator Tat, have been shown to interact with HATs. Several other TFs that bind to the LTR, including unliganded nuclear hormone receptors (such as GR), E-box binding proteins, YY1, Sp1, TCF-1 α /LEF-1, AP-4, and NF- κ B, have been shown to interact with HDACs.

The observed redundancy in transcription activation pathways and the presence of many different transcription binding sites in HIV-1 favor productive viral infection in several cellular environments with large differences in the pool of TFs.

Role of the Chromatin Environment

Evidence has accumulated that the HIV-1 provirus is able to determine its local nucleosomal organization and that chromatin is an integral and dynamic component of the HIV-1 transcriptional regulation. Among these nucleosomes, a single one, nuc-1, is specifically and rapidly disrupted during transcriptional activation (see above). Nuc-1 disruption is independent of DNA replication and also of RNAPII transcription. Nuc-1

itself mediates HIV-1 transcription inhibition either by enhancing sequence-specific pausing or by blocking the binding of a TF. The mechanisms underlying the maintenance of a repressive chromatin state of the HIV-1 provirus and factors implicated in nuc-1 remodeling will be further discussed in association with epigenetic modifications of the HIV-1 promoter (see below). Epigenetic modifications are heritable modifications, which do not involve changes in the DNA sequence itself. They are dynamic, rapidly changing depending on the signaling conditions within the cell, and function sequentially or in combination to form "a code," which is read by effector proteins to produce distinct biological outcomes. These modifications principally include histone posttranslational modifications and DNA methylation.

Chromatin condensation is critical for the regulation of transcription initiation and elongation since it determines the DNA accessibility to regulatory TFs. The chromatin condensation status can be modulated through a variety of mechanisms that can work together: DNA methylation, ATP-dependent chromatin-remodeling events, and histone posttranslational covalent modifications. Indeed, DNA methylation is recognized by methyl-CpG-binding proteins (MBDs) that "read" DNA methylation patterns and allow the recruitment of other chromatin-modifying enzymes. ATP-dependent chromatin-remodeling complexes couple the hydrolysis of ATP to structural changes of the nucleosome. Histone posttranslational modifications, including acetylation, methylation, phosphorylation, sumoylation, ADP-ribosylation, and ubiquitination, are all reversible and mainly localized to the amino-terminal histone tails. These modifications influence gene expression patterns by two different mechanisms: (i) by directly altering chromatin packaging (electrostatic charge modifications or internucleosomal contacts might emphasize or reduce the access of DNA to TFs (such as histone acetylation)) and (ii) by generating interactions with chromatin-associated proteins (such as histone methylation). These modifications function sequentially or act in combination to form the "histone code" and serve as extremely selective

recruitment platforms for specific regulatory proteins that drive different biological processes. The major histone marks involved in HIV-1 repression are detailed below and in Van Lint et al. (2013).

Histone Posttranslational Modifications

Histone Acetylation An acetylation reaction consists in the transfer of an acetyl group from acetyl coenzyme A onto the ϵ -amino group of the lysine residue, resulting in the neutralization of the positive charge on the histone that promotes destabilization of histone-DNA interactions in the nucleosome. The histone acetylation levels are the result of a dynamic equilibrium between competing enzymes: HATs and HDACs. Generally, chromatin deacetylation by HDACs diminishes the accessibility of the nucleosomal DNA to TFs, thereby generating repressive heterochromatin, whereas acetylation by HATs promotes chromatin opening, resulting in increased accessibility of the chromatin to the transcription machinery. Moreover, histone acetylation marks enable the recruitment of bromodomain-containing proteins, such as chromatin-remodeling complexes and some TFs, which in turn regulate gene expression.

To date, numerous studies have demonstrated that HDAC1, HDAC2, HDAC3, and HDAC6 are recruited to the HIV-1 5' LTR (Van Lint et al. 2013). Following activation, cellular HATs, including p300/CBP, PCAF, and Gcn5, are recruited to the promoter. Several TFs have been shown to interact with HDACs and HATs (see above and Fig. 3). By altering histones, recruiting other chromatin-remodeling factors, and modifying the activity of certain TFs, HDACs appear to be critical for the epigenetic repression of HIV-1 transcription. Following the recruitment of HATs and chromatin-remodeling complexes, nuc-1 disruption allows viral transcriptional activation to occur (Van Lint et al. 1996; Verdin et al. 1993).

Histone Methylation Histone methyltransferases (HMTs) catalyze the transfer of one to three methyl groups from the cofactor S-adenosylmethionine to the lysine and arginine residues of histone tails. Histone methylation has

no effect on DNA/histone interactions but serves as a recognition template for effector proteins modifying the chromatin environment. Lysine methylation has been linked to both transcriptional activation and repression, depending on the site of modification. In general, methylation of histone 3 at lysine residue 4 (H3K4) is associated with transcriptional activation, while methylation of H3K9, H3K27, and H4K20 is associated with transcriptional repression.

Histone methylation marks (including H3K9me2, H3K9me3, and H3K27me3) have been shown to be associated with HIV-1 transcriptional silencing (reviewed in Van Lint et al. 2013; Fig. 3). The HMTs Suv39H1, G9a, GLP(-G9a-like protein), and EZH2, which are involved in H3K9me3, H3K9me2, H3K9me2, and H3K27me3, respectively, are recruited to the HIV promoter and are rapidly displaced following proviral reactivation (Fig. 3). In microglial cells, the main central nervous system HIV-1 reservoirs, Suv39H1 is recruited to the HIV-1 promoter via CTIP2, leading to a multi-enzymatic complex including HDAC1/HDAC2. CBF-1 (C-promoter binding factor-1) is responsible for the recruitment of EZH2 and other chromatin-modifying enzymes of the Polycomb complex to the HIV-1 promoter (Van Lint et al. 2013).

The histone demethylase (HDMT, which removes histone methylation with both lysine-site and methyl-state specificity) LSD1 is also recruited to the HIV-1 promoter and associated with both H3K4me3 and H3K9me3 marks. LSD1 functionally cooperates with CTIP2 to repress both viral replication and transcription in microglial cells (Van Lint et al. 2013).

Implication of SWI/SNF Complex

Remodeling of nuc-1 during transcriptional activation required ATP-dependent SWI/SNF chromatin-remodeling complexes (Mahmoudi et al. 2006; Treand et al. 2006). Two biochemically distinct SWI/SNF subcomplexes exist, the BAF and the PBAF complexes. The defining subunit of the BAF complex is BAF250a/ARID1a, and those of the PBAF complex are BAF180, BAF200, and BRD7. The presence of the distinct SWI/SNF subcomplexes BAF and PBAF has

been critical to distinguish between SWI/SNF-mediated LTR repression and Tat-mediated SWI/SNF recruitment. Possible mechanisms of BAF recruitment to the HIV promoter are (i) nuc-1-associated repressive TFs such as ATF3, (ii) LTR-bound TFs such as AP-1 and NFAT, (iii) distinct LTR pattern of histone modifications such as histone acetylation, and (iv) a unique DNA structure within the LTR. BAF is required for LTR repression and maintenance of latency. Upon transcriptional activation, BAF dissociates from the LTR, while the PBAF complex is selectively recruited by acetylated Tat to facilitate LTR transcription. Thus, BAF and PBAF, recruited during the different stages of the HIV-1 life cycle, display opposite functions in regulating LTR activity.

Implication of DNA Methylation

In mammalian cells, DNA methylation occurs as 5-methylcytosine predominantly in the context of CpG dinucleotides and is achieved by the specific recruitment of DNA methyltransferases (DNMTs). DNA methylation in transcriptional regulatory regions is generally associated with gene silencing, either by directly blocking binding of TFs to their recognition sequences or by indirectly preventing TFs from accessing their target sites through attachment of MBDs. These MBDs recruit HDACs and HMTs, thereby resulting in the formation of a closed repressive chromatin structure. During latency, the HIV-1 promoter is hypermethylated at two CpG islands surrounding the HIV-1 transcription start site as demonstrated in the latently infected cell lines (Blazkova et al. 2009; Kauder et al. 2009; Fig. 3). MBD2 and HDAC2 are recruited to the promoter via the second CpG island. Treatment with the demethylating agent 5-aza-2'-deoxycytidine decreases cytosine methylation in the two HIV-1 CpG islands, resulting in the loss of MBD2 and HDAC2 from CpG island 2 of the viral promoter region and in partial transcriptional reactivation (Kauder et al. 2009). However, to date, the DNA methylation status of the HIV-1 promoter in vivo in patient cells is still controversial (Van Lint et al. 2013).

Role of Tat and Its Cofactors

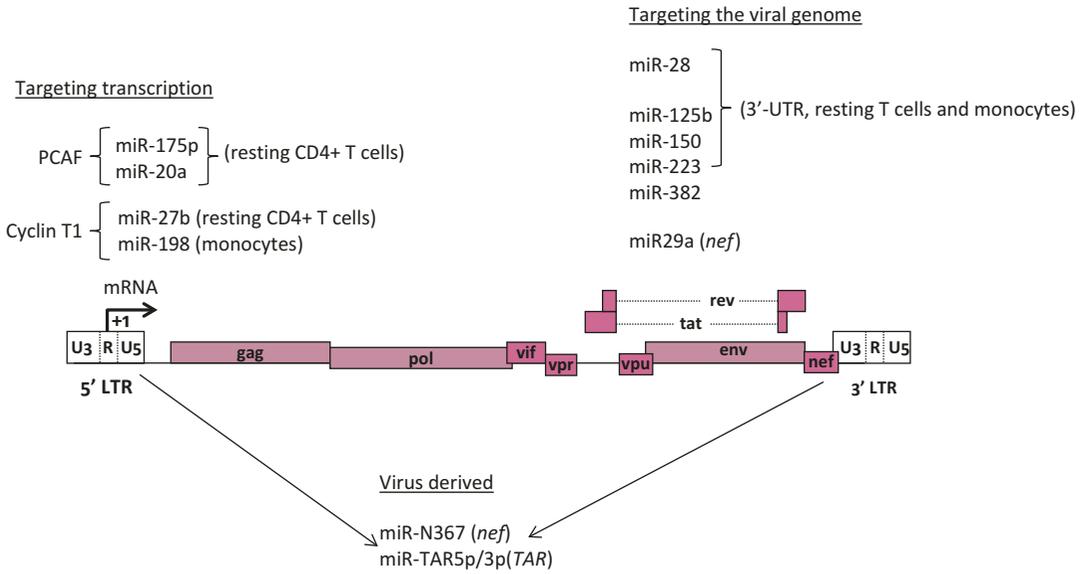
The intricate regulation of Tat function by post-translational modifications and its relationship with P-TEFb and several host cofactors are largely implicated in HIV-1 transcriptional regulation (see above and ► [Tat](#)).

Posttranscriptional Regulation

Considerable attention has focused on the role of chromatin and transcription (co)factors in the control of HIV-1 transcription. However, mechanisms operating at the posttranscriptional level such as inefficient viral mRNA transport or implication of miRNA are also implicated in HIV-1 gene expression.

The generation of infectious retroviral progeny requires the synthesis and export to the cytoplasm of the partially spliced and unspliced genomic RNAs. The viral protein Rev promotes the export of these RNAs (Rev). In resting T cells from HIV-1-infected individuals on cART, both Tat and Rev transcripts are retained in the nucleus. The defect in viral RNA export principally results from insufficient levels of Rev.

MicroRNAs (miRNAs) are short single-stranded noncoding RNAs that act as gene regulators. Primary miRNAs are sequentially processed via the nuclear RNases III Droscha and Dicer to generate mature miRNAs which interact with a complementary sequence in the 3' untranslated region of target mRNAs by partial sequence matching, resulting in degradation of the mRNA and/or translational inhibition. miRNAs can also regulate gene expression at the epigenetic level, by specifically inducing methylation along the promoter region or by directly generating the remodeling of the surrounding chromatin. Upregulation and downregulation of cellular miRNAs induced by HIV-1 infection have been observed in patient cells (Triboulet et al. 2007). Indeed, Tat and possibly Vpr function as RNA silencing suppressors, being able to modulate miRNA expression levels in infected cells. Cellular miRNAs can inhibit HIV-1 gene expression by decreasing the levels of essential cellular cofactors such as PCAF (regulated by miR175p and miR20a) and Cyclin T1 (regulated by miR27b)



Transcription (Initiation, Regulation, Elongation), Fig. 4 Modulation of HIV-1 replication by miRNA. HIV-1 impacts the cellular miRNA pathways in several ways. The figure shows a schematic representation of the HIV-1 provirus with the indicated viral genes and LTRs, including the transcription start site in the 5' LTR. PCAF and Cyclin T1, which are both essential cofactors for viral transcription, are targeted by the indicated miRNAs in

resting T cells and monocytes. A cluster of miRNAs is targeted directly toward the 5' end of the HIV-1 genome, and their inhibition rescues infectious virus from resting T cells. Finally, two sets of miRNA that are derived from the HIV-1 genome are shown. Their location within the LTR (TAR and *nef*) is duplicated (arrows) (From Van Lint et al., *Retrovirology* 2013, with permission from BioMed Central)

and are involved in the susceptibility to HIV-1 infection of different cell types. Alternatively, miRNAs may participate in repressing HIV-1 gene expression by directly targeting HIV-1 mRNAs in resting CD4⁺ T cells. Five cellular miRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) recognize the 3' end of HIV-1 mRNAs and are upregulated in resting, but not in activated CD4⁺ T cells. Moreover, the HIV-1 *nef* gene contains a miR-29a-targeted site that interferes with HIV-1 replication. Finally, several HIV-1-derived miRNAs exist and include TAR-derived miRNA-TAR5p/3p and the *nef*-derived miR-N367. TAR-derived miRNAs may target HIV-1 transcription directly. A summary of the principal miRNAs affecting HIV-1 is shown in Fig. 4 and in Van Lint et al. (2013).

Impact of HIV Transcriptional Regulation

HIV-1 transcription repression is crucial for the establishment and maintenance of latency

(Siliciano and Greene 2011; Trono et al. 2010; Van Lint et al. 2013). Elements implicated in HIV-1 transcriptional regulation described above represent potential targets for anti-latency strategies since they can be pharmacologically modulated (reviewed in Van Lint et al. (2013)). Indeed, a major approach to eradicating HIV-1 involves reactivation of HIV viral gene expression in cART patients. Cells harboring induced proviruses could then be lysed by HIV-1-specific cytolytic T lymphocytes (CTL), while new rounds of infection are blocked by cART. HIV-1 reservoirs containing stably integrated, transcriptionally silent but replication-competent proviruses are a permanent source for virus reactivation and could be responsible for the rebound of viremia observed after cART interruption. Persistence of HIV-1 latency certainly represents the major challenge to finding a cure. Indeed, the levels of HIV-1 reservoirs appear as one of the major factors influencing the duration of a “functional cure”



(long-term control of HIV-1 replication and disease progression in the absence of cART) after cART cessation. Indeed, a recent and elegant study described “posttreatment controllers,” patients who durably control HIV-1 after stopping a treatment initiated at primary infection, but, unlike natural controllers, have rather unfavorable HLA genotypes (Saez-Cirion et al. 2013). This study suggests that a low reservoir of latent HIV-1 could be controlled by the patient immune system. Consequently, a decline of the HIV-1 latent reservoir to a level sufficient to permit an efficient control of the infection by the host immune system might allow for interruptions in therapy (“treatment-free windows”) and would represent important progress in AIDS treatment. Reactivation of HIV gene expression in latently infected cells together with an efficient or intensified cART could serve as an adjuvant therapy aimed at eliminating/decreasing the pool of latent viral reservoirs. Several studies have identified individual compounds that are capable of reversing HIV-1 latency. Upregulating cellular transcription to induce HIV-1 gene expression has opened the way of an HIV cure by testing strategies to purge the HIV-1 reservoirs. Several clinical trials have started with HDAC inhibitors (VPA, SAHA, panobinostat, romidepsin), with PKC inducers (bryostatin) and with disulfiram (for a review see Van Lint et al. (2013)). However, results from these clinical trials question the efficiency of these drugs used alone and underly the need to evaluate other classes of HIV-1 inducers and to test combinatory strategies in order to reduce the size of the HIV-1 reservoirs. Indeed, the combined use of two drugs to activate latent HIV could cause a synergistic reactivation of HIV-1 production, i.e., a higher reactivation than the sum of the reactivations produced by each drug individually. In this regard, proof of concepts has been reported for the coadministration of two different types of therapeutically promising HIV-1 inducers together with efficient cART as a therapeutic perspective to decrease the pool of latent HIV-1 reservoirs (Van Lint et al. 2013). Consequently, further understanding of the molecular

mechanisms regulating HIV-1 transcription will identify new targets and should help to develop novel pharmacological therapeutic strategies aimed at eliminating latent HIV-1 infection.

Conclusion

HIV-1 gene expression is a complex process that results from the convergence of multiple mechanisms, including the chromatin environment of the site of HIV-1 integration, transcriptional interference, the chromatin structure of the HIV-1 promoter, the epigenetic control of the HIV-1 promoter, and the varying levels of cellular transcription factors and of the viral trans-activator Tat protein and its associated cofactors. The relative importance of these mechanisms is probably dependent on the physiological state of the cell undergoing infection. Understanding the complexity of the mechanisms involved in HIV-1 transcriptional repression and the numerous links between different control levels has to be continued. They will provide fundamental new insights into the process of HIV-1 transcriptional latency and reactivation from latency and ultimately should contribute to an increased understanding of AIDS pathogenesis. Different forms of latency vary from one patient to the other and even from one cell to the other in single patient. This underlies the importance to test in reactivation strategies the combination of various compounds used simultaneously for targeting HIV-1 transcriptional repression at multiple levels. These combinatory anti-latency strategies could facilitate the escape from latency and the clearance of viral reservoirs. Alternatively, a deeper understanding of the mechanisms regulating HIV-1 transcription could lead to strategies aimed at maintaining the virus latent. In addition to latency, the failure to cure HIV-1 infection is believed to result from T-cell dysfunction stemming from persistent immune activation. Therefore, new therapeutic strategies should also include reversal of immune exhaustion and/or increase of immune clearance to kill the productively infected cells.

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Translational Interventions (Intervention Technology Transfer)

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Definition

Translational interventions aim to bridge the gap between prevention science and widespread adoption and diffusion of research-based practices in real-world, uncontrolled community settings. *Intervention technology transfer* is the process that supports the dissemination and implementation of translational interventions, including effective behavioral HIV prevention interventions (EBIs).

Introduction

Advances in prevention science have resulted in the identification of interventions with demonstrated effectiveness or potential to impact behavioral outcomes related to HIV transmission, including reduced sexual risk taking, uptake of

HIV testing, and medication adherence and linkage to clinical care. Yet the availability of proven programs has not guaranteed their dissemination to their intended audiences. Lessons from the field show that innovative prevention strategies are neither naturally diffused nor automatically adopted by program providers. The difficulties of successful technology transfer are not unique to the field of HIV prevention; they reflect the “long and winding road” from research to practice that too often keeps the best prevention from reaching the populations that might benefit most. To address barriers to successful intervention technology transfer, increased attention is being given to understanding intervention technology transfer, which includes the process of *dissemination*, or the “spread of knowledge and intervention materials” to providers and communities, as well as the process of *implementation*, or “the use of strategies to adopt and integrate interventions and to change practice patterns within specific settings” (National Institutes of Health 2012a).

Evidence-based HIV prevention interventions vary in size and scope, as well as the populations they target. Whether they entail modest practice changes, such as the integration of brief motivational counseling for HIV testing and linkage into care, provider-delivered messages to encourage medication adherence, and recruitment of individuals from risk groups to participate in small group sessions, or multicomponent community-level strategies, significant barriers have limited the wide-scale dissemination that is critical for reducing HIV incidence. Even what appear to be relatively simple changes in practice may not be easily adopted or maintained. Common barriers to dissemination and implementation include lack of knowledge, recruitment and retention challenges, staff and resource constraints, lack of interest by or support from agency administrators, and concerns about how to adapt interventions for different target populations (Solomon et al. 2006; Gandelman and Dolcini 2012).

Multiple theoretical perspectives inform scientific understanding of the transfer process and identify key factors that may influence success. Diffusion of an innovation is supported by program designers and early adopters, who can serve

as champions and role models to later users (Rogers 2003). Stages of change theory have been applied to both organizations and individual providers and depict how issues and challenges evolve as a new practice is considered, adopted, implemented, and maintained (Lehman 2002). Operations and systems research identifies key agency inputs and resources that may influence dissemination and implementation success (Sobo et al. 2008). These theoretical perspectives provide a framework for identifying what factors influence intervention technology transfer. Understanding these processes helps identify what types of training, technical assistance, and capacity-building support facilitate dissemination and implementation; it can also identify characteristics of interventions that are most likely to be successful in reaching their intended audiences.

Phases of Intervention Technology Transfer

Intervention technology transfer is commonly divided into distinct phases. From the perspective of changes within agencies (described below), these have been labeled *pre-implementation*, *implementation*, and *maintenance and evolution phase* (Kraft et al. 2000). The Society for Prevention Research has proposed a similar framework scaled to a broader community framework, including Translation stage-setting during pre-adoption phases, Institutional or individual adoption of EBIs, Effective implementation of EBIs, and Sustainability of EBIs, or TIES (Society for Prevention Research 2008). A variety of technology transfer activities are required during all phases for a program to be successfully transferred to real-world settings.

The *pre-implementation phase* focuses on agency identification and selection of an effective behavioral HIV prevention intervention (EBI). Dissemination of knowledge about the benefits, advantages, and characteristics of different interventions is critical at this stage. Providers assess the fit and feasibility of potential interventions by considering their organizational and staff capacity, mission, resources, client characteristics and

needs, and compatibility with existing services. Once they have selected an intervention and have allocated or received resources for its adoption, agencies prepare for implementation by providing staff training, mobilizing support among administrators and providers, and, as necessary, adapting or tailoring the intervention for their local populations.

The *implementation phase* starts with the provider's first use of the intervention with clients. While staff may receive training in the pre-implementation phase, there often is a continued need for assistance on how to best adapt the intervention so that it smoothly integrates into their organizational and programmatic structure. In addition, to inform their own efforts and meet the requirements of funders, providers need to begin collecting data about the implementation process, including documenting numbers and risk profiles of clients reached, how often the intervention was provided, fidelity to the core elements of the EBI, and adaptations made. Monitoring and evaluation is critical for assuring the intervention is being delivered as intended to achieve desired behavioral outcomes, identifying challenges and barriers to reaching the intended audiences, and providing evidence that can support requests for the ongoing resources required for sustainability.

In the *maintenance and evolution phase*, attention turns to how the intervention can be sustained and if it should be altered or replaced. Agencies must put in place strategies for sustaining staff and administrator support, addressing staff turnover and retraining needs, determining ongoing costs and resources, and reviewing evaluation data to see how well the intervention is meeting its objectives. These efforts help determine if and how the prevention intervention can and should be embedded into the organization's routine operations and budget.

Key Components of Intervention Technology Transfer

There are multiple components or activities involved in comprehensive intervention technology transfer. These components include user-

friendly packaging of intervention materials, training, and technical assistance (TA).

User-Friendly Packaging of the Intervention as Replication Kits

An intervention with demonstrated efficacy in a research-controlled setting may not be easily transferrable for a number of reasons. For community-based providers to be able to adopt and implement an intervention with ease, they must not only have adequate training on the intervention but also be supplied with intervention materials that are user friendly and visually appealing. These materials should try to address providers' needs during all three phases of technology transfer (e.g., information about the intervention's objectives for pre-implementation, guidance and tips for implementation, and evaluation measures during maintenance/evolution). In the United States, federally funded initiatives such as Program Archive on Sexuality, Health, and Adolescence (PASHA) and Replicating Effective Programs (REP) have worked with original researchers and program developers to produce program packages or replication kits with materials such as curriculum guides, videos, workbooks, and survey instruments that allow prevention providers to not only easily offer the program to their clients but also to collect information for evaluation purposes (Card et al. 2007; Eke et al. 2006). These materials typically contain information about the program's history and evidence base as well as the materials needed for implementation and easy-to-understand guidelines for use.

Training

Government agencies have played a role in promoting technology transfer through the provision of funding for training and technical assistance programs on effective HIV prevention interventions. In 1999, the Centers for Disease Control and Prevention (CDC) published a Compendium of HIV Prevention Interventions with Evidence of Effectiveness to respond to prevention service providers who requested evidence-based interventions that work. Since then, it has launched the Diffusion of Effective Behavioral Interventions project (DEBI), a national-level strategy to

provide high-quality training and ongoing technical assistance on selected evidence-based HIV/STI/viral hepatitis prevention interventions to state and community HIV/STI program staff.

Because the selection of an EBI is often made by agency administrators and/or is contingent on receipt of funding, attending a training on the intervention is often the first time staff who are responsible for implementing a program learn about a program's objectives and core elements. Quality, standardized training to increase knowledge and skills needed for intervention delivery is a critical component of successful technology transfer. Training activities include the development of formal training curricula, the logistical coordination of training activities, and the delivery of training to two groups of trainees – trainers who will implement training activities (e.g., through a training of trainers) and agency staff. At the agency level, training is usually required for staff who will be implementing the program and often recommended for agency administrators whose support is essential to success and sustainability. Training objectives may focus not only on specific program elements but also general interpersonal skills that program providers need to successfully implement a prevention intervention such as facilitation skills, group management skills, and recruitment skills.

Technical Assistance

While training activities on EBIs often occur over a relatively short period, ranging from 1 to 5 days, literature on intervention technology transfer has demonstrated that after training has been completed, many intervention providers require access to quality technical assistance to support them through the implementation and maintenance and evolution phase (Fuller et al 2007). Surveys of community-based agencies that receive technical assistance suggest that technical assistance (TA) needs are varied and the role of TA providers can change during the different phases of technology transfer. During the pre-implementation phase, for example, agencies may require TA to help staff plan for an intervention and adapt it to fit into agency schedules and existing services. Some providers may need TA

during implementation to overcome specific barriers such as difficulty in recruiting and retaining clients (O'Donnell et al 2000). TA may also be utilized to enhance organizational infrastructure (e.g., fiscal management, strategic planning) that allows the agency to continue to receive funding for program implementation and maintenance.

Online Strategies for Intervention Technology Transfer

Literature on translational interventions has focused primarily on the use of in-person strategies for transference of knowledge and skills, through printed curricular materials and user-friendly packages, face-to-face trainings, in-person or telephone consultations, and site visits. More recently, intervention technology transfer activities have also been offered through the Internet so providers are able to access them through their computers, tablets, or phones. These additional methods include synchronous events, such as webinars and real-time discussion forums, web-based services to link community-based organizations (CBO), and TA requests to TA providers, and asynchronous events, such as interactive, web-based training courses which use video as a means of modeling intervention best practices. Advantages of online methods include decreased costs for travel, increased flexibility for users, and decreased disruption to agency staffing and services. More research is needed on the acceptability and effectiveness of these online strategies for technology transfer.

Fidelity and Adaptation During Intervention Technology Transfer

One of the challenges for translational interventions is maintaining fidelity to the "core," or required, elements of an effective intervention and the need to adapt or tailor the intervention to best fit an agency's circumstances and local needs and its target population. Models of technology transfer highlight the importance of tailoring or customizing delivery of interventions to agency circumstances and ensuring that messages are appropriate for agency target populations without

altering, deleting, or adding to the intervention's core elements. Without sufficient TA and training, HIV prevention providers may unintentionally risk altering intervention effectiveness by modifying interventions in ways that either eliminate or change core elements. Therefore, such guidance needs to be offered proactively before an intervention has been substantially changed and is well into the implementation phase. By working to increase providers' ability to systematically adapt HIV prevention interventions without losing fidelity to the core elements, TA providers and researchers can help ensure their continued use and acceptability in real-world settings.

Translational Research on Implementation in Real-World Settings

While intervention technology transfer activities to successfully embed EBIs into "real-world" settings often follow rigorous randomized control trials (RCTs), fewer HIV/AIDS behavioral health studies have conducted what NIH defines as "Type II translational research" to determine how effective these interventions are when implemented by practitioners and not researchers, with or without intervention technology transfer support (National Institutes of Health 2012b). Some recent studies that have been conducted on the implementation of EBIs by practitioners, however, have shown continued evidence of success and have provided a possible framework for operationalizing criteria to help ensure effectiveness (Neumann et al. 2011; Harshbarger et al. 2012).

Conclusion

Translational interventions seek to provide HIV prevention program providers with the knowledge and skills they need to successfully transfer effective HIV prevention programs to their target populations. Literature on technology transfer shows that to be successful, program providers must have access to user-friendly intervention materials, quality training, and ongoing technical assistance. Effective practitioner-scientist

collaboration is also critical to the success of technology transfer. In addition, supporting technology transfer activities must address a range of issues outside of program implementation, including organizational infrastructure development, adaptation, and evaluation, to help providers overcome common barriers to sustainable implementation.

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Transmission HIV-2: Origin, Epidemiology, and Phenotype

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Definition

This entry focuses on the current knowledge about how HIV-2, the second and more benign form of the human immunodeficiency virus, is transmitted

(“► [HIV-2 Transmission](#)”). First, the origin and geographical distribution of HIV-2 transmission over time will be discussed. Second, the different and most important transmission pathways will be described, and a few well-described transmission chains will also be dissected. Finally, HIV-2 transmission on a cellular level during primary infection and the importance of different HIV-2 phenotypes in transmission and pathogenesis will be discussed.

Origin, Epidemiology, and Subtypes

Current evidence indicates that HIV-2 is the result of several cross-species transmission events and the most likely route of transmission of HIV-2 to humans involves contact with blood of different primates hunted for bushmeat in Africa. Phylogenetic analysis have indicated that HIV-2 is related to SIV from wild sooty mangabeys (SIVsm), and to date eight genetically distinct clades of HIV-2 (“► [Phylogeographic Insights into the Origins and Epidemic History of HIV-2](#)”) have been described (subtypes A–H). However, only subtypes A and B has spread considerably in humans, and groups C–H most likely represent dead-end infections since they only have been detected in one individual, respectively.

The geographic origin of HIV-2 (“► [Phylogeographic Insights into the Origins and Epidemic History of HIV-2](#)”) was initially thought to be Guinea-Bissau where the highest prevalence (ranging from 2% in healthy controls to 64% in commercial sex workers) was recorded in the initial screenings of blood samples obtained between 1985 and 1987 from six West African countries (Kanki et al. 1987). However, the oldest documented HIV-2 cases have been recorded in samples collected in Côte d'Ivoire during 1966, and it has been shown that both group A and B share the most common ancestry with SIV strains collected from communities found in the Tai forest, in western Côte d'Ivoire (Santiago et al. 2005). Furthermore, studies of the timing of the HIV-2 origin have found that group A and B arose within the same time interval, likely during the 1930s and 1940s

(combined interval for group A, 1906–1956; group B, 1907–1961).

Considering that phylogenetic analyses have pointed towards Côte d’Ivoire as the origin of HIV-2 (“► [Phylogeographic Insights into the Origins and Epidemic History of HIV-2](#)”) whereas Guinea-Bissau showed the highest prevalence numbers during the 1980’s, it is possible that the migration of HIV-2 to Guinea-Bissau occurred at a very early stage in the epidemic (Santiago et al. 2005). Transmission in Guinea-Bissau is supposed to have coincided with the prolonged war of liberation against the colonial power Portugal in 1963–1974. HIV-2 has also been recorded in the West African countries (“► [The Epidemiology of HIV-2 Infection in West Africa](#)”) of Cape Verde, Mauritania, Senegal, The Gambia, Guinea, Sierra Leone, Liberia, Ghana, Togo, Benin, Mali, Burkina Faso, Nigeria, Niger, São Tomé and Príncipe, and Cameroon and in patients originating from Equatorial Guinea.

Being the country with the highest HIV-2 prevalence outside West Africa (“► [Epidemiology of HIV-2 Infection in Europe](#)”), Portugal had 1,813 registered individuals with HIV-2 by the end of 2008. As in France (another non-West African country with relatively high HIV-2 prevalence), the majority of these individuals were immigrants of West African origin. From Portugal and France, further spread has occurred to other European countries. Direct immigration from West African countries has likely also occurred, as seen in the United Kingdom and Spain in Europe and the United States and Canada in North America. A Portuguese link has also been seen in HIV-2 transmission with cases in other former Portuguese colonies such as Angola, Mozambique, Brazil, and parts of India (Schim van der Loeff and Aaby 1999). HIV-2 has also been recorded in Japan.

HIV-2 group A (“► [Phylogeographic Insights into the Origins and Epidemic History of HIV-2](#)”) has been the dominant group both in West African countries, including Guinea-Bissau, and subsequently also in countries influenced by Portuguese heritage and other countries outside West Africa. In West Africa, group B has primarily been recorded in Côte d’Ivoire but also in Ghana, Sierra

Leone, and Nigeria. Outside West Africa, group B has been found in France immigrants originating from Côte d’Ivoire and Mali and in Spanish immigrants from Equatorial Guinea.

HIV-2 Transmission Routes

As for HIV-1, HIV-2 can be transmitted both horizontally (e.g., sexually or through contaminated blood) and vertically (e.g., during pregnancy and childbirth or via breast milk) in humans (“► [HIV-2 Transmission](#)”; “HIV-1 Transmission Dynamics”; “► [HIV-1 Sexual Transmission](#)”). However, HIV-2 has consistently been shown to be less transmissible than HIV-1 for all studied transmission pathways (Schim van der Loeff and Aaby 1999). The lower transmission rates for HIV-2 correspond to lower levels of viremia and lower shedding in semen and in the female genital tract. Heterosexual spread was early shown to be substantially lower in HIV-2 compared with HIV-1, estimated at 10–20% of the transmission frequency of HIV-1 (Schim van der Loeff and Aaby 1999). Mother-to-child transmission of HIV-2 (“► [HIV-2 Transmission](#)”; “► [MTCT HIV-1 Transmission Update](#)”) has been demonstrated to be directly linked to maternal viremia, as in HIV-1 infection. Vertical transmission in untreated HIV-2 infection has been recorded at levels between 0% and 4% compared with up to 25% for HIV-1 infection in the same studies (Table 1).

Risk Factors of Transmission

Documented risk factors for HIV-2 transmission are similar to those for HIV-1 including history of or serological proof of sexually transmitted infections including genital ulcer disease, multiple sex partners, and having sex with or being a commercial sex worker. In contrast to HIV-1, where usually a younger population is affected, older age is a risk factor for HIV-2 acquisition. Already in the first community-based survey in Guinea-Bissau in 1987, where an adult HIV-2 prevalence of 8.9% was found, there was an overrepresentation in the

Transmission HIV-2: Origin, Epidemiology, and Phenotype, Table 1 Mother-to-child transmission of HIV-2

Country	Study period	Frequency of HIV-2 transmissions ^a
Côte d'Ivoire	1990–1992	1.2 %, <i>N</i> = 132 (HIV-1: 25 %, <i>N</i> = 138)
Guinea-Bissau	1987–1988	0 %, <i>N</i> = 51
The Gambia	1993–1995	4.0 %, <i>N</i> = 294 (HIV-1: 24 %, <i>N</i> = 144)
Portugal	1999–2005	1.5 %, <i>N</i> = 131 (HIV-1: 3.4 %, <i>N</i> = 1315) ^b
France	1986–2007	0.6 %, <i>N</i> = 531 (HIV-1: 5.2 %, <i>N</i> = 10122) ^c

^a*N* equals the number of children born to HIV-infected mothers in the studies

^b22 % of HIV-1- and 44 % of HIV-2-infected mothers did not access MTCT prevention

^c20 % of HIV-1- and 43 % of HIV-2-infected mothers did not access MTCT prevention

40–65-year-old age group. An overrepresentation especially of older women has suggested that women become more susceptible to HIV-2 at an older age. Later observations also concluded that as part of coinfection with other retroviruses, HIV-2 infection was more common in older women than in men.

Being less transmissible than HIV-1, HIV-2 reached surprisingly high prevalence levels in certain areas of West Africa (“► [The Epidemiology of HIV-2 Infection in West Africa](#)”). It has been hypothesized that a substantial parenteral spread occurred early on in the epidemic, primarily in the 1960s, due to activities such as vaccination and treatment campaigns for trypanosomiasis and tuberculosis as well as ritual acts of female excision (Pepin et al. 2006). Support for this hypothesis is given by the fact that HIV-2 prevalence has been diminishing in several West African countries while at the same time HIV-1 prevalence has been increasing, in particular during the 1990s. The trends of declining HIV-2 prevalence also continued between 2000 and 2010, in spite of HIV-1 prevalence keeping at steady levels. The early expansion of the HIV-2 epidemic has also been hypothesized to coincide with outbreaks of genital ulcer disease, occurring earlier in areas with lower frequency of male circumcision.

Transmission HIV-2: Origin, Epidemiology, and Phenotype, Table 2 Phylogenetically verified HIV-2 transmission chains or clusters

Route	Country	HIV-2 transmissions
MTCT	Portugal	Mother and child
MTCT	Portugal	Mother and two children
Sexual	Denmark	Index case and two partners
Sexual	India	Between a couple
Sexual	South Korea	Transmission cluster (4–6 individuals)
Sexual	Spain	Transmission cluster (4 individuals)

HIV-2 Transmission Chains and Clusters

HIV-2 transmissions have been identified in a number of case and population studies, and some have also been characterized by phylogenetic analysis. This has allowed detailed studies of the genetic relationship between index-recipients viral strains (Table 2).

One study reported evidence of HIV infection in two females in Denmark who had been in sexual contact with an HIV-1 and HIV-2 dual-infected man (Christiansen et al. 1997). Serology from the two female partners indicated that partner one was infected with both HIV-1 and HIV-2, whereas partner two was infected with HIV-2 only. To confirm the serology data, nucleic acid was extracted and amplified from plasma and peripheral blood mononuclear cells (PBMC) of the suspected index case and the two partners. Partner one was positive for both HIV-1 and HIV-2, and female partner two was only positive for HIV-2. Phylogenetic analyses showed that both HIV-1 and HIV-2 sequences of the index case and the two female partners were closely related. The monophyletic clusters retained after analyses with both global and local HIV-2 sequences, strongly suggesting that the index case had transmitted HIV-1 and/or HIV-2 to his female partners.

A Spanish study reported of eight Caucasian men, living in northern Spain, positive by HIV-2 serology and infected during a 5-year period (Cilla et al. 2001). Despite extensive screening, no other HIV-2 infections could be identified in the region. Specimens from four of the eight

infected men were available for PCR and sequence analysis. Sequences were obtained from the HIV-2 *env* and *pol* regions, and phylogenetic analysis showed that sequences of both genome regions from the four patients were highly related. They formed monophyletic clusters in comparison to epidemiologically unlinked HIV-2 reference sequences and also from other Spanish HIV-2 sequences. Epidemiological data could not identify a likely index case; however, sex with other men was the only identified risk factor among the eight patients, suggesting a possible link between them and/or an unknown index case.

Another study identified ten HIV-2-infected individuals in South Korea between 1991 and 2002 (Nam et al. 2006). Among those, nine were residing in the southern part of the country. Phylogenetic analyses using a part of the HIV-2 *env* region indicated that six of the patient samples formed a monophyletic cluster that was distinguishable from other foreign and South Korean sequences. The earliest infected patient (a man) in the cluster reported that he had likely been infected in the Canary Islands, whereas the remaining five (one man and four women) reported that they most likely were infected in South Korea. Among those five, the man had been a co-resident of the earliest infected. The results indicate that the virus of the six patients had a common source, perhaps introduced into South Korea from the Canary Islands followed by local dissemination.

The above studies suggest the existence of several different HIV-2 transmission chains where HIV-2 had been transmitted sexually from a common source. These studies also indicate that HIV-2 infections are spreading in countries where HIV-2 prevalence is low or even not recognized. Because disease progression rate (“► [Natural History and Clinical Features of HIV-2 Infection](#)”) is much slower in the average HIV-2-infected patient compared to those infected with HIV-1, HIV-2 may stay unrecognized in infected individuals for a much longer time compared to HIV-1, resulting in slow and unrecognized spread in some populations, even outside the endemic regions. Although HIV-2 infection is not considered to be

a large global health problem (as HIV-1), HIV-2 is clearly spreading outside its traditional borders.

Primary HIV-2 Infection

The characteristics of virological and immunological markers of an acute infection following either blood-borne, sexual, or vertical HIV-2 transmission between humans are not known due to the paucity of studies reporting results from primary HIV-2 infections. To date, the only well-documented case was a 19-year-old white woman that displayed symptoms of primary HIV infection (Besnier et al. 1990). The woman was negative for HIV-1 but displayed a clear HIV-2-specific serological profile. Her symptoms appeared approximately 3 weeks after infection and disappeared within a few days. However, the knowledge from studies of HIV-1 primary infections (“Modeling Early HIV-1 Infection and Dissemination”) has clearly shown that virological and immunological events taking place during the primary phase of the infection can be associated with rate of disease progression. Thus, it is also likely that the primary phase of an HIV-2 infection differs from that of an HIV-1 infection since the natural disease courses of HIV-1 and HIV-2 infections (“► [Natural History and Clinical Features of HIV-2 Infection](#)”) in general are so distinct.

It is well established that the plasma viral load (“► [HIV-2 Diagnosis and Viral Load Measurements](#)”) set point after an acute HIV-2 infection is significantly lower than that of an HIV-1 infection (de Silva et al. 2008). In fact, the HIV-2 viral load set point is more than ten times lower than for HIV-1. Conceivably, the lower viral load set point of an HIV-2 infection may be the result of differences in acute phase virus replication and/or virus controlling host response. As in HIV-1 infection, the transmission of HIV-2 may occur via the mucosa, even though a major early transmission route is thought to be blood-borne (de Silva et al. 2008). During mucosal transmission (“HIV-1 Transmission Dynamics”), it is known that HIV-1 may target several different cell types, including CD4⁺ T cells, Langerhans cells,

submucosal dendritic cells (DC), and possibly also tissue macrophages. In vitro studies have shown that peripheral blood-derived DCs are less efficiently infected by HIV-2 compared to HIV-1 (Hodges-Mameletzis et al. 2011). However, the susceptibility of Langerhans cells and submucosal DCs to HIV-2 infection and replication is not well established. Acute viremia may also be influenced by intracellular viral restriction factors (“► [Interactions Between HIV-2 and Host Restriction Factors](#)”), including the tripartite motif-containing protein (TRIM)-5 α (“► [TRIM5 Alpha and HIV-2 Infection](#)”). Studies on variations in the HIV-2 p26 capsid that interact with TRIM-5 α have revealed associations with plasma viral load levels (Hodges-Mameletzis et al. 2011).

As for virus controlling immune responses in HIV-2 infection, magnitude of Gag-targeted CD8+ T-cell response (“► [The Cellular Immune Response to HIV-2 Infection](#)”) has been linked to viral control (Hodges-Mameletzis et al. 2011). Broad and potent contemporary autologous neutralizing antibody response (“► [The Antibody Response to HIV-2](#)”), which is common in HIV-2 infection and rare in HIV-1 infection, may also contribute to viral control limiting the primary HIV-2 infection (Hodges-Mameletzis et al. 2011). In addition, better antigen presentation by myeloid cells (“► [Role of Dendritic Cells in HIV-2 Pathogenesis](#)”) has been implied due to the HIV-2-encoded accessory protein Vpx (“► [Molecular Biology of HIV-2](#)”) (Vpx is not present in HIV-1) that counteracts the cellular restriction factor SAM- and HD-domain-containing protein 1 (SAMHD1 [MJ22]) (Hodges-Mameletzis et al. 2011) (“► [Interactions Between HIV-2 and Host Restriction Factors](#)”).

HIV-2, in contrast to HIV-1, can establish infections in Asian macaques and has also been used as a nonhuman primate model for AIDS (“► [Non-human Primate Model - For HIV-1 Transmission](#)”). The natural course of pathogenic HIV-2 infection in macaques appears similar to that of SIVmac macaque infections (Joag 2000). Accordingly, it has been noted that the viremia of acute HIV-2 macaque infections may peak around 2 weeks after infection and can reach up to 10⁸ RNA copies/ml. However, virus replication is

only partially controlled after 4–8 weeks, with a viral set point significantly higher than that observed in humans. Thus, the translation of findings from acute HIV-2 infections in macaques to acute HIV-2 infection in humans has its limitations, possibly due to differences in host genetics, including intracellular restriction factors and immune response.

Coreceptor Use of HIV-2

Early work performed during the 1980s showed that replicative capacity of HIV-2, like HIV-1, correlates with immunodeficiency. Such that syncytia formation (formation of multinuclear cells) in peripheral blood mononuclear cell (PBMC) cultures and the ability to replicate in cell lines frequently characterized viruses obtained from AIDS patients but not viruses obtained from asymptomatic individuals, allowing classification of HIV-2 isolates into rapid/high and slow/low phenotype, respectively (Albert et al. 1989). In fact, the asymptomatic phase of HIV-2 infection rarely yielded virus upon isolation, and the viruses, if obtained, had the slow/low phenotype. Among 24 HIV-2-infected subjects, CD4+ T-cell counts in blood were lowest in AIDS patients compared to asymptomatic or symptomatic but non-AIDS cases. Conversely, it was easier to isolate virus from patients with HIV-2 disease, and the isolated virus had a higher replicative capacity both in PBMC and cell lines than viruses isolated from asymptomatic subjects. This established the link between HIV-2 viral phenotype and clinical progression and suggested that HIV-2 with slow/low phenotype is more likely to be transmitted and/or dominate early in infection. The phenotypic change, if it occurs at all, occurs later in infection. It is known that high HIV-1 viral load in plasma increases the risk of transmission, whereas effective control of viral load by antiretroviral therapy decreases the risk of HIV-1 transmission. The prolonged asymptomatic period in HIV-2 infection with low viral load (“► [HIV-2 Diagnosis and Viral Load Measurements](#)”) and low, if any, replicative capacity of HIV-2 may be linked to the low transmission rate.

Transmission HIV-2: Origin, Epidemiology, and Phenotype, Table 3 Comparison of HIV-1 and HIV-2 coreceptor use

		HIV-1					HIV-2					
Number of coreceptors used		1	1	2	2	3	1	2	2	3	3	5
Tropism		R5	X4	R5X4	R3X4	R3R5X4	R5	R1R5	R3X4	R1R3R5	R1R2bR5	R1R2bR3R5X4
Phenotype	Rapid/high	0	2	1	1	5	1	0	1	1	0	1
	Slow/low	11	0	0	0	0	1	1	0	2	2	1

Phenotype: rapid/high, replication in both PBMC cultures and cell lines; slow/low, replication in PBMC cultures but not in cell lines. X4, CXCR4 use only; R5, CCR5 use only; all other coreceptors CCR1, CCR2b, and CCR3 used in combinations with CXCR4 and/or CCR5. Coreceptor use was tested in human cell lines engineered to express different chemokine receptors

The discovery that HIV-1 uses CD4 and a chemokine receptor, mainly CCR5 and CXCR4 (“► [CXCR4, Coreceptors](#)”; “► [Attachment/Binding](#)”), to enter cells led to the question whether the difference in pathogenic potential of HIV-1 and HIV-2 is reflected by a difference in coreceptor use. Comparison of HIV-1 and HIV-2 coreceptor use revealed that CCR5 use (“Selection of CCR5 Using Viruses Transmission”; “Transmission of HIV-1 CCR5 Phenotypes”), with few exceptions, is a general property of viruses in both groups (Table 3) (Morner et al. 1999). Viruses not using CCR5 used CXCR4 alone or in combination with CCR3. What distinguished HIV-1 and HIV-2 was the ability to use other coreceptors in addition to CCR5. Whereas HIV-1 isolates with slow/low phenotype seem to exclusively use CCR5, the majority of HIV-2 isolates seems to be able to use one or several additional coreceptors. Furthermore, most HIV-1 isolates with a rapid/high phenotype uses CXCR4 alone or in combination with CCR3 and/or CCR5, whereas coreceptor use of HIV-2 isolates with this phenotype does not seem to be as distinct (Morner et al. 1999; Neil et al. 2005). An overview of HIV-2 coreceptor use reported in the literature is presented in Table 4, and in comparison with HIV-1, the use of CXCR5 appears to be unique for HIV-2.

Both HIV-1 and HIV-2 use two receptors for cell entry (“► [Attachment/Binding](#)”; “► [Molecular Biology of HIV-2](#)”). In a first step, binding occurs to CD4 that functions as a “key” to open up the closed viral envelope structure. This will

expose the hidden coreceptor binding site and allow binding of the viral envelope to the coreceptor in a second step. Viruses that can infect cells without first binding to CD4, like some HIV-2 isolates, appear to have a more open envelope conformation than HIV-1, which is with few exceptions CD4 dependent for cell entry. Conceivably, the relative CD4 independence of HIV-2 entry into cells may enable a wider range of coreceptors to be exploited for infection and may even assist in adaptation or switching to new coreceptors *in vivo* (Reeves et al. 1999).

The *in vitro* studies, on which broad coreceptor use of HIV-2 is based, were carried out with human cell lines engineered to express CD4 and chemokine receptors. Expression of receptors on transfected target cells *in vitro* may not reflect expression of receptors on HIV-2 target cells *in vivo*, such that infection of cells *in vivo* may not involve other than CCR5 and CXCR4. In line with this, it has been shown that broad coreceptor use as detected *in vitro* is not associated with the generally slower disease progression caused by HIV-2. Marchant and colleagues observed that both HIV-1 and HIV-2 infected human macrophage cultures, although they differed in the kinetics of replication. After an initial burst of replication, HIV-1 continued to replicate, while HIV-2 seemed to establish latency. It was suggested that this feature of HIV-2 might be responsible for the delayed disease progression.

Alternative receptors have been described for HIV-2 (Neil et al. 2005). D6 (CCR9) is a broad-

Transmission HIV-2: Origin, Epidemiology, and Phenotype, Table 4 Coreceptors used by HIV-2

Coreceptor	Alternative names ^a	Ligand (s)	Cellular distribution ^b
Human chemokine receptors			
CCR1	CD191	CCL3, CCL5, MCP-3	T (effector)
CCR2b	CD192	MCP-1, MCP-2, MCP-3, MCP-4, CCL11	M, T, B
CCR3	CD193	CCL5, CCL15, MCP-2, MCP-3, MCP-4, MCP-5	T, DC, As
CCR5	ChemR13, CD195	CCL5, CCL3, CCL4, MCP-2	M, T, DC
CCR8	ChemR1, CDw198	CCL1	M
CCR9	D6	Broad range of inflammatory proteins	M, T, E, placenta
CXCR4	LESTR, Fusin, CD184	CXCL12	M, T, B, DC
CXCR5	BLR-1	BCA-1	T, B
CXCR6	BONZO, STRL33, CD186	CXCL16	T, NK
Human orphan chemokine receptor			
gpr15	BOB	Not known	M, T
Undefined ^c			
To be determined		In the presence of TAK-779 and AMD3100	Replication in PBMC

^aFor all alternative names, see www.ncbi.nlm.nih.gov/gene/

^bM monocyte/macrophage, T T lymphocyte, B B lymphocyte, DC dendritic cell, NK natural killer cell, As astrocytes, Ne neutrophil, E endothelial cells

^cUndefined, receptor defined by replication of one HIV-2 isolate in PBMC in the presence of inhibitors to CCR5 and CXCR4 (TAK-779 and AMD3100, respectively)

specificity epithelial chemokine receptor, probably involved in regulating the concentrations of inflammatory chemokines and their endothelial transport. D6 expression is also found in the placenta, on myeloid and lymphoid precursor cell lines, and on macrophages in inflamed tissue, and it has been suggested that it may facilitate transmission and have a role early in infection, perhaps in dissemination of virus infection. For HIV-2, the role of D6 still remains to be clarified.

Conclusion

HIV-2 originates from several cross-species transmission events between wild sooty mangabeys and humans. This likely occurred in or close to Côte d'Ivoire from where HIV-2 has spread mostly within West Africa but also to other parts of the world (in particular to countries with colonial links to West African countries). HIV-2 can be transmitted both vertically and horizontally, and studies of transmission chains have indicated that

HIV-2 can give rise to local spread both among heterosexual couples and men who have sex with men. As in HIV-1 infection, the transmission of HIV-2 may occur via the mucosa, even though the major early transmission route is thought to be blood-borne. Although HIV-2 seems to be more promiscuous in the use of different coreceptors in vitro, most studied HIV-2 isolates favor the use of CCR5 and CXCR4, alone or in combination with CD4, for cellular entry. Finally, despite some efforts, many of the cellular targets and mechanisms involved in transmission and establishment of an HIV-2 infection are not known, and further studies of this virus are clearly needed.

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Treatment Failure and Resistance

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Definition

HIV treatment failure can be defined in three different ways. Virologic failure is the inability to suppress the viral load to <200 copies/mL after 24 weeks of therapy or to <50 copies/mL after 48 weeks. Immunologic failure is the inability to attain normal CD4 counts despite viral suppression. Clinical failure is defined as the development of opportunistic infections after at least 3 months of antiretroviral therapy. Clinical failure rarely occurs in patients who are taking their antiretrovirals faithfully and are virologically suppressed. Because the management of immunologic failure is not well defined, virologic failure is the most clinically relevant definition of treatment failure and will be the focus of this chapter.

Development of Virologic Failure

Virologic failure occurs in three main patient groups and the outcomes and management of each of these populations differs. There are patients who fail with no resistance mutations predominantly due to poor adherence, either from never taking their medications or taking them successfully and then abruptly stopping. There are patients who fail with one or two class resistance that is easily salvageable; this is usually due to intermittent adherence. Finally, there are patients who fail with extensive multiclass resistance as a result of being treated sequentially with less potent drugs in the early years of combination ART, generally the period from 1996 to 2001.

Patients with sporadic adherence and treatment-experienced patients with prior

suboptimal therapy are more likely to develop HIV resistance than those who never take medications or abruptly stop them after virologic suppression. When patients intermittently take antivirals, they have subinhibitory drug levels allowing for continued viral replication and the selection of resistance mutations. Treatment-experienced patients with prior virologic failure due to suboptimal regimens are also at risk of selection of additional resistance, as the new regimen may not have sufficiently activated drugs to achieve or maintain virologic suppression. Patients can fall into more than one of these categories, but in general the pattern of adherence and the treatment history determines the degree of drug resistance and the optimal approach to virologic failure.

Work Up of Virologic Failure

Virologic failure is the inability to achieve virologic suppression after initiating treatment or to maintain virologic suppression while on an HIV regimen. The viral load should be <200 copies/mL after 24 weeks of starting therapy or <50 copies/mL by 48 weeks. Virologic rebound is defined as confirmed HIV RNA >200 copies/mL after attaining virologic suppression. When patients are found to have detectable HIV viral RNA on treatment, the first goal is to determine the etiology. The most important thing to assess is adherence and whether they are never, intermittently, or consistently taking their antiretrovirals (ARVs.)

There are many ways to ascertain adherence information. You can ask the patient in a nonjudgmental way about how often they are taking their medications; it is important to ask because often they will not spontaneously admit to treatment noncompliance. You can have them bring in their active pill bottles to assess dispensing dates; even more accurate is to contact their pharmacy to obtain renewal records. You then want to ask about and identify the underlying causes for nonadherence or intermittent adherence (e.g., side effects, insurance issues, pill burden, substance abuse, mental illness). If the patient is consistently

taking their medications you want to review food requirements for their regimen and their medication list, including dietary supplements, all of which could be altering the pharmacokinetics of the ARVs.

The next step is to evaluate for resistance. Resistance testing should be performed on all patients with virologic failure. Ideally it should be ordered while the patient is still taking the failing regimen, as this maximizes the chances of identifying drug resistance. When the selective pressure of HIV therapy is withdrawn, some resistance mutations will no longer be detectable as they are overgrown by wild-type virus. However, even when the patient has stopped antivirals, many resistance mutations will persist – in particular those that do not impair viral fitness, such as the mutations conferring resistance to NNRTIs. As a result, genotype testing is indicated in essentially all patients with virologic failure.

Resistance testing is reliably performed if the viral load is greater than 1000 copies/mL and often is successful between 500 and 1000 copies/mL. If a resistance test is not possible based on viral load <500 copies/mL there are different management approaches. One option would be to increase adherence and recheck the viral load in a short time frame. Another option is to change the regimen based on previous resistance testing or inferred resistance patterns based on the current regimen. A third option that became available recently is to check for “archived” resistance mutations; this advanced diagnostic procedure is more costly than standard genotyping and has not been validated in prospective clinical studies. However, it could provide valuable information in select circumstances.

There are two types of resistance testing: genotypic and phenotypic. Genotype tests report the mutations that are known from both in vitro and clinical samples to cause resistance to particular drugs. Phenotype tests create a recombinant virus from the patient’s blood and test the ability of individual drugs to suppress the growth rate as compared to wild-type virus. Genotype testing is more commonly used as it is cheaper, easily standardized, and has a faster turnaround time; it is also more sensitive for detection of low-levels of

Treatment Failure and Resistance, Table 1 Categories of virologic failure based on resistance pattern

Resistance pattern	Cause of virologic failure	Management
No mutations	Non adherence <i>or</i> Adherence then abrupt stop of medications	Assess for adherence and tolerability Resume regimen unless the cause of poor adherence was drug toxicity; consider treatment simplification if possible
1 or 2 class resistance	Intermittent adherence <i>or</i> Administration/absorption errors (drug-drug interactions, incorrect dosing, dietary effects)	Assess for adherence and inquire about pharmacokinetic factors Change to boosted PI regimen plus either NRTIs or (if no integrase resistance) an integrase inhibitor
Multiclass resistance	Treated previously with suboptimal regimens <i>or</i> History of intermittent adherence to multiple regimens	Review all previous resistance testing Obtain supplemental testing if warranted and not yet available, such as integrase resistance genotype, resistance phenotype, or viral tropism assay Goal is to find regimen with 2–3 active drugs

resistance virus. Phenotype testing is usually reserved for treatment-experienced patients who have complicated resistance patterns, in particular to protease inhibitors.

A standard genotype resistance test checks for mutations to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and to protease inhibitors (PIs.) Some tests also include resistance assessments for integrase stand transfer inhibitors (INSTIs) but more commonly this requires a separate genotype specifically assessing for integrase-inhibitor resistance.

Mutations are described with letters and numbers. The first letter is code for the wild-type amino acid. The numbers refer to the position of the amino acid and the second letter is code for the new amino acid in the mutant virus. One example is M184V, which denotes a substitution of valine for methionine at position 184. This mutation is selected by lamivudine (3TC) or emtricitabine (FTC). Of note, the resistance mutations and their clinical implications have been determined based on HIV-1 subtype B, which is the predominant strain in the United States, West and Central Europe, Japan, and Australia. There is still ongoing study to determine the effect of the identified resistance mutations in non-B subtypes of HIV.

In assessing virologic failure, it is critical to obtain all of a patient’s prior resistance tests. Some mutations previously detected may no longer be evident if the specific selective pressure exerted by antiretroviral therapy is stopped. However,

these mutations are “archived” and, though no longer detectable, are not completely cleared, and will reemerge rapidly with nonsuppressive treatment.

The next three sections will describe the management of virologic failure in three different patient populations based on their resistance patterns and treatment history. See Table 1 for a summary.

Management of Patients with Virologic Failure and No Resistance

In all patients with new virologic failure, the first step is to assess their adherence. This is optimally done by nonjudgmental questioning and, if necessary, determining the frequency of pharmacy refills. While clinicians’ estimates of patient adherence are notoriously poor, if patients report suboptimal adherence this alone is highly predictive; likewise, irregular refill rates at the pharmacy are strongly predictive of noncompliance.

If it is determined that a patient is not taking medications, the next goal is to figure out why this is the case. Adherence can be affected by a multitude of factors, including but not limited to drug side effects, cost, medication access, housing, food security, patient safety, health literacy, mental illness, and substance abuse. Ideally all involved factors should be addressed with the assistance of a multidisciplinary team including social workers, case managers, pharmacists, and



psychiatrists. If available, the use of community health workers for directly observed therapy can increase adherence in certain patient populations (Chaiyachati et al. 2014).

Although resistance mutations are rare in these nonadherent patients, the testing is still of value since a negative genotype prevents unnecessary switching to potentially more expensive, toxic, or complex regimens. If no resistance mutations are found, then the patient should be started on the simplest and most tolerable regimen, preferably one of the recommended or alternative regimens in the most recent version of the DHHS Guidelines (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015). There are currently four single-pill once daily regimens and one of these would seem the natural choice. However, three of them – elvitegravir/cobicistat/tenofovir/emtricitabine, efavirenz/tenofovir/emtricitabine, or rilpivirine/tenofovir/emtricitabine – have a relatively low barrier to resistance compared with regimens consisting of boosted PIs or dolutegravir. As a result, clinicians must weigh the benefits of simplicity with these treatments with potential consequences of selecting for resistance if the treatments fail. The patterns of resistance with these regimens are described in more detail below.

Regardless of what new regimen is selected, close clinical and laboratory follow-up is recommended. A repeat viral load should be checked 2–4 weeks after restarting treatment, which should demonstrate at least a 1 log or greater HIV RNA decline. Relatively frequent virologic monitoring (for example, every 6–12 weeks) should be done until the HIV RNA is <50 copies/mL.

Management of Patients with Single- or Dual-Class Resistance

Patients develop single- or dual-class resistance most commonly from intermittent adherence to NNRTI or INSTI-based first-line regimens. In rare cases, treatment failure with resistance can also develop from patient errors (i.e., only taking one drug instead of the prescribed three), pharmacy errors (i.e., not dispensing ritonavir with a protease

inhibitor based regimen), or drug-drug interactions (i.e., a patient taking a proton-pump inhibitor with rilpivirine.) The type and frequency of resistance mutations depends on the specific regimen.

Data on patterns of resistance after failure of first-line regimens have been derived both from clinical trials and treatment cohorts; the former give a more precise estimate, as resistance testing has been incorporated into the evaluation of treatment failure for many years. For example, in the 934 study, patients were randomized to receive either TDF/FTC or ZDV/3TC, along with the NNRTI EFV (Gallant et al. 2006). In the TDF/FTC + EFV arm, 12 patients (out of 244 enrolled) with virologic failure underwent genotype testing. Three had no resistance detected, 9 had EFV resistance (mostly K103N), and an additional 2 also had the M184V mutation of FTC and 3TC. Numerous other studies since then of NNRTI-based therapy demonstrate similar results – namely, that a significant proportion of treatment failures will have no resistance and those that do have mutations will show a predominance of NNRTI resistance, sometimes accompanied by M184V. Triple class resistance – meaning inclusion also of the K65R mutation of TDF – is relatively rare.

The pattern of resistance after failure of initial integrase-inhibitor based treatments differs based on the specific agent chosen. For first-line regimens containing raltegravir or elvitegravir, drug resistance occurs in approximately half the patients with virologic failure (Lennox et al. 2009; DeJesus et al. 2012; Sax et al. 2012). Unlike with NNRTI-based first-line regimens, here dual-class resistance usually ensues, with both the M184V mutation of FTC and 3TC and mutations conferring resistance to EVG and RAL. In the three treatment-naïve studies evaluating dolutegravir plus 2 NRTIs, by contrast, no patient with virologic failure assigned to the dolutegravir strategy has developed resistance to this drug (Raffi et al. 2013; Walmsley et al. 2013; Clotet et al. 2014).

Similarly, virologic failure with initial treatment regimens of a boosted PI with 2 NRTIs is rarely associated with genotypic resistance. When such resistance occurs, it is usually limited to

M184V with no detected PI resistance. This property of boosted PI regimens is the primary reason that TDF/FTC with DRV/r is maintained as a recommended regimen in the most recent revision of the DHHS HIV Treatment Guidelines. Specifically, the guidelines state: “For patients who are at high risk for intermittent therapy because of poor adherence or have transmitted NRTI drug resistance, a PI/r-based treatment is preferred given the PIs high genetic barrier to resistance.” (Panel on Antiretroviral Guidelines for Adults and Adolescents 2015)

The optimal management of patients with single- or dual-class resistance can be inferred from the results of the EARNEST and SECOND LINE studies (Boyd et al. 2013; Paton et al. 2014). These two trials enrolled patients who failed initial therapy with 2 NRTIs and an NNRTI, many of whom had resistance to NNRTIs and NRTIs. In the SECOND LINE study, eligible study subjects were randomized to LPV/r plus NRTIs or RAL; in the EARNEST study, to LPV/r plus either NRTIs or RAL, with a third arm of 12 weeks of LPV/r and RAL induction, followed by LPV/r monotherapy. Both studies found that the RAL and the NRTI arms were virologically noninferior, despite extensive resistance sometimes present at baseline (The LPV/r monotherapy strategy was inferior.).

Based on these results, patients with single- or dual-class resistance after failure of an initial NNRTI-based regimen should be treated with a boosted PI plus either NRTIs or an integrase inhibitor. While the studies cited above used LPV/r and RAL, it is reasonable to assume that similar results would be seen with DRV/r as the boosted PI component and DTG as the integrase component.

In patients who develop virologic failure while on boosted PIs, they are unlikely to develop resistance mutations to the PI but might have NRTI resistance. The most common NRTI mutation to develop while on lamivudine or emtricitabine is M184V. This confers high in vitro resistance to these two agents but increases the in vitro-susceptibility to tenofovir, stavudine, and zidovudine. However, studies have demonstrated that lamivudine retains activity and decreases viral fitness when continued even in the setting of the

M184V mutation (Campbell et al. 2005; Castagna et al. 2006). Given this benefit and that M184V also increases tenofovir susceptibility, many clinicians will continue a regimen of tenofovir/emtricitabine in the presence of M184V. So in general for patients who develop virological failure on a boosted PI and NRTI regimen, studies have shown that virologic suppression can be achieved in these patients by keeping the same regimen and increasing adherence (Zheng et al. 2014). If protease inhibitor resistance mutations are found then phenotype testing should be sent.

Management of Patient with Extensive Multiclass Resistance

Some patients were treated with nonsuppressive NRTI-only regimens before more potent combinations were available. Furthermore, as new agents were introduced, they were often added as single active drugs to failing regimens, a practice sometimes referred to as “serial monotherapy.” Despite excellent medication adherence – or, ironically, *because* of excellent adherence – these strategies led to extensive multiclass drug resistance that makes finding a suppressive regimen challenging. Not surprisingly, most of the patients in this category started treatment in the 1990s or earlier. Virologic failure in this patient population requires meticulous attention to current and past resistance testing. Supplemental assessments, including a resistance phenotype (especially in those with complex PI resistance), integrase genotypes, and a viral tropism assay may be required. Once this information is obtained, the goal is to find a regimen that has at least two, and preferably three, fully active drugs.

In the NNRTI category, etravirine often retains viral activity even in those with resistance to the first generation NNRTIs. Genotypic scores are available to help determine if etravirine will still be active based on the NNRTI mutations present (Vingerhoets et al. 2010). Among the protease inhibitors with activity against PI-resistant virus, darunavir is the most potent and well tolerated and has been shown to be active even in the presence of certain protease mutations (Clotet et al. 2007).

In the integrase inhibitor category, dolutegravir retains activity against virus that has become resistant to raltegravir and elvitegravir (Castagna et al. 2014; Eron et al. 2013). Patients can also undergo tropism testing to determine if the CCR5 antagonist maraviroc could be used as a fully active agent.

The above approach generally leads to virologic suppression even in patients with extensive multiclass resistance. In the TRIO study, the combination of raltegravir, etravirine, and darunavir/ritonavir (often with inclusion of NRTIs at the discretion of the investigators) yielded virologic suppression in 90% of study subjects at 24 weeks, a rate comparable to that seen in treatment-naïve patients (Yazdanpanah et al. 2009). A more recent randomized clinical trial demonstrated that in this patient population, inclusion of NRTIs did not improve virologic suppression rates if the background regimen consisted of more than two active drugs by genotypic scoring (Tashima et al. 2015). Eliminating the NRTIs can limit drug toxicities, cost, and decrease the pill burden.

Immunologic Failure in the Setting of Viral Suppression

Immunologic failure is the inability to attain normal CD4 counts despite viral suppression although there is no clear consensus on the CD4 cut off for this definition. Most patients will recover their CD4 counts to >500 cells/mm³ with viral suppression, but some patient's CD4 counts will remain <200 cells/mm³. The key risk factor for immunologic failure is starting HIV therapy with severely depleted CD4 cell counts. These patients have a 2.6 fold greater risk of mortality compared to those with better immunologic recovery (Engsig et al. 2014). Adjunctive therapy with IL-2, while effective at raising the CD4 cell count, does not improve clinical outcomes (Abrams 2009).

Regimens containing zidovudine or the combination of TDF and ddI are known to impair CD4 response. If a patient is receiving these drugs, the regimen should be changed, preferably to one currently recommended in treatment guidelines. In addition, patients should be evaluated for other

conditions that may be associated with low cd4 cell counts, including hepatitis C, cirrhosis, and malignancy. Aside from the examples cited above, modifying treatment in an already virally suppressed regimen does not consistently improve the CD4 count and there is no evidence that it improves clinical outcomes. (DHHS ART)

Conclusions

The most common reason for virologic failure is poor adherence. It is crucial to uncover and then address the obstacles that prevent patients from taking their medications. The next step is to assess for resistance mutations by understanding each patient's treatment history, their resistance history, and patterns of adherence. Treatment decisions can be tailored to each individual based on these data. The goal is to treat each patient with at least two active agents in order to attain virologic suppression, which is possible in the majority of cases.

Cross-References

- ▶ [Entry Inhibitors](#)
- ▶ [Integrase Inhibitors](#)
- ▶ [Non-Nucleoside Reverse Transcriptase Inhibitors for Treatment of HIV Infection](#)
- ▶ [NRTIs](#)
- ▶ [Protease Inhibitor](#)

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Treatment of Histoplasmosis, Coccidioidomycosis, and Paracoccidioidomycosis in Patients with HIV Infection

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Definitions

Endemic mycoses	Fungal infections that occur within specific geographic regions
Immune reconstitution	Improvement in cellular immune function consequent to potent antiretroviral therapy

Prevention of disease	Antimicrobial therapy or other methods to prevent initial infection
Prevention of relapse	Antimicrobial therapy given after clinical disease has resolved

patients who present with an endemic fungal infection at the time of their HIV diagnosis.

Specific recommendations about managing these three fungal diseases are made below. A summary of these recommendations can be seen in Table 1.

Introduction

This chapter will discuss three endemic mycoses, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis, which have been associated with HIV-related infections. All are dimorphic and reside as molds in the environment, and each occurs in specific geographic regions in the Western Hemisphere, although histoplasmosis can be found worldwide.

As the HIV epidemic spread and involved new geographic regions, endemic fungi became important causes of opportunistic infections. Because they exist in the environment, limiting exposure to these fungi is not easily achieved. While these fungi cause illness among hosts with normal immune function, most infections are sub-clinical and many are self-limited. However, in those with impaired T-lymphocyte responses, clinical infection is both more prevalent and more severe. In those with HIV infection, the severity of illness caused by infection with these fungi is dependent on the degree of cellular immune deficiency of the host and can be estimated by the peripheral blood CD4 lymphocyte concentration.

Because potent antiretroviral therapy increases the CD4 lymphocyte population and results in an improvement of the cellular immune response, effective antiretroviral therapy is the most important tool in preventing and managing clinical disease due to these fungal infections. Improvement in the cellular immune response may be accompanied by inappropriate inflammation, known as the immune response inflammatory syndrome (IRIS). This itself has been associated with significant morbidity and mortality. Fortunately, IRIS appears to be an uncommon complication of the endemic fungi discussed here. Because of this, it appears to be reasonable to start antiretroviral therapy concurrently with antifungal treatment in

Histoplasmosis

Background. Histoplasmosis is due to infection with *Histoplasma capsulatum*. This thermally dimorphic fungus exists as a mold at room temperature and as a yeast within the host. Most cases are due to *H. capsulatum* var. *capsulatum*. Infection is endemic to the Mississippi and Ohio River Valleys of the United States. However, histoplasmosis is seen throughout the world, particularly in Central and South America, Africa, and Asia. Infection occurs by inhalation of fungal microconidia. Because the mold has a propensity to grow in nitrogen-rich environments, exposure to chicken coops, bird nests, caves, and similar areas may increase the risk of infection.

In the immunocompetent host, most infections are asymptomatic or result in a mild pulmonary syndrome. However, in the patient with cellular immunodeficiency due to HIV infection, severe and disseminated infection is common. In an early review of histoplasmosis among patients with AIDS, fever and weight loss were the most common symptoms. Hepatosplenomegaly, splenomegaly, pulmonary infiltrates, skin lesions, and anemia were frequent findings (Wheat et al. 1990). Skin lesions, gastrointestinal ulcers, and lymphadenopathy are also observed. Clinical disease may result from acute infection or from reactivation of a previously quiescent infection as cellular immunodeficiency wanes.

The diagnosis of histoplasmosis can be elusive. The fungus may take weeks to grow from clinical sources. Histopathology may reveal the yeast forms, often within macrophages. Serologic tests are frequently not helpful in disseminated disease. However, urine and serum assays for *Histoplasma* galactomannan antigen have been found to have both diagnostic and prognostic utility in patients with progressive disseminated histoplasmosis.

Treatment of Histoplasmosis, Coccidioidomycosis, and Paracoccidioidomycosis in Patients with HIV Infection, Table 1 Summary recommendations for treatment of histoplasmosis, coccidioidomycosis, and

paracoccidioidomycosis in patients with HIV infection based on disease activity and severity. IV indicates intravenous route; po indicates per oral

Fungal infection	Disease state	Severity	First choice antifungal	Alternatives
Histoplasmosis	Active	Severe	L-AmB ^a IV 3 mg/kg daily	Other lipid formulations of AmB IV 3–5 mg/kg daily; AmBd ^b IV 0.7 mg/kg daily
		Mild-moderate	Itraconazole po 200 mg twice daily	Fluconazole po 400 mg daily ^c
	Relapse prevention		Itraconazole po 200 mg daily	Fluconazole po 200 mg daily ^c
Coccidioidomycosis	Active	Severe	IV AmB, any formulation	Fluconazole IV 400 mg daily
		Mild-moderate	Fluconazole po 400 mg daily	Itraconazole po 200 mg twice to three-times daily ^d
	Relapse prevention		Fluconazole po 400 mg daily	Itraconazole po 200 mg twice daily ^d
Paracoccidioidomycosis	Active	Severe	IV AmB, any formulation	
		Mild-moderate	TMP-SMX ^e po twice daily	Itraconazole po 200 mg daily
	Relapse prevention		TMP-SMX po daily	Itraconazole po 100 mg daily ketoconazole po 400 mg daily sulfadiazine po 6 g daily

^aLiposomal amphotericin B

^bDeoxycholate amphotericin B

^cInferior to itraconazole

^dItraconazole preferred for bone and joint disease

^eTrimethoprim–sulfamethoxazole 160/800 mg

With the advent of potent antiretroviral therapy, the incidence of severe histoplasmosis among patients with HIV infection has declined. However, in resource-limited settings, including areas of Africa and South America, histoplasmosis continues to be an important opportunistic infection.

Treatment of Active Disease. Early studies documented that the deoxycholate formulation of amphotericin B (AmBd) is effective in treating severe histoplasmosis in patients with AIDS (Wheat et al. 1990). However, the renal, hematopoietic, and infusion-related toxicities of AmBd made this regimen problematic. A subsequent randomized, double-blind multicenter study demonstrated that a 2-week course of liposome-formulated amphotericin B (L-AmB) at a dosage of 3 mg/kg daily resulted in superior clinical improvement, lower mortality, and less infusion-related toxicity compared to AmBd at 0.7 mg/kg

daily (Johnson et al. 2002). Other lipid formulations of amphotericin B at a dosage of 3–5 mg/kg daily could also be used.

For patients with mild to moderate disseminated histoplasmosis, an alternative is oral itraconazole. In a nonrandomized, open-label study, 50 of 59 (85%) patients responded to therapy after receiving a 12-week induction course of 200 mg twice daily oral itraconazole with food preceded by a loading dose of 300 mg twice daily for 3 days. Clinical improvement was associated with a drop in the urine and serum *Histoplasma* antigen concentration. The median plasma concentration of itraconazole was 6.1 µg/mL (Wheat et al. 1995). Fluconazole appears to be less effective than itraconazole for the treatment of histoplasmosis. In a nonrandomized, open-label study of 49 patients with mild to moderate disseminated histoplasmosis and AIDS, only 36 (74%) of

patients responded to the initial 12 weeks of induction therapy of 800 mg of daily oral fluconazole (Wheat et al. 1997).

Prevention of Relapse. In the era prior to potent antiretroviral therapy, relapse of histoplasmosis was frequent once induction therapy was completed (Wheat et al. 1990). To prevent this, prolonged courses of antifungals were proposed. McKinsey and colleagues treated seven patients with disseminated histoplasmosis and HIV infection with an induction course of 1000 mg of AmBd followed by 50–80 mg weekly until a cumulative dose of 1000 mg was achieved. Then, 50–80 mg was given every other week indefinitely. Another nine patients received a cumulative induction dose of 2000 mg AmBd followed by 80 mg infusions weekly. Combining both treatment groups, 13 of 14 patients who did not die of other causes remained free of relapse (McKinsey et al. 1989).

Oral itraconazole is an alternative to prevent relapse. In an open-label, nonrandomized trial of 46 patients with mild to moderate histoplasmosis who completed induction therapy, 42 patients received oral itraconazole 200 mg daily. The four other patients received 400 mg daily. Patients were followed for a median of 64 weeks. There were only two relapses, one after 16 weeks and another after 29 weeks of maintenance therapy. The first appeared to be due to medication nonadherence and the second was associated with the addition of rifampin, which likely increased the metabolism of itraconazole (Hecht et al. 1997).

Fluconazole was less effective than itraconazole in preventing relapse. Among 36 patients who responded to 12 weeks of induction therapy with 800 mg of daily fluconazole and were given 200 mg of daily fluconazole for maintenance, 11 (31%) recurred (Wheat et al. 1997). A follow-up study suggested that relapses may be due to lower activity and drug resistance. In 65 subjects, the median minimum inhibitory concentration (MIC) of fluconazole against *H. capsulatum* was significantly lower among the 37 who responded to fluconazole compared to the 28 subjects who did not respond. Moreover, the median MIC for fluconazole among the entire

group was much higher than for itraconazole. Finally, a fourfold increase in MIC of fluconazole occurred over time in isolates from 10 of 17 patients, while the MIC for itraconazole remained unchanged (Wheat et al. 2001).

Discontinuing Therapy After Immune Reconstitution. With effective suppression of HIV replication by potent antiretroviral therapy, cellular immune function improves. This has led to the concept that maintenance antifungal therapy could at some point be discontinued. In a multisite, prospective, observational study, 32 subjects who had received at least 6 months of antiretroviral therapy and 12 months of antifungal therapy for histoplasmosis were studied. All had negative fungal blood cultures, urine and serum *Histoplasma* antigen concentrations <4.1 µg/mL, and a peripheral blood CD4 lymphocyte count >150/µL. After 24 months of follow-up, no instances of relapse were observed.

A recent retrospective study (Myint et al. 2014) of 97 patients with histoplasmosis and HIV infection identified by chart review demonstrates the role of suppression of HIV infection and immune reconstitution on relapse. In this group, antifungal therapy was discontinued by the physician in 38 patients, while it was continued in the other 59. The group continued on antifungal therapy was significantly less adherent to their antiretroviral regimen and had higher HIV RNA concentrations, lower CD4 cell counts, and higher urinary *Histoplasma* urinary antigen concentrations. While none of the patients in the physician-discontinued group had a relapse of histoplasmosis, 29 (36%) of those in the continued group did. The authors conclude that maintenance antifungal therapy for histoplasmosis can be safely discontinued in those who have received at least 1 year of antifungal therapy; had a CD4 cell count $\geq 150/\mu\text{L}$, an HIV RNA <400 copies/mL, and a urinary *Histoplasma* antigen level of <2 ng/mL; and did not have central nervous system histoplasmosis.

Histoplasma Antigen Monitoring. A useful tool for monitoring the clinical response to treatment of disseminated histoplasmosis is the *Histoplasma* galactomannan antigen test. Myint

and coworkers (Myint et al. 2014) found that those with a urinary antigen concentration >2.0 ng/mL after 1 year of treatment had a risk of relapse nearly 13 times that of those with levels below this.

Therapeutic Drug Monitoring and Drug Interactions. The capsule formulation of itraconazole is variably absorbed from the gastrointestinal tract and affected by food, acid, and even grapefruit juice. The oral suspension is more uniformly absorbed in the fasting state. Itraconazole is metabolized principally by the P450 isoenzyme CYP3A4, and its major metabolite is hydroxy-itraconazole, which has equipotent antifungal activity. Because of this, there is variability in metabolism as well as significant drug–drug interactions. Plasma levels <2 $\mu\text{g/mL}$ of itraconazole have been associated with clinical failure in some patients with histoplasmosis (Wheat et al. 1995). Because of this, a random plasma sample should be obtained after 2 weeks of therapy to assure adequate itraconazole concentrations. If the bioassay is used, a level of at least >1 $\mu\text{g/mL}$ should be obtained. If high-pressure liquid chromatography (HPLC) is employed, the sum of itraconazole and hydroxy-itraconazole concentrations should reach this level (Wheat et al. 2007).

Azole antifungals, including itraconazole, both inhibit and are metabolized by the CYP isoenzyme system. Their use may result in bidirectional drug effects. For itraconazole, drugs that induce CYP3A4 may result in subtherapeutic itraconazole levels and treatment failure. This has been observed among patients with HIV infection on efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI). Conversely, HIV-1 protease inhibitors inhibit CYP3A4, and increases in the levels of both itraconazole and protease inhibitors can be expected. Because of this, therapeutic drug monitoring is useful when such drug combinations are used.

Prevention of Disease. It has long been recommended to avoid tasks associated with an increased risk of exposure to the fungus. These include avoiding areas with high concentrations of bird and bat excrement. While reasonable, many infections occur in the absence of such exposures.

For medical prophylaxis, in a double-blind, placebo-controlled trial in several US cities endemic for histoplasmosis, oral itraconazole at 200 mg daily among 295 HIV-infected patients who had a CD4 count $<150/\mu\text{L}$ was associated with a reduction in the number of cases of histoplasmosis from ten to four over a median of 16 months. However, this benefit was only seen in those with CD4 $\leq 100/\mu\text{L}$ and no survival benefit was observed (McKinsey et al. 1999). Moreover, no comment was made regarding the role of potent antiretroviral therapy. Current guidelines recommend prophylaxis only in those living in highly endemic regions (>10 cases per 100 patient-years) and recommend discontinuing when the CD4 count $\geq 150/\mu\text{L}$ (Wheat et al. 2007).

Coccidioidomycosis

Background. Coccidioidomycosis is caused by the dimorphic fungus *Coccidioides*, which is recognized as two genetically distinct species, *C. immitis* and *C. posadasii*. These species are not distinguishable on culture nor by the clinical disease they cause. Coccidioidomycosis is endemic to the San Joaquin Valley of California, the south-central region of Arizona, and northern Mexico. However, it can be acquired in other areas of southern California, certain regions of Nevada, Utah and Texas, as well as areas of Central America and South America, including northeast Brazil and northern Argentina.

Coccidioides spp. exist as molds in the environment. While they reside in the upper 10 cm of the soil, the precise ecological niche is not known. In most cases, inhalation of arthroconidia, small hydrophobic fungal cells, is the cause of infection. In immunocompetent hosts, most infections are asymptomatic. However, in those with cellular immunodeficiencies such as AIDS, severe and disseminated infections are common.

The diagnosis of coccidioidomycosis can be established in several ways. Unlike the other endemic fungi, *Coccidioides* spp. usually grow within 7 days on routine bacteriological media. It can also be seen in biopsy specimens in its unique tissue phase, the spherule. Serologic

tests are very useful in the diagnosis of coccidioidomycosis. In particular, the titer of complement fixation (CF) antibody response is reflective of fungal growth and can be used both for diagnosis and for monitoring response to therapy. Finally, similar to histoplasmosis, a galactomannan antigen test for both urine and serum exists. However, its usefulness appears limited to extrathoracic disseminated disease and has not been studied extensively as a tool to assess response to therapy.

During the late 1980s and early 1990s, coccidioidomycosis emerged as an important opportunistic infection among HIV-infected persons living in the coccidioidal endemic region. In a retrospective study of 77 patients from 1990, a wide range of manifestations were seen, including diffuse pulmonary disease and liver and lymph node involvement. Death occurred in 32 (42%) over a median of 7 months (Fish et al. 1990). In a prospective study of 147 patients followed in a single HIV clinic beginning in 1988, the cumulative risk of developing coccidioidomycosis was nearly 25% over 41 months. Risk factors included a CD4 cell count $<250/\mu\text{L}$ and a previous AIDS diagnosis. Five (38%) of the 13 patients died during the follow-up period. Prior infection did not appear to be a risk, suggesting that most disease was associated with recent infection (Ampel et al. 1993). The incidence of clinically active coccidioidomycosis declined with the advent of potent antiretroviral therapy. A follow-up study in 2010 from the same clinic revealed that 29 (11%) of 257 patients had coccidioidomycosis and the annual incidence was estimated to be only 0.9% (Masannat and Ampel 2010).

Treatment of Disease. Unlike histoplasmosis, there are no placebo-controlled, randomized studies regarding the treatment of coccidioidomycosis in patients with HIV infection. For severe pulmonary and extrathoracic disseminated coccidioidomycosis in the acutely ill patient, amphotericin B is recommended as initial therapy. There are no comparative trials of AmBd and lipid formulations for coccidioidomycosis. Because of this, the formulation used should be based on the risk of renal disease and cost. For initial therapy, AmBd at 0.7 mg/kg daily is recommended,

while 3 mg/kg daily of a lipid preparation is appropriate. Many experts would also start a triazole antifungal at 400 mg daily at the same time. As the patient improves, the amphotericin B preparation can be reduced in frequency and finally discontinued when the patient becomes clinically stable, leaving the patient only on the triazole antifungal.

For less severe disease, initial therapy can be with an oral triazole antifungal at 400 mg daily. For pulmonary disease, most experts would choose fluconazole, given its better absorption and tolerance compared to itraconazole. However, for extrathoracic disease, particularly that involving bones and joints, itraconazole appears to have better activity. There are limited data on the use of posaconazole and voriconazole, but these agents appear appropriate in patients not responding to fluconazole or itraconazole.

Prevention of Relapse. For patients with focal pulmonary disease, treatment with at least 400 mg daily of a triazole antifungal should continue for a minimum of 6 months. For patients with diffuse pulmonary disease and those with extrathoracic dissemination, antifungal therapy is usually much longer and based on clinical and immunological response. There are no data on using lower doses of triazole antifungals to prevent relapse.

Discontinuation of Therapy After Immune Reconstitution. Data indicate that the specific cellular immune response to coccidioidal antigens is maintained when the peripheral blood CD4 cell count is $\geq 250/\mu\text{L}$ (Ampel 1999). Moreover, a prospective study of patients with coccidioidomycosis and HIV infection demonstrated that less severe disease occurs in those with higher CD4 cell counts and lower HIV RNA levels (Masannat and Ampel 2010). Based on this, for those patients on potent antiretroviral therapy with undetectable HIV RNA and CD4 $\geq 250/\mu\text{L}$, the HIV patient with coccidioidomycosis can be managed similarly to the patient without immunodeficiency. For those with focal pulmonary disease, this is usually 6 months of treatment. For extrathoracic disseminated disease, it is longer and is based on the clinical response and the CF titer. Patients with coccidioidal meningitis should receive lifelong therapy.

Prevention of Disease. For those living in the endemic region, it is difficult to avoid exposure to *Coccidioides* spp. Remaining inside during dust storms or similar conditions seems prudent but is unproven. A retrospective study suggested that in highly immunosuppressed patients living in the coccidioidal endemic area, fluconazole resulted in a reduced risk of coccidioidomycosis (Woods et al. 2000). However, the benefit was small. Initiation of potent antiretroviral therapy with its consequent immune reconstitution is the best method to reduce the risk of severe coccidioidomycosis for those living in the endemic region (Masannat and Ampel 2010).

Paracoccidioidomycosis

Background. Caused by the thermally dimorphic *Paracoccidioides brasiliensis*, paracoccidioidomycosis appears to be acquired only in Central and South America and is the most common systemic mycosis seen there. The vast majority of cases are reported from Brazil, with fewer reported from Venezuela, Colombia, and Argentina. The natural habit of the fungus is not known, but most infections are presumed to be acquired by inhalation of mycelial conidia from the environment.

There are several distinct clinical presentations of paracoccidioidomycosis. Subclinical infection is asymptomatic and usually accompanied by delayed-type hypersensitivity to antigen skin testing. Progressive disease is defined as an inability of the host to control fungal growth and results in diverse clinical presentations. It has been divided into two major forms. The chronic, adult form accounts for 90% of progressive cases of paracoccidioidomycosis. It occurs overwhelmingly among men, usually from 35 to 60 years. Pulmonary symptoms and signs are common as are lesions of the oral mucosa and skin. The acute juvenile form occurs principally in patients under 30 years with less male predominance. Pulmonary findings are uncommon and skin lesions of the face, neck, and trunk, with gastrointestinal ulcers and bone lesions, are frequent. The third major manifestation of paracoccidioidomycosis,

residual disease, is organ damage as a result of fibrosis from a previously active infection.

Like histoplasmosis and coccidioidomycosis, the diagnosis of paracoccidioidomycosis can be made in several ways. Biopsy of infected tissue may reveal the characteristic configuration of multiple attached yeasts in a pattern resembling a steering wheel. Cultures may take up to 30 days for the organism to grow as a mold without distinguishing characteristics. Several serologic tests are available but are not standardized.

In 1995, Goldani and Sugar reviewed the first 27 cases of paracoccidioidomycosis associated with HIV infection reported in the medical literature (Goldani and Sugar 1995). The median age was 31 years and 21 were male. Only one patient was receiving trimethoprim–sulfamethoxazole prophylaxis for pneumocystosis and the overall mortality was 30%. Paniago and colleagues reported 21 cases of paracoccidioidomycosis occurring during HIV infection from a single medical center in Mato Grosso, Brazil, in 2005 (Paniago et al. 2005). All were male with a mean age of 36 years. The clinical presentations seen, with lymph node and bone marrow involvement, corresponded to the acute juvenile form of paracoccidioidomycosis. A later retrospective case–control study compared the epidemiological and clinical data of 53 cases of paracoccidioidomycosis among patients with HIV infection to a control group of 106 patients without HIV (Morejon et al. 2009). Those with HIV infection were younger, more likely to be female, and were less likely to be involved in agricultural work compared to the control group. In the HIV group, 80% had CD4 < 200/μL and only 15 (28%) were on trimethoprim–sulfamethoxazole prophylaxis. After 24 months of follow-up, only 63% of those with HIV infection were improved or in remission, compared to 93% of those with HIV.

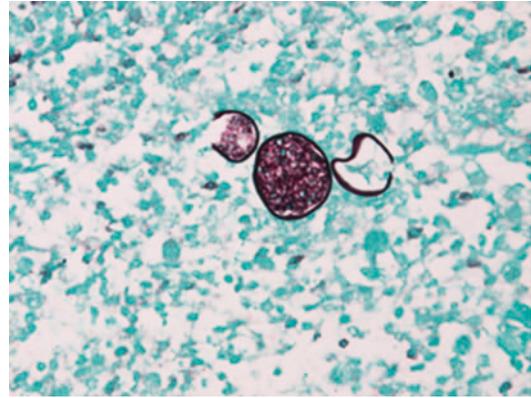
Treatment of Disease. There are no comparative trials of different therapies for paracoccidioidomycosis in the HIV-infected patient. Moreover, unlike histoplasmosis and coccidioidomycosis, sulfa drugs have been successfully used to treat various forms of this infection. In the case–control study cited above, therapy of HIV-infected patients included trimethoprim–sulfamethoxazole in 60%, some formulation of

amphotericin B in 45%, and a triazole antifungal in 32% (Morejon et al. 2009).

For severe disease, amphotericin B is recommended as initial therapy, either as AmBd at 0.7/kg daily or as a lipid preparation at 3 mg/kg daily. The choice of formulation should be based on cost and risk of renal toxicity. Amphotericin B therapy should be continued until the patient is clinically stable. For milder disease, trimethoprim–sulfamethoxazole or itraconazole can be employed.

Prevention of Relapse. Once clinical stability has been achieved, an azole antifungal or sulfa antimicrobials may be started. In a randomized trial, itraconazole up to 100 mg daily, ketoconazole up to 400 mg daily, and sulfadiazine up to 6 g daily were compared among 42 patients both acute and chronic progressive paracoccidioidomycosis. All three regimens led to statistically comparable rates of clinical cure (Shikanai-Yasuda et al. 2002). More recently, de Souza Calvacante and colleagues compared the efficacy of itraconazole 200 mg daily to trimethoprim–sulfamethoxazole in a quasi-experimental study among 177 patients with chronic progressive paracoccidioidomycosis. None were reported as having HIV infection. While overall results were comparable, the time to clinical cure was shorter for itraconazole (de Souza Cavalcante et al. 2014). Other agents used to treat paracoccidioidomycosis include sulfadoxine and voriconazole. Data are limited regarding use of any of these agents in HIV-infected patients with paracoccidioidomycosis. However, itraconazole at 200 mg daily would seem reasonable. An alternative would be trimethoprim–sulfamethoxazole given as up to 1200 mg of sulfamethoxazole twice daily (Marques 2013).

Discontinuation of Therapy After Immune Reconstitution. There are no studies regarding stopping therapy once immune reconstitution has occurred, but it would seem reasonable to continue therapy at least until the CD4 cell count exceeds 200/ μ L, based on the retrospective case review (Morejon et al. 2009) and observations in histoplasmosis and coccidioidomycosis. Given that therapy is recommended to be continued for



Treatment of Histoplasmosis, Coccidioidomycosis, and Paracoccidioidomycosis in Patients with HIV Infection, Fig. 1 Coccidioidal spherules in tissue. Gomori methenamine silver stain

up to 12 months for mild disease and up to 2 years for more severe illness among those without HIV infection, therapy for at least this long beyond the time from immune reconstitution should be considered.

Prevention of Disease. There are no environmental means to prevent infection. However, the use of trimethoprim–sulfamethoxazole as prophylaxis for pneumocystosis in those with CD4 cell counts $<200/\mu$ L likely has had a significant effect in either preventing infection with *Paracoccidioides* or ameliorating clinical infection. There are no studies on the use of azole antifungals in preventing paracoccidioidomycosis in the HIV-infected person.

Conclusion

Three endemic mycoses, histoplasmosis, coccidioidomycosis, and paracoccidioidomycosis, which can cause disease in the immunocompetent host, have been found to also act as opportunistic infections in those with HIV infection, particularly at low peripheral blood CD4 counts. Therapy of acute and severe disease frequently requires the use of an amphotericin B preparation. While the liposomal formulation is favored in histoplasmosis, no data exist for either coccidioidomycosis or paracoccidioidomycosis. Subsequent therapy

or therapy for milder disease can employ an azole antifungal or, in the case of paracoccidioidomycosis, a sulfa antimicrobial. With the use of potent antiretroviral therapy, the frequency and severity of these three fungal infections in the HIV-infected population are diminishing, and immune reconstitution allows discontinuation of antifungal therapy in many instances.

Cross-References

- ▶ [Cryptococcosis and HIV](#)
- ▶ [Talaromyces \(Penicillium\) marneffei and HIV](#)

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TRIM Protein Family and Viral Restriction

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Definition

Tripartite motif (TRIM) proteins constitute a vast family of proteins characterized by a common motif, known as RBCC, as it is composed, from N- to C-terminal, by a RING (R) domain, one or two zinc finger domains, known as B-boxes (B) and a coiled coil (CC). TRIM genes are found in all metazoans and have rapidly expanded during vertebrate evolution. Although TRIM proteins are implicated in a plethora of biological processes, several lines of evidence indicate that many of them are key actors of cellular defense against viruses. In particular, many TRIM proteins have been found to interfere with various stages of HIV life cycle. Furthermore, an increasing number of TRIMs are found to be key

modulators of interferon (IFN) response and can therefore further impact viral spread and clinical course of infection.

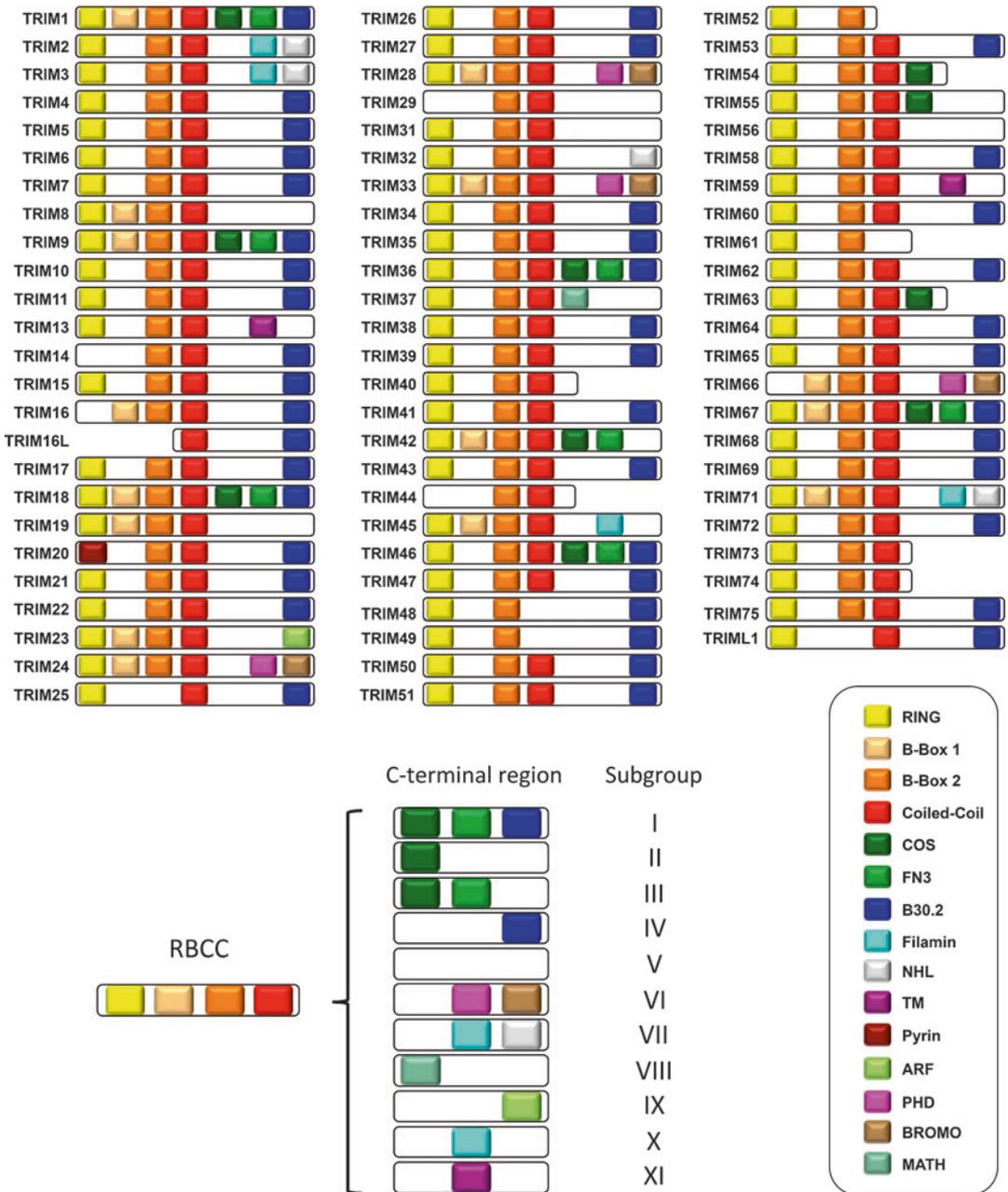
Introduction

Whereas there are a relatively small number of TRIM genes in lower eukaryotes, vertebrates and especially mammals have a vast TRIM repertoire, suggesting an extensive evolution of the TRIM family. In humans, the TRIM protein family consists of over 70 members that all harbor the RBCC motif, but differ from each other by the nature of their C-terminal region (Fig. 1). Short and Cox have proposed a classification of TRIM proteins into nine different subgroups, based on the domain composition of their C-terminal region (Short and Cox 2006). This classification was slightly modified, leading to the definition of 11 classes (C-I to C-XI) (Fig. 1).

However, this classification does not entirely reflect the complexity of this protein family, since most TRIM genes can generate multiple alternatively spliced transcripts encoding TRIM proteins with different C-terminal domains and thus different functions.

TRIM proteins have strong self-association ability, mainly mediated by their CC domain, which results in the formation of large protein complexes that in some cases form discrete nuclear and/or cytoplasmic subcellular structures (Reymond et al. 2001). TRIM proteins are implicated in a broad range of biological processes, such as transcriptional regulation, apoptosis, and development. As a result, TRIM protein dysregulations are implicated in numerous pathologies including genetic, developmental, or neurological disorders and cancer. Among these various cellular functions, one has rapidly emerged as an important and possible common feature of all TRIM proteins: their involvement in antiviral innate immunity (Rajsbaum et al. 2014).

In addition to their implication in various biological processes, the heterogeneity of TRIM proteins is further illustrated by the fact that they exhibit diverse expression patterns. They can be found either in the nucleus or in the cytoplasm,



TRIM Protein Family and Viral Restriction, Fig. 1 Schematic representation of the human TRIM protein family. The domain organization of individual human TRIM proteins together with their classification based on the domain composition of their C-terminal region proposed by Short and Cox is represented. Note that only “real” TRIM proteins are represented in the Short and Cox classification. Thus, proteins that do not possess a RBCC motif per se are absent from this classification. This is, for example, the case of TRIM20 that has a pyrin domain in place of the RING, or TRIM29 and TRIM44,

which have no RING. *RING* really interesting new gene, *COS* C-terminal subgroup one signature, *FN3* fibronectin type 3, *PHD* plant homeodomain, *NHL* NCL-1, HT2A, and LIN-41, *MATH* meprin, and TRAF homology, *ARF* ADP ribosylation factor-like, *TM* transmembrane. B30.2 domains are also called PRYSPRY as they are composed of a SPRY with an additional PRY domain. Although some TRIM proteins only have a SPRY domain, PRYSPRY and PRY domains are all referred to B30.2 in this representation. Also, the coiled-coil region is shown systematically, although its computer-based prediction is variable

some with a diffuse distribution and some others forming punctate structures. But the common N-terminal RBCC motif of TRIM proteins also confers them with common features, such as the capacity to homo- and hetero-oligomerize. Another general feature of this protein family is common enzymatic activity conferred by their RING domain, since TRIM proteins have emerged as a family of E3 ubiquitin ligases (Rajsbaum et al. 2014). Ubiquitination is a post-translational modification that consists in the covalent conjugation of ubiquitin moieties to lysine residues of specific substrate proteins. Conjugated ubiquitin itself can serve as a substrate for ubiquitination, leading to the formation of poly-ubiquitin chains. All seven lysine residues in ubiquitin contribute to the synthesis of poly-ubiquitin chains, and each type of chain has specific consequences for the substrate protein. Most TRIM proteins promote either K48 ubiquitination that is usually followed by the proteasomal degradation of target proteins or K63 ubiquitination, which is involved in protein activation. Furthermore, several TRIM proteins have been reported to function as E3 ligases for small ubiquitin-like modifier (SUMO) or ISG15.

TRIM Proteins and Intrinsic Immunity

In addition to the two main branches of immunity (i.e., innate and adaptive immunity), the notion of the so-called intrinsic immunity appeared with the discovery of antiretroviral restriction factors. These factors confer cells with a protection against specific viruses by inhibiting their replication independently of the immune system. Thus, intrinsic immunity constitutes a cell autonomous form of innate antiviral defense.

Since this intrinsic defense directly targets viral components, they have coevolved with virus, each providing a selection pressure to the other. Thus, a common feature shared by TRIM proteins with antiviral intrinsic properties is the fact that they have evolved under positive selection, especially in the regions that interface with the virus (Sawyer et al. 2005, 2007). These signatures can be detected through phylogenetic comparison of

synonymous and nonsynonymous substitution rates.

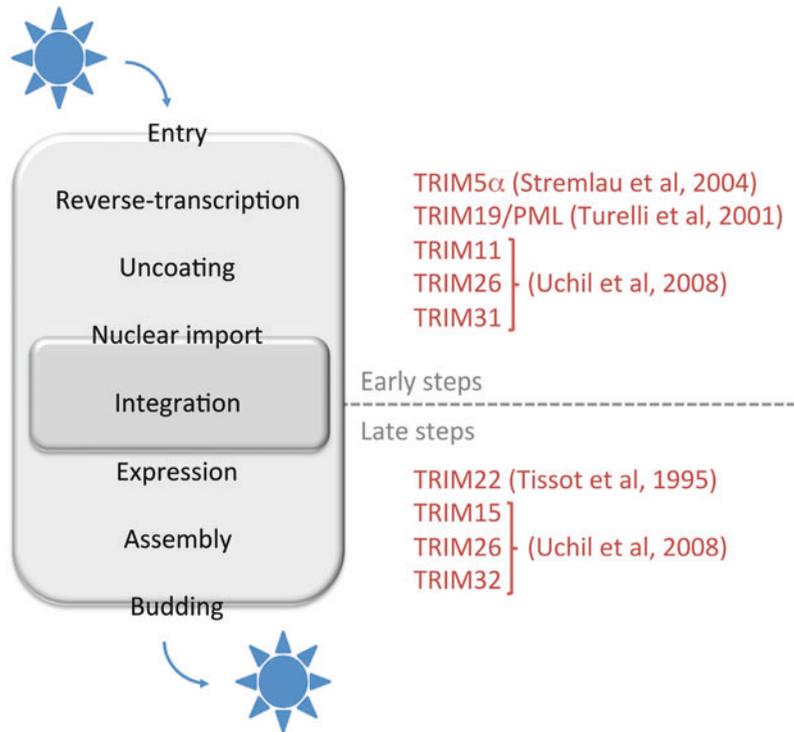
Many TRIM proteins have been identified as restriction factors. Although TRIM proteins can inhibit various viruses, HIV-1 is the most exemplified target (Fig. 2).

One of the best characterized TRIM proteins with intrinsic antiviral properties is TRIM5 α , the longest transcript encoded by the TRIM5 gene. Initially discovered as the protein that protects rhesus macaques from HIV-1 infection (Stremlau et al. 2004), TRIM5 α was later shown to display a broad antiretroviral intrinsic immunity in other primates, including humans. The C-terminal region of TRIM5 α , composed of a PRYSPRY (or B30.2) domain, allows the protein to recognize retroviral capsids. Due to a high degree of polymorphism within this domain of TRIM5 α between species, restriction patterns are species specific (Sawyer et al. 2005). For example, TRIM5 α from rhesus macaque inhibits HIV-1, but not SIVmac, whereas in humans, TRIM5 α restricts N-tropic murine leukemia virus (N-MLV) and equine infectious anemia virus (EIAV). Unlike those encoded by Old World monkeys, human TRIM5 α has only a modest restriction activity toward HIV-1, although more potent anti-HIV activity can be artificially conferred to the human protein by a single point mutation within the PRYSPRY domain (Yap et al. 2005). The targeted residue is located within a patch subject to intense selective pressure and presumably located at the interface with the viral capsid (Sawyer et al. 2005). TRIM5 α interferes with an early postentry step of retroviral replication, since its expression inhibits reverse transcription. Mechanistically, TRIM5 α binds the capsid protein lattice of incoming viruses and promotes their disassembly, thus inhibiting reverse transcription and subsequent steps of replication (Stremlau et al. 2006). The RING domain of TRIM5 α acts as an E3 ubiquitin ligase, but it is still unclear whether this activity is implicated or not in retroviral restriction. TRIM5 α is expressed constitutively in virtually all cells in the organism, where it is found both diffuse in the cytoplasm and within large dots, named cytoplasmic bodies. TRIM5 α was also recently found to shuttle

TRIM Protein Family and Viral Restriction,

Fig. 2 Human TRIM proteins interfering with HIV-1 replication.

The main steps of HIV-1 replicative cycle are schematically represented, along with TRIM proteins that have been found to interfere either with early or late stages of HIV cycle



between the nucleus and the cytoplasm, but the function of nuclear TRIM5 α is unknown (Diaz-Griffero et al. 2011).

Although TRIM genes are distributed throughout the human genome, TRIM5 gene resides in a cluster of four closely related TRIM genes that also include TRIM6, TRIM34, and TRIM22. While TRIM6 and TRIM34 have evolved under purifying selection, TRIM5 and TRIM22 underwent strong positive selection in primates (Sawyer et al. 2007). Accordingly, TRIM22, also known as Staf50, has also been identified as a potent antiviral protein. TRIM22 is an interferon-stimulated gene (ISG) product that inhibits transcription from the HIV-1 LTR (Tissot and Mechti 1995). In addition, it was later found to interact with the HIV-1 Gag protein and to interfere with its trafficking to the plasma membrane (Barr et al. 2008). Unlike TRIM5 α whose antiviral activity only targets retroviruses, TRIM22 also confers resistance to hepatitis B virus (HBV), EMCV, and influenza A virus.

Another TRIM protein with a broad antiviral activity is TRIM19, better known as PML

(promyelocytic leukemia). PML can interfere with the replication of members of many viral families, including *Arenaviridae*, *Herpesviridae*, *Orthomyxoviridae*, *Parvoviridae*, *Picornaviridae*, *Rhabdoviridae*, and *Retroviridae* (Nisole et al. 2013). In the case of retroviruses, PML was first shown to inhibit foamy virus transcription, by complexing the viral transactivator Tas, thereby preventing its binding to viral DNA (Regad et al. 2001). PML was also reported to interfere with HIV-1 replication. More specifically, HIV-1 infection was found to rapidly trigger the translocation of PML from the nucleus to the cytoplasm where it co-localizes with pre-integration complexes (Turelli et al. 2001). Interestingly, arsenic-induced degradation of PML has been reported to cause an enhancement of HIV-1 infection, suggesting that PML cytoplasmic export is deleterious for the virus (Turelli et al. 2001). However, this latter observation was later contradicted by other studies, and the implication of PML in HIV restriction remains controversial.

A study investigated the potential antiretroviral activity of 36 human and 19 mouse TRIM

proteins (Uchil et al. 2008). From their screen, they identified 20 TRIM proteins that interfere either with early or late stages of the retroviral replication cycle. In particular, murine TRIM8, TRIM10, TRIM11, and TRIM56 and human TRIM11, TRIM26, and TRIM31 were found to interfere with early steps of HIV-1 infection (Fig. 2). In contrast, HIV-1 release was affected by murine TRIM11, TRIM25, TRIM27, and TRIM56 and by human TRIM15, TRIM26, and TRIM32. This first large screen of antiretroviral TRIM proteins confirms that antiviral activity is a general feature of many (if not all) TRIM proteins.

However, it is difficult to tell from these studies whether the majority of TRIM proteins are targeting retroviruses because this particular viral family has coevolved with mammals for millions of years or whether this is simply due to the fact these viruses are more widely studied.

Besides HIV, however, some other TRIM proteins have been shown to display a direct antiviral activity toward other viruses (Rajsbaum et al. 2014). This is the case of the murine protein TRIM79 α , which restricts tick-borne encephalitis virus, and human TRIM56, which blocks pestivirus infection. Whereas TRIM21 was also identified as a potent antiviral protein, its activity could not be considered as intrinsic per se, since it facilitates the cytoplasmic detection of antibody-opsionized non-enveloped viruses, through the capacity of its PRYSPRY domain to act as an IgG Fc receptor.

Since positive selection is a hallmark of TRIM proteins implicated in intrinsic immunity, it should be possible to predict the potential antiviral activity of the entire family through a computational screen. Such a screen has been performed and revealed that ten uncharacterized human TRIM genes showed signatures of positive selection (Malfavon-Borja et al. 2013).

TRIM Proteins and Innate Immunity

In addition to their implication in intrinsic immunity, an increasing number of TRIM proteins have been found to control viral replication by regulating innate immune pathways (Rajsbaum et al.

2014). Once a virus enters a cell, its nucleic or proteic components are sensed by cellular pattern recognition receptors (PRRs), which trigger transduction pathways leading to the activation of transcription factors such as NF- κ B and interferon regulatory factors (IRFs). Once activated, these factors translocate into the nucleus and induce the expression of many cytokines, including IFNs and pro-inflammatory cytokines. Type I IFNs are the main inducers of innate immunity in the case of viral infections, since they initiate an antiviral program in infected and surrounding cells. Most recently identified restriction factors, such as TRIM5 α , APOBEC3G, and tetherin/BST-2, are induced by type I IFN. Furthermore, many TRIM proteins, in particular those known to display antiviral activity, are among the many antiviral factors whose expression is induced by type I IFN (Carthagen et al. 2009). In particular, some TRIM proteins have been identified as key mediators of the anti-HIV activity of type I IFN, such as TRIM5 α and TRIM22 (Barr et al. 2008; Carthagen et al. 2009).

IFNs exert both antiviral activity and immunostimulatory functions and are a key bridging mechanism between innate and adaptive immune responses. Despite their crucial role during acute viral infections, however, the prolonged stimulation of IFN has detrimental consequences. In this context, HIV-1 infection represents an example of how chronic IFN production could compromise long-term immune protection and contribute to the pathogenesis of the disease. Thus, since many TRIM proteins are not only able to interfere with HIV replication but also to modulate IFN synthesis, they are likely to have a major implication in the development of viral spread and pathogenesis. However, the global impact of TRIM proteins on the outcome of HIV infection will require a better understanding of their respective mode of action.

The list of TRIM proteins modulating innate immune pathways is expanding so rapidly that it is already almost impossible to review them all in detail (Rajsbaum et al. 2014). Therefore, only a few examples will be developed here to illustrate the heterogeneity of their mode of action. TRIM proteins can act directly on PRRs or on

downstream signaling pathways. Among the TRIM proteins that act as positive regulators of innate immune signaling, one of the best examples is TRIM25, which was identified as a positive regulator of retinoic acid-inducible gene-I (RIG-I) activation. RIG-I is a cytoplasmic dsRNA helicase that senses viral RNA within the cytoplasm of infected cells. During a viral infection, TRIM25 induces the K63-linked ubiquitination of RIG-I, thus allowing its binding to mitochondrial antiviral-signaling protein (MAVS, also known as VISA, IPS-1, or Cardif). This results in a marked increase of RIG-I downstream signaling activity and IFN production (Gack et al. 2007). TRIM25 can also catalyze the synthesis of unanchored K63-ubiquitin chains, which can activate RIG-I. Another TRIM protein, TRIM4, was also recently proposed to promote K63 ubiquitination-dependent activation of RIG-I. K63 ubiquitination seems to be a general mechanism used by TRIM proteins to activate innate immune signaling. Not only PRRs can be targeted, but virtually all intermediates of downstream signaling pathways. For example, TRIM56 targets STING for K63 ubiquitination to promote its dimerization and subsequent TBK1 activation, thus increasing dsDNA-induced IFN- β production (Fig. 2). Similarly, TRIM8 promotes TAK1 K63 ubiquitination to activate the NF- κ B pathway. TRIM23 is an exception to the rule as it activates NEMO through a K27-linked polyubiquitination. While most TRIM proteins modulate immune pathways leading to IFN production, some other TRIMs directly target IFN- β transcription. This is the case of TRIM19/PML, which enhances IFN- β transcription during viral infections through the sequestration Pin1, an enzyme that normally terminates IFN transcription (El Asmi et al. 2014). In addition to these various activators, some other TRIM proteins have been identified as negative regulators of innate immune signaling. Given the pleiotropic and opposite effects of TRIM proteins on innate immune pathways, evaluating the global impact of the whole TRIM family on innate sensing, IFN response and viral spread will need further intensive work once the mechanism of each individual TRIM has been deciphered.

In most cases, intrinsic antiviral activity and modulation of innate immunity are mutually exclusive. This can easily be explained by the fact that TRIM proteins that target viral proteins are under a strong selection pressure and thus evolve rapidly, whereas TRIMs that target cellular proteins to modulate innate immune signaling are likely to be well conserved among species. They are exceptions, including TRIM5 α and TRIM19/PML. TRIM5 α directly interferes with retroviral replication and also activates immune pathways, acting as a PRR (Pertel et al. 2011), whereas TRIM19/PML is able to restrict many different viruses from unrelated families and, at the same time, enhance IFN- β transcription through the inhibition of a cellular protein. However, despite its multiple roles in antiviral defense, the PML gene does not show any signature of positive selection (Malfavon-Borja et al. 2013), suggesting that PML does not directly interact with viral components.

Conclusion

It is now established that TRIM proteins constitute an ancient protein family that emerged during an early step of metazoan evolution and rapidly diversified by gene duplications in vertebrates driven by selective adaptation. In the case of TRIM proteins acting as restriction factors, this adaptation was imposed by continuous viral pressure. However, even if a growing number of TRIM proteins have been identified as antiviral proteins or as activators of innate immune response, the vast majority of TRIMs remain uncharacterized. Further investigations will be required to draw a complete picture of the implication of the whole TRIM protein family in viral infections, including HIV.

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TRIM5 Alpha and HIV-2 Infection

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Definition

This entry discusses the restriction of HIV-2 by the host restriction factor TRIM5 α . Although most published studies focus on HIV-1, we looked at the parallels between the two viruses, paying particular attention to the differences between HIV-1 and HIV-2 and to studies that specifically focused on HIV-2, to put together a current synthesis of our understanding of HIV-2 restriction by TRIM5 α .

Introduction

HIV-2 is the second human retrovirus that can cause AIDS. While very similar to HIV-1 in terms of genomic sequence and protein function, HIV-2 infection usually leads to an attenuated disease course. About 80% of HIV-2 infections do not lead to AIDS; they are instead characterized by a longer asymptomatic stage, lower

plasma viral loads, slower decline in CD4 count, decreased mortality rate, and lower rates of vertical and sexual transmission (see “► [HIV-2 Transmission](#)”). Nonetheless, a significant proportion of HIV-2-infected individuals eventually progress to AIDS, with high viral loads, low CD4 counts, and a clinical picture indistinguishable from HIV-1 disease progression (see “► [Natural History and Clinical Features of HIV-2 Infection](#)”). The HIV-1 epidemic is characterized by extensive genetic variation, with four groups, several subtypes, and many circulating recombinant forms (CRF) (see “► [Origin and Distribution of HIV-1 Subtypes](#)”), while the HIV-2 epidemic consists of only two groups (A and B) and six non-epidemic groups (C–H) (see “► [HIV-2, Phylogeographic Insights into the Origins and Epidemic History](#)”). HIV-1 has spread throughout the world with high transmission rates, while HIV-2 is restricted mainly to West Africa, where prevalence has been declining (Tienen et al. 2010). The main difference between HIV-1 and HIV-2 is in their origin. HIV originated from SIV from chimpanzees (*Pan troglodytes troglodytes*), but HIV-2 arose from zoonotic transfer of SIV from the sooty mangabeys (*Cercocebus atys*) to humans (see “► [HIV-2, Phylogeographic Insights into the Origins and Epidemic History](#)”): consequently HIV-2 and SIV from sooty mangabeys (SIV_{sm}) and SIV-infected macaques (SIV_{mac}) share about 75% sequence homology compared to 40–50% homology with HIV-1 (Hirsch et al. 1989). Several studies have revealed factors, immunological, virological, and molecular, that contribute to the attenuated course of HIV-2 infection. More recently, studies on host genetic factors, such as TRIM5 α , have provided genetic contributions to understanding the reduced HIV-2 pathogenicity.

TRIM5 α is a member of the tripartite motif family of proteins that has the three protein domains: RING, B-box, and coiled coil (see “► [TRIM Protein Family and Viral Restriction](#)”). TRIM5 α has an additional domain, at its C-terminal, the PRYSPRY or B30.2 domain that interacts with the retroviral capsid and is responsible for species specificity (Stremlau et al. 2005). The retroviral restriction properties of TRIM5 α were discovered because HIV-1 could not grow

in certain Old World monkey cells. TRIM5 α can mediate a postentry block of lentiviral replication either before or after reverse transcription, in a species-specific manner. Although the exact mechanism of retroviral restriction is not fully understood, several mechanisms, some of which are interdependent, have been proposed, with supporting experimental data. Oligomeric TRIM5 α recognition of and binding to the incoming intact capsid destabilize the capsid core, leading to accelerated or premature uncoating, which perturbs reverse transcription. The E3 ligase activity of the RING domain can add ubiquitin molecules to itself and to other proteins, leading to proteasome-mediated protein degradation of the capsid-TRIM5 α complex. TRIM5 α forms occlusion bodies, where the virus is essentially trapped, and it also inhibits nuclear transport of the replicating virus (Veillette et al. 2013). TRIM5 α is a pattern recognition receptor that recognizes and binds to the viral capsid, causing a cascade of innate immune signaling that leads to an antiviral state, restricting retroviral replication (Pertel et al. 2011).

TRIM5 α and the Viral Capsid

HIV capsid was previously thought to be an inert packaging shell that protects the genomic material of the virus. However, it has emerged that it has several domains that allow interaction with host proteins, such as cyclophilin A, a peptidylprolyl isomerase. Cyclophilin A catalyzes the cis/trans isomerization of a surface-exposed proline (Pro-90) in a loop (residues 85–93) between helices 4 and 5 of the HIV-1 gag capsid (Gamble et al. 1996). Despite HIV-1 and HIV-2 capsids being very similar both in sequence and structure, HIV-1 has a high binding affinity for CypA, but HIV-2 does not. HIV-1 incorporates CypA into incoming and nascent virions, where they enhance viral infectivity. At the same time, this capsid-CypA interaction also increases HIV-1 sensitivity to restriction by Old World monkey TRIM5 α . Unlike HIV-1, HIV-2, as well as SIV_{mac}, and murine leukemia viruses (MLV) neither incorporate CypA into nascent

virions nor require it for infectivity. HIV-2 binds very weakly to CypA, mainly because its proline (Pro-88) is not positioned correctly in the catalytic site of CypA, unlike Pro-90 of HIV-1 (Lahaye et al. 2013). Despite weak binding and independence of CypA for infectivity, mutational analysis has revealed that the determinants of HIV-2 TRIM5 α sensitivity lie in the region of the HIV-2 capsid equivalent to the CypA binding loop of HIV-1, mapping specifically to the glycine-proline (Gly-Pro) motif at positions 87–88 on the HIV-2 capsid (Price et al. 2009). This Gly-Pro motif in HIV-1 has been linked to TRIM5 α sensitivity, and the G87A (glycine to alanine) mutation in HIV-2 was associated with reduced HIV-2 titers in cell lines from different species (Ylinen et al. 2005). Available crystal structures of retroviral capsids from different species show an exposed loop structure, like HIV-1, in the region equivalent to the HIV-1 CypA binding loop. It was suggested that this structure can be recognized by TRIM5 α from different species (Ylinen et al. 2005).

In at least two independent events, retrotransposition of the *CypA* gene into the C'-terminal into the *TRIM5 α* gene has occurred, essentially replacing the PRYSPRY/B30.2 domain to form the *TrimCyp* gene. In New World monkeys of genus *Aotus* (owl monkey), the TrimCyp replaces exon 8, accounting for most of the PRYSPRY domain, and this protein is able to restrict CypA-binding retroviruses like HIV-1 and FIV. In Old World monkeys of genus *Macaca* (macaques), sequences from *CypA* in the rhesus macaque replace both exons 7 and 8, removing the entire PRYSPRY domain, to produce a protein that can potentially inhibit HIV-2 and FIV, but not HIV-1 (Wilson et al. 2008). There are at least six species of macaques that express TrimCyp; some express this gene exclusively, while others express both TrimCyp and TRIM5 α (Dietrich et al. 2011). It was shown that the ability of the rhesus macaque (*Macaca mulatta*) RhTrimCyps to restrict HIV-2 was due to the presence of two amino acid changes, D66N and R69H, in the CypA domain of the TrimCyp, allowing it to bind strongly to the HIV-2 capsid (Price et al. 2009). In the longtail macaque

(*Macaca fascicularis*), HIV-2 specificity is mapped to amino acids 66 and 143 of the LtTrimCyp. The protein expressed by the 66 N-143E genotype binds to and potently restricts HIV-2 but not HIV-1, while that from the 66D-143 K genotype restricts HIV-1 and not HIV-2, while the 66D-143E protein restricts both viruses (Dietrich et al. 2011). Recent studies into host restriction factors have also shown that the capsid serves as a pathogen-associated molecular pattern (PAMP) for TRIM5 α which can therefore be thought of as a pattern recognition receptor (PRR) (Pertel et al. 2011).

TRIM5 α and HIV-2

Human TRIM5 α potently restricts N-MLV and can moderately limit HIV-2 replication but has an insignificant effect on HIV-1. TRIM5 α restriction has been shown to be mapped to the B30.2/PRYSPRY domain. Restriction of N-MLV is different from that of HIV-1, in that a larger area of the PRYSPRY domain is involved, as well as the coiled coil for restriction of the N-MLV mutant L117H (Yap et al. 2005). HIV-1 restriction by human TRIM5 α maps to the V1 region of the PRYSPRY domain of TRIM5 α , where a change from arginine (R) or any positively charged residue at position 332 to a proline (P) or any uncharged residue results in potent restriction of both HIV-1 and SIVmac (Yap et al. 2005; Li et al. 2006). This R332P change has been shown to lead to potent restriction of HIV-2 as well (Kono et al. 2008). In a study on HIV-2 restriction, another area of the V1 region, 337–339 (in human TRIM5 α), has been shown to be important for both HIV-2 and HIV-1 restriction. The change from QTF in the V1 region of human TRIM5 α to TRP found in some rhesus macaque (Rh) Trim5 α (339–341) has been shown to result in potent restriction of both HIV-2 and HIV-1 even in the presence of arginine at position 332 (Kono et al. 2008).

In addition to the TRIM5 α protein, the viral capsid also contains polymorphic determinants of TRIM5 α restriction. In HIV-1, capsid mutations in the CypA binding region, such as V86M, result

in resistance to RhTrim5 α (Veillette et al. 2013). Human TRIM5 α restriction of HIV-2 was mapped to a single amino acid at position 119 of HIV-2 ROD capsid that has no known link with CypA binding. HIV-2 viruses with a proline at this position were much more sensitive to human TRIM5 α restriction than those without (glutamine or alanine) (Song et al. 2007). Further studies showed that the presence of hydrophobic amino acids or those with ring structures was associated with sensitivity to TRIM5 α restriction and those with small side chains or amide groups were linked to TRIM5 α resistance (Miyamoto et al. 2011). A study from our group showed that the presence of three prolines at positions 119, 159, and 178 in the HIV-2 capsid was significantly associated with low viral load or viral control (Onyango et al. 2010). This report further showed that the presence of these prolines was predicted to reduce the dimer binding energies of the “PPP” capsid, resulting in a weaker core and more unstable protein (Onyango et al. 2010). Three-dimensional modeling of the HIV-1 and HIV-2 capsids showed that the N-terminal domain consists of seven α -helices from which three loops protrude. Position 119 of the HIV-2 capsid is located in the loop between helices 6 and 7. When a proline is present at position 119, the loop between helices 6 and 7 (L6/7) is closer to the loop between helices 4 and 5 (L4/5), a region that directly interacts with CypA in HIV-1 (Miyamoto et al. 2011). The presence of hydrophobic or ring-structure residues at position 119 of the capsid maintains a certain conformation at L4/5 that is characteristic of TRIM5 α sensitive viruses. Curiously, the equivalent position in N-MLV, at position 110, also determined N-MLV susceptibility to human TRIM5 α . It was suggested that while HIV-1 and HIV-2 are restricted by different mechanisms, human TRIM5 α utilizes a similar mechanism of recognition for N-MLV and HIV-2 (Miyamoto et al. 2011).

Studies utilizing the HIV-1 pNL43 backbone with the HIV-2 capsids from lab-adapted (HIV-2 ROD, HIV-2 GL) and primary isolates from different HIV-2 groups have shown that human TRIM5 α can restrict all these capsids with similar efficiency (maximum of fourfold median

difference) and restricts HIV-1pNL43 chimeras with an HIV-2 capsid (HIV-2 groups A, B, and AB) to a significantly higher extent than HIV-1pNL43 (mean range \sim 4.5–7-fold) (Takeuchi et al. 2013). However, chimeric viruses containing capsid sequences from non-epidemic HIV-2 groups C to H showed a wide range of susceptibilities to human TRIM5 α , ranging from 1.3 to 7.5 (Takeuchi et al. 2013). In contrast, capsid sequences from patients infected with circulating recombinant forms from group A and B (HIV-2A/B) were found to be highly resistant to human TRIM5 α (Miyamoto et al. 2012). Most HIV-2 infected individuals (\sim 75%) have asymptomatic disease, but interestingly, all three reported patients infected with HIV-2A/B presented at an advanced disease stage of AIDS (Miyamoto et al. 2012). Although the numbers were small, mainly due to very few reports of HIV-2A/HIV-2B, it may be that faster disease progression is a hallmark of HIV-2A/HIV-2B recombinant virus infection. The capsids of this recombinant virus were shown to be unique, possessing a glycine at position 119, instead of proline/glutamine/alanine, but most of the differences were observed in the C-terminal domain (CTD) (Miyamoto et al. 2012).

Studies on the association of human TRIM5 α single nucleotide polymorphisms (SNPs) with HIV-1 and HIV-2 disease progression are limited, and most show few or no differences between genotypes. A study in Japan showed that an SNP in the L2 linker region, G249D, was more prevalent in Asians and Africans compared to Caucasians (Nakayama et al. 2013). Our unpublished data supports this information; we found a minor allele frequency (MAF) of 27.6% for G249D, the second most frequent SNP in an HIV-2 cohort in Guinea-Bissau. The presence of this SNP was associated with reduced viral control for both HIV-1 and HIV-2 (Nakayama et al. 2013).

Conclusions

It has been postulated that the greater ability of human TRIM5 α to restrict HIV-2 may be partially responsible for the slower disease progression observed in HIV-2-infected individuals; however,

that does not fully account for the apparent dichotomy in outcome between progressors and non-progressors with HIV-2. Potentially, progression status may reflect particularly advantageous or disadvantageous combinations of TRIM5 α sequence and the capsid sequence of the infecting virus. Although currently rare, the few HIV-2 group A/B recombinants that have been reported are all associated with rapid disease progression. The fact that the capsids from these recombinants are more resistant to human TRIM5 α may indicate that viral evolution has occurred to provide escape from human TRIM5 α restriction, further supporting the *in vivo* role of human TRIM5 α in at least partial restriction of HIV-2. Further studies on the mechanism of restriction of HIV-2 may provide a better overall understanding of TRIM5 α restriction in humans.

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TRIM5alpha

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Definition

Tripartite motif 5 alpha also known as TRIM5alpha (TRIM5 α) or TRIM5a is a cellular restriction factor that blocks the early stages of infection by retroviruses. TRIM5 α is a cytoplasmic E3 ubiquitin ligase protein (~55 kDa) expressed from *TRIM5* gene in most primate species and some mammals. Shortly upon infection of a cell by a retrovirus, TRIM5 α interacts with the incoming viral particle and precipitates its degradation, therefore interrupting precociously the viral replication cycle before reverse transcription (► [HIV Life Cycle: Overview](#)). The antiviral activity of TRIM5 α relies on its ability to recognize in a species-specific manner the capsid (CA) protein forming the shell of the retroviral core. TRIM5 α also acts as a pattern recognition receptor and elicits innate immune signals upon CA recognition. Thus TRIM5 α mediates antiviral activities through a dual function of restriction and innate sensing.

Introduction: Tripartite Motif Family, TRIM5 and TRIM5alpha

TRIM5 α belongs to the tripartite motif (TRIM) family, also referred to as RBCC family, with nearly 100 TRIM paralog genes scattered all over the human genome (a catalog of TRIM genes can be found on HGNC website <http://www.genenames.org/>). Clusters of TRIM genes resulting from tandem duplication are located on human chromosomes 5, 6, 11, and 17. The archetype TRIM protein is defined by an N-terminal tripartite motif called “TRIM” or “RBCC” composed of three motifs: a RING (really interesting

new gene) E3 ubiquitin ligase domain, one or two zinc-finger B-boxes (B-box1 and B-box2), and an α -helical coiled-coil motif mediating oligomerization. The tripartite motif coordinates homo- or heteromultimerization and functions as E3 ubiquitin ligase. In addition, most TRIM proteins carry a C-terminal domain (i.e., B30.2/SPRY, PHD, BROMO, COS, NHL, FN3, IGFLMN) with binding properties that dictates the specificity to a cellular substrate. Members of the TRIM family are implicated in a vast array of cellular processes. Remarkably, the expression of numerous TRIM genes is stimulated by interferons, and many TRIMs intervene in innate signaling and antiviral responses (► [TRIM Protein Family and Viral Restriction](#)). Like other families of genes involved in immunity (MHC, TLR, APOBEC, Mx, IFITM), the TRIM family has undergone gene expansion through duplications and rearrangements during evolution.

In the human genome, *TRIM5* gene is located on the short arm of chromosome 11 at position 11p15.4 as part of a cluster of closely related genes comprising TRIM6, TRIM34, and TRIM22. The cluster TRIM6/34/5/22 is also found on chromosome 11 and 14 in chimpanzee and rhesus macaque, respectively. Human *TRIM5* gene is about 22 kb long and is segmented into 8 exons. Multiple spliced variants, designated as TRIM5alpha, -beta, -gamma, -delta, -epsilon, -iota, and -kappa, are generated from TRIM5 mRNA. The most abundant 3-kb-long alpha isoform represents up to 50% of TRIM5 mRNAs and carries a 1.5-kb-long coding sequence for TRIM5 α protein (~55 kDa, 493aa) (Johnson and Sawyer 2009).

The prototypical TRIM5 α protein expressed in primates and some mammals contains four major domains, from N-terminus to C-terminus: a RING domain, a B-box2, a coiled-coil, and a B30.2/SPRY domain. The B30.2/SPRY domain, also referred to as B30.2, PRYSPRY, and RFP-like domain, corresponds to the fusion of PRY and SPRY (Sp1A and ryanodine receptor) motifs. Linker regions L1 and L2, respectively, separate the RING and B-Box2 motifs and the coiled-coil and B30.2/SPRY domains. Other human spliced variants encode for TRIM5 isoforms lacking the

B30.2/SPRY and deprived of antiviral activity; however, these isoforms can interfere with TRIM5 α activity through dominant negative effects by multimerizing via the coiled-coil. Some primates express an unusual *TRIM5* allele called *TRIMCyp* that encodes a TRIM5-cyclophilin A fusion protein. TRIM5 α is constitutively expressed in most tissues, and its expression is upregulated by type I interferons through an IFN-stimulated response element in its promoter. TRIM5 α localizes mainly in the cytoplasm under a diffuse form or in aggregates designated as cytoplasmic bodies; it can also shuttle between the nucleus and the cytoplasm.

Discovery of TRIM5 α and TRIMCyp

Restriction factors are germline-encoded factors engaged in cell-intrinsic immunity, building a frontline defense against viruses (► [Cell Intrinsic Immunity](#)). The first reports of restriction against a retrovirus date back to the early 1970s from studies that pinpointed an inheritable resistance to murine leukemia virus (MLV) strains in mice ascribed to *Fv1* gene (*Friend virus susceptibility gene 1*). *Fv1^b* and *Fv1ⁿ* alleles respectively conferred protection to mice against NIH-tropic MLV (N-MLV) and BALB/c-tropic MLV (B-MLV) strains but not against NB-MLV (NB-tropic) strains. *Fv1* gene is exclusively found in mice and derives from *gag* gene of the murine endogenous retrovirus type L (MERV-L). The restriction factor Fv1 imposes a postentry block of N- or B-MLV infection after reverse transcription but before integration. The restriction block targets specifically the viral CA and is abrogated by using high doses of virus, which can saturate Fv1 activity. The precise mechanism of restriction is still poorly understood (Sanz-Ramos and Stoye 2013).

Evidence for the existence of Fv1-like restriction factors in nonmurine mammalian cells accumulated in the 1990s and early 2000s. In human cells, an unidentified factor named Ref1 (restriction factor 1) potentially blocked of N-MLV infection but not B-MLV. Likewise, a factor termed Lv1 (lentivirus factor 1) restricted

lentiviruses including HIV-1 in simian cells originating from Old World monkeys (Asian and African species) and some rare New World monkeys (American species). Like Fv1, Ref1 and Lv1 mediated a restriction block, which was dominant and saturable, targeted the CA protein, and occurred prior reverse transcription. In 2004, TRIM5 α was identified as the postentry restriction factor blocking HIV-1 in rhesus macaque cells through a screen using a rhesus macaque cDNA library (Stremlau et al. 2004). TRIM5 α was subsequently shown as responsible for activities previously ascribed to Ref1 and Lv1. While TRIM5 α was identified in Old World monkeys, a TRIM5-cyclophilin A fusion protein called TRIMCyp was independently discovered by investigating HIV-1 restriction in New World primates (Sayah et al. 2004). Cyclophilin A (CypA) is a cellular chaperone (peptidyl-propyl isomerase) that interacts with HIV-1 CA (► [Cyclophilin A and HIV-1 Replication](#)). In human cells, CypA acts as a cofactor of HIV-1 since disrupting CypA-CA interaction by cyclosporine A treatment impairs HIV-1 replication. In contrast, in owl monkey cells normally refractory to HIV-1 infection, cyclosporine A treatment or CypA knock down alleviate the restriction against HIV-1. TRIMCyp was discovered by screening an owl monkey cDNA library in an effort to identify an isoform of CypA endowed of anti-HIV-1 activity. Owl monkey *TRIMCyp* gene originates from the retrotransposition of CypA mRNA into *TRIM5* gene leading to an in-frame coding sequence. The resulting protein contains TRIM5 RBCC fused to CypA (Nisole et al. 2004; Sayah et al. 2004). The mechanism of restriction of TRIMCyp is similar to that of rhesus TRIM5 α apart from the fact that the CypA domain mediates CA recognition.

TRIM5 α -Mediated Restriction Against Retroviruses

TRIM5 α intercepts incoming virions by recognizing specifically motifs of the CA assembled into viral cores and triggers their premature uncoating (► [Uncoating and Nuclear Entry](#)) (Stremlau et al.

2006). TRIM5 α from Old World monkeys, like rhesus macaque TRIM5 α , strongly restricts HIV-1. TRIM5 α orthologs from New World monkeys broadly counteract simian immunodeficiency viruses (SIVs). Human TRIM5 α does not significantly inhibit HIV-1 and SIVs, but it restricts lentiviruses from nonprimate mammals such as FIV (feline immunodeficiency virus) and EIAV (equine infectious anemia virus). Most TRIM5 α orthologs, including human TRIM5 α , are active against N-MLV but not against B-MLV and NB-MLV. Investigations on the spectrum of activity of TRIM5 α in primates and mammals indicate that TRIM5 α restricts a wide range of retroviruses (i.e., lentiviruses, gammaretroviruses, betaretroviruses, and spumaretroviruses) in a species-specific manner.

All protein domains of TRIM5 α are required for restriction. The RING E3 ubiquitin ligase domain recruits E2-conjugating enzymes involved in restriction and innate signaling (Pertel et al. 2011; Fletcher et al. 2015). The coiled-coil mediates antiparallel dimerization, and the B-Box-2 domain coordinates higher-order self-assembly of TRIM5 α dimers with enhanced avidity for viral CA (Goldstone et al. 2014; Sanchez et al. 2014). Species-specific recognition of CA is mainly determined by sequence variations in the B30.2/SPRY domain. Major determinants for recognition of retroviral CA have been mapped to variable regions (v1, v2, v3, v4) of the B30.2/SPRY that display strong interspecies sequence diversity and high rate of positive selection, typically observed in protein domains of restriction factors at binding interface with viral proteins (Sawyer et al. 2005). The B30.2/SPRY has a conserved hydrophobic core structure formed by a 13-stranded β -sandwich. Variable regions fold into flexible loops connecting β -stands and forming the CA-binding surface of the B30.2/SPRY that is reminiscent of binding sites of antibodies (Grutter et al. 2006; Biris et al. 2012).

The mature retroviral CA protein contains two independently folded α -helical domains, the N-terminal domain (NTD) and the C-terminal domain (CTD), that are connected by a flexible linker. The mature HIV-1 core is built with

multimers of CA proteins, namely, 12 pentamers and approximately 250 hexamers, forming a cone-shaped fullerene shell enclosing the viral RNA genome and proteins necessary for infection (► [HIV-1 Virion Structure](#)). CA NTDs form hexameric and pentameric rings exposed at the outer surface of the lattice, while CA CTDs connecting the rings are included in the inner face of the shell. Viral determinants for TRIM5 α restriction were mapped to CA NTD forming a binding interface on the outer CA lattice. Exposed surfaces of CA NTD, namely, the β -hairpin, the CypA-binding loop between helix 4 and 5, and the loop between helix 6 and 7, modulate the sensitivity of HIV-1, SIVs, and N-MLV to TRIM5 α . Direct interaction between TRIM5 α and viral CA has been difficult to establish because TRIM5 α recognizes the oligomeric CA lattice but not CA monomers. Electron microscope imaging allowed visualizing spontaneous self-assembly of TRIM5 α dimers into hexagonal arrays promoted by the presence of preassembled HIV-1 CA-NC crystals and matching the symmetry and spacing of the hexagonal CA lattice (Ganser-Pomillos et al. 2011). The resulting model of recognition suggests that molecules of TRIM5 α build a hexagonal scaffold surrounding viral core. This scaffold is formed by antiparallel TRIM5 α dimers stabilized via the coiled-coil and interconnected through the B-box2; the RING domain localized at the outer surface can recruit E2 ubiquitin enzymes and the B30.2/SPRY may directly interact with multiple epitopes of CA.

The mechanism underlying TRIM5 α -mediated restriction and by which TRIM5 α precipitates the disassembly of viral cores to terminate the infection has been extensively investigated. Multiple mechanisms have been envisaged including direct damage of core, recruitment of cofactors, proteasome-dependent degradation involving TRIM5 α E3 ubiquitin ligase activity and proteasome-independent activity. TRIM5 α constructs containing the coiled-coil and B30.2/SPRY domains are sufficient to induce structural damages on CA lattices in vitro. However in cells, binding between TRIM5 α and CA is necessary but not sufficient for restriction, and efficient restriction requires the B-box2 domain. TRIM5 α

may trigger a proteasome-dependent degradation of retroviral cores. Indeed, TRIM5 α is an E3 ubiquitin ligase and recruits E2 ubiquitin-conjugating enzymes via its RING domain. TRIM5 α -mediated inhibition of reverse transcription requires the recruitment of E2 enzymes Ube2W and Ube2N-Ube2V2 that anchor K63-linked polyubiquitin chains to process TRIM5 α auto-ubiquitination (Fletcher et al. 2015). TRIM5 α auto-ubiquitination, which leads to its rapid degradation by the proteasome, may precipitate TRIM5 α -bound viral cores to proteasomal degradation. However, proteasome inhibitors or depletion of E2 enzymes rescue the block of reverse transcription but do not restore viral infection, which remains impaired before integration, implying that TRIM5 α may restrict viral infections through multiple ways. TRIM5 α has also been proposed to act as an autophagic cargo and target viral cores to autophagic degradation (Mandell et al. 2014).

Role of TRIM5 α in Innate Immunity

The expression of human and simian TRIM5 α orthologs, including owl monkey TRIMCyp, is upregulated by type I interferons which may potentiate TRIM5-mediated antiviral activity. In addition to a direct antiviral effect, TRIM5 α functions as a pattern recognition receptor (PRR) by triggering a cascade of innate immune signals that lead to inflammatory cytokine production and contribute to the establishment of the antiviral state upon retroviral infection. In the absence of viral infection, TRIM5 α can promote innate immune signaling by triggering NF- κ B and AP-1 activation. To do so, TRIM5 α recruits E2 ubiquitin-conjugating enzymes UBC13-UEV1A (aka Ube2N-Ube2V1) via its RING domain to catalyze the synthesis of free K63 ubiquitin chains that activate TAK1 complex and downstream AP-1 and NF- κ B signaling (Pertel et al. 2011). This cascade of activation is enhanced in the presence of CA lattice that is sensed as a pathogen-associated molecular pattern (PAMP) by TRIM5 α . A large number of human TRIM proteins have the ability to activate NF- κ B in a

TAK1-dependent manner, AP-1 and interferons (Versteeg et al. 2013). An emerging concept is that TRIM E3 ubiquitin ligases can both play antiviral effector functions and enhance the innate antiviral response by inducing interferons and proinflammatory cytokines.

TRIM5 Evolution

The assembly of a RING, a B-Box, and a coiled-coil domain into a TRIM motif originates from metazoans. TRIM genes with SPRY and B30.2/SPRY domains are exclusively found in vertebrates, and from an evolutionary point of view, these genes are more recent, divergent, and dynamic than TRIM genes with other C-termini. SPRY-encoding TRIM genes have evolved through rapid expansions in vertebrates most likely as the result of selective pressure imposed by pathogens; they may constitute a reservoir of new antiviral genes.

TRIM5 gene originates from eutherian mammals about 90–180 million years ago (Johnson and Sawyer 2009). *TRIM5* locus has undergone many events of duplication, insertion, deletion, degeneration, and positive selection likely driven by ancient infections during mammalian evolution. *TRIM5* homologs are found in primates, scandents, rodents, lagomorphs (i.e., rabbit, hare, pika), and laurasiatheria (including cow, pig, horse, sheep, goat, cat, dog). The cow genome has expanded a cluster of eight *TRIM5* paralogs including three pseudogenes and five full-length genes. At least one of them encodes a functional TRIM5 α protein that is able to restrict several viruses but not the bovine immunodeficiency virus (BIV) that naturally infects cows. In carnivores, *TRIM5* locus was independently disrupted twice during evolution. On one hand, the dog genome has lost *TRIM5* due to an insertion between the exons 2 and 8 of *TRIM5* of which the remnants degenerated into pseudogenes. On the other hand, feliforms encompassing species such as cats, leopards, lions, and hyenas encode a truncated TRIM5 protein lacking the B30.2/SPRY domain due to a stop codon in 5' of exon 8. Lagomorphs (including rabbits, hares, pikas) express

an active TRIM5 α protein that is able to restrict several lentiviruses and N-MLV. The genome of mouse and rat respectively contain clusters of eight and three TRIM5-like genes (termed TRIM30 and TRIM12 in rodents) encoding proteins with no apparent restriction activity.

All primate species (hominoids, Old World monkeys, New World monkeys, and prosimians) encode a functional *TRIM5* gene, occasionally under the form of *TRIMCyp* fusion gene as in the case of owl monkeys and macaques. Like other restriction factors (APOBEC3G, SAMHD1, BST2/Tetherin) engaged in evolutionary arm races with retroviruses, TRIM5 α has rapidly evolved in primates and carry signatures of positive selection in domains interacting directly with viral proteins (► [Virus/Host Coevolution – Positive Selection Essay](#)). The molecular evolution of TRIM5 in primates is characterized by recurrent events of positive selection resulting in strong interspecies sequence diversity, balancing selection maintaining intraspecies allelic diversity, and genetic innovations providing selective advantages such as lineage-specific expansions of variable regions of the B30.2/SPRY domain and TRIM5-cyclophilin A gene fusions. Analyses of selective pressures show that *TRIM5* locus experienced positive selection in a majority of branches of the primate phylogeny and residues under positive selection localize predominantly the coiled-coil and the CA-interacting B30.2/SPRY domain (Sawyer et al. 2005; Johnson and Sawyer 2009). In addition, TRIM5 α B30.2/SPRY domain has also experienced lineage-specific expansion of the variable loops v1–v3. Hominoid and Old World monkey TRIM5 α proteins are characterized by expansions of the v1 loop (~10–30 residues), while in New World monkey TRIM5 α proteins, the v3 loop is 10–60 residues longer than in other orthologs. Similarly, v2 expansion (up to ~35 residues) is observed in prosimian TRIM5 α and some mammalian TRIM5 α and TRIM5-like proteins. Such adaptations may have been fixed during evolution as they brought beneficial advantages in combating infections by ancient viruses such as PSIV (prosimian immunodeficiency virus) in lemurs and RELIK (rabbit endogenous lentivirus

type K) in lagomorphs (Rahm et al. 2011; Yap and Stoye 2013).

Remarkably, events of LINE-1-dependent retrotransposition of *CypA* into *TRIM5* locus occurred independently in several lineages of primates, giving birth to *TRIMCyp* genes. In owl monkeys, which belong to New World primates, *TRIMCyp* gene results from an in-frame retrotransposition of *CypA* cDNA in intron 7 of *TRIM5* gene. Owl monkey TRIMCyp protein is therefore made of TRIM5 RBCC, encoded by exons 2–7, fused to CypA protein (Nisole et al. 2004; Sayah et al. 2004). First identified in *Aotus trivirgatus*, *TRIMCyp* exists in other owl monkey species from *Aotus* genus but not in other New World primates, meaning that the gene arose about 4–6 million years ago prior to *Aotus* radiation. Owl monkeys are homozygous for *TRIMCyp* and do not express other TRIM5 isoforms. Owl monkey TRIMCyp protein efficiently restricts HIV-1. A TRIM5-CypA chimeric gene also exists among Old World primates in macaques (genus *Macaca*), i.e., rhesus macaque (*Macaca mulatta*), pig-tailed macaque (*Macaca nemestrina*), and crab-eating macaque (*Macaca fascicularis*). In that case, *CypA* cDNA retrotransposed in the 3' untranslated region of *TRIM5* about 5–6 million years ago, and a single G-to-T point mutation additionally occurred at 3' splice acceptor site of *TRIM5* intron 6 resulting in exon skipping. Thus in macaques, TRIMCyp expression is the result of alternative splicing between *TRIM5* exon 6 and *CypA* exon, while exons 7 and 8 of *TRIM5* are skipped. Owl monkey TRIMCyp and macaque TRIMCyp proteins differ by the sequence (L2 linker) linking the coiled-coil and CypA. Traces of a third retrotransposition leading to an ancient *TRIMCyp* gene were detected in the genomes of Old World monkeys. The gene, which likely encoded an active antiviral protein, arose 43 million years ago and decayed into a pseudogene about 10 million years ago (Malfavon-Borja et al. 2013). A fourth event of *CypA* retrotransposition into exon 8 of *TRIM5* has also occurred outside the primate lineage, in the genome of tree shrews which contain a cluster of four TRIM5 paralogs and a functional *TRIMCyp* gene. *CypA* is one of the most common processed

pseudogene found in the human genome possibly due to the abundance of CypA mRNA. *TRIM5* is the only gene that experienced recurrent and independent *CypA* retrotranspositions leading to functional gene fusions.

Role of TRIM5 in Controlling Viral Infections in Nonhuman Primates

African primates are natural hosts of SIVs responsible of nonpathogenic infections in their natural host in most cases, with the exception of chimpanzees (► [SIV Infection of African Green Monkeys](#); ► [Nonpathogenic SIV Infection of Sooty Mangabeys](#); ► [SIV Infection in Mandrills](#)). Asian rhesus macaques (*Macaca mulatta*) are not natural carriers of SIVs, but cross-species transmission of SIV from sooty mangabeys (SIVsm) to captive Asian rhesus macaques occurred in US Primate Research Centers in the 1960s. SIVmac viruses isolated from captive macaques in the 1980s, including laboratory-adapted SIVmac239 and SIVmac251 strains, result from decades of adaptation of SIVsm in rhesus monkeys (► [SIVmac Infection of Macaques, Immunopathogenesis of](#)). Both SIVmac and SIVsm induce AIDS-like symptoms in rhesus macaques, which have become an invaluable animal model for AIDS research.

Rhesus macaque *TRIM5* gene is highly polymorphic; multiple alleles have been maintained in macaques as a result of long-term balancing selection. Strong intraspecies sequence diversity is especially detected in the coiled-coil and the B30.2/SPRY domains. An important six-nucleotide insertion/deletion TFP339-341Q polymorphism in variable region v1 of the B30.2/SPRY provides differential degrees of restriction against lentiviruses. Rhesus *TRIM5* alleles can be classified into three major functional alleles, from the most to the least frequent: TRIM5^{TFP} (TFP339-341), TRIM5^Q (Q339), and TRIM5^{CypA}. Rhesus TRIM5^{TFP} and TRIM5^{CypA}, but not TRIM5^Q, restrict efficiently SIVsm strains in vitro, while rhesus-adapted SIVmac viruses are relatively resistant to the three variants; in

some cases, moderate inhibition by TRIM5^{TFP} and TRIM5^{CypA} has been observed in vitro. Both protective TRIM5^{TFP} and TRIM5^{CypA} alleles are associated with a very drastic impact on viral acquisition and replication of SIVsm in rhesus macaques and with a moderate but significant in vivo control of SIVmac251. TRIM5^{TFP/TFP} homozygous macaques display lower SIVmac251 viral replication (1.3 log median difference in set point viral loads), an attenuated loss of CD4⁺ T cells and significant survival advantage compared to TRIM5^{Q/Q} homozygous animals (Lim et al. 2010). But restrictive rhesus *TRIM5* alleles may not provide protection against mucosal transmission of SIVmac251.

The most remarkable effect of rhesus TRIM5 in vivo is observed in animals infected with SIVsm strains (i.e., SIVsmE543, SIVsm041, SIVsmE660) that are less adapted to rhesus macaques than SIVmac. The viremia of SIVsm-infected macaques carrying two restrictive TRIM5^{TFP} and TRIM5^{CypA} alleles is about 2–3 log lower than that of TRIM5^{Q/Q} homozygous animals (Kirmaier et al. 2010). Animals with one restrictive allele display intermediate viral load. Efficient restriction also delays disease progression and increases the survival rate of macaques infected with SIVsm. Multiple studies support the role of restrictive *TRIM5* alleles in protecting rhesus macaques from mucosal transmission (i.e., penile and rectal) of SIVsm. These findings emphasize the requirement of considering *TRIM5* genotype, as well as protective MHC class I alleles, in the design of challenge studies and vaccine trials using rhesus monkeys. Restrictive rhesus *TRIM5* alleles select the emergence of escape mutations in CA NTD of SIVsm at late stages of infection. Mutations P37S and R98S in SIVsm CA are associated with escape from TRIM5^{TFP} restriction, and mutations in the CypA loop of CA (LPA89-91QQ) allow escaping TRIM5^{CypA} (Kirmaier et al. 2010; Wu et al. 2013). SIVmac strains isolated from captive macaques carry similar R-to-S and LPA-to-QQ adaptations, which likely result from selective pressure imposed by TRIM5^{TFP} and TRIM5^{CypA}. Thus, TRIM5 α efficiently modulates viral acquisition,

in vivo replication, and cross-species transmission of SIVs in rhesus macaques.

Polymorphisms in Human TRIM5 Gene and HIV Infection

Unlike its rhesus counterpart, human TRIM5 α seemingly fails to counteract HIV infection in vitro and in vivo. Various studies evaluated the impact of *TRIM5* polymorphisms on HIV-1 acquisition and in vivo infection by surveying single-nucleotide polymorphisms (SNPs) on *TRIM5* locus in HIV/AIDS cohorts. The most frequent nonsynonymous SNPs in human *TRIM5* lead to amino acid changes in RING (H43Y), B-Box2 (V112F), coiled-coil (R136Q), linker L2 (G249D), and B30.2/SPRY (H419Y). H43Y and R136Q TRIM5 α display respectively impaired and enhanced antiviral activities in vitro, while V112F, G249D, and H419Y polymorphisms do not affect the function of human TRIM5 α . Overall naturally occurring SNPs in human *TRIM5* gene have been only associated with weak effect or no statistically significant effect on HIV-1 susceptibility, infection, and AIDS progression (An and Winkler 2010). Homozygous H43Y individuals may progress faster to AIDS, and R136Q polymorphism has been associated with a protective effect and also with an increased risk of viral acquisition. Genome-wide association studies (► [Host Genetics and Genomics](#)) have not detected any common genetic in human *TRIM5* gene as important to the control of HIV-1 infection and to disease progression. Primary HIV-1 isolates, especially strains carrying CTL (cytotoxic T lymphocyte) escape mutations in Gag/CA, exhibit an increased susceptibility to human TRIM5 α -mediated restriction in vitro compared to laboratory-adapted strains, which have a low sensitivity (twofold) to human TRIM5 α . TRIM5 α has been proposed to contribute to the control of CTL-escape HIV-1 strains in patients carrying protective MHC class I alleles (► [HIV-1 Mutational Escape from Host Immunity](#)). Likewise, HIV-2 subtypes are more sensitive to restriction by human TRIM5 α compared to

HIV-1. The susceptibility to human TRIM5 α , even though moderate, may partially account for the overall lower infectivity and pathogenicity of HIV-2 in humans (► [TRIM5alpha and HIV-2 Infection](#)).

TRIM5-Based Therapeutic Strategies

Rhesus monkey TRIM5 α is a powerful inhibitor of HIV-1. Due to the possible antigenicity of rhesus TRIM5 α , recombinants based on the human protein have been engineered for potential application in gene therapy; these include punctual mutants, rhesus-human chimeras, and artificial human TRIM-CypA fusions. Single mutation at residue R332 or modifications in v1 of the B30.2/SPRY domain are sufficient to render human TRIM5 α active against HIV-1. Thus, restrictive human TRIM5 α mutants (R332, R335) have been optimized for the purpose of gene therapy. Another approach has consisted in generating an artificial human TRIMCyp protein, based on human TRIM5 α and CypA, able to elicit potent anti-HIV-1 activity in vitro and in vivo. Humanized Rag2^{-/-} γ c^{-/-} mice engrafted with human TRIMCyp-expressing CD4⁺ T cells display robust protection against HIV-1 infection, reduced HIV-1-induced loss of CD4⁺ T cells, and survival advantage (Neagu et al. 2009). This proof-of-concept study reveals that a TRIM5-derived transgene may confer protection against HIV-1 infection in vivo and highlights human TRIM5-based constructs as promising candidates for anti-HIV-1 gene therapy. Additional TRIMCyp chimeras based on human TRIM1, TRIM18, TRIM19, or TRIM21 have been developed and exhibit potent anti-HIV-1 activity in vitro. Alternative TRIM5-based strategies involve the delivery of a rhesus-human TRIM5 α chimera in human hematopoietic stem cells (HSC). Humanized mice engrafted with HSCs stably expressing rhesus-human TRIM5 α chimera, a CCR5 shRNA, and a TAR decoy display a survival advantage upon HIV-1 infection and maintenance of CD4⁺ T cells. HIV-1-resistant macrophages have also been generated by

reprogramming induced pluripotent stem cells (iPSCs) that express rhesus-human TRIM5 α chimera and a CCR5 shRNA (Anderson 2013). Despite promising results, the delivery of exogenous TRIM5 recombinants for gene therapy raises concerns such as the immunogenicity of the transgene, undesirable immune activation due to innate signaling properties of TRIM5 α , and the risk of insertional activation of neighboring genes. The genetic fragility of HIV-1 CA lattice also makes CA an attractive target for drug design and antiviral therapy. Small compounds mimicking the premature disassembly of HIV-1 capsid elicited by TRIM5 α may represent a new generation of antiviral agents (Bhattacharya et al. 2014).

Conclusions

Studies of the susceptibility of primate cells to retroviral infections led the identification of the intrinsic restriction factor TRIM5 α that inhibits retroviruses in a species-specific manner. TRIM5 α efficiently modulate SIV infection in rhesus macaques and has likely constituted a barrier against cross-species transmission of viruses in primates. To the contrary, human TRIM5 α has no significant impact on HIV infection. TRIM5 α mediates antiviral activities through restriction and innate sensing. This dual function illustrates how host retroviral restriction factors can act both as cell-intrinsic antiviral effectors and mediators of the innate immune response. Research on restriction factors has significantly expanded our knowledge on the strategies employed by viruses to escape and counteract restriction and has greatly contributed to improvement of models of HIV/AIDS infection and the development of therapeutic applications.

Cross-References

- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [Cyclophilin A and HIV-1 Replication](#)
- ▶ [HIV Life Cycle: Overview](#)

- ▶ [HIV-1 Mutational Escape from Host Immunity](#)
- ▶ [HIV-1 Virion Structure](#)
- ▶ [Host Genetics and Genomics](#)
- ▶ [Nonpathogenic SIV Infection of Sooty Mangabeys](#)
- ▶ [SIV Infection in Mandrills](#)
- ▶ [SIV Infection of African Green Monkeys](#)
- ▶ [SIVmac Infection of Macaques, Immunopathogenesis of](#)
- ▶ [TRIM Protein Family and Viral Restriction](#)
- ▶ [TRIM5 Alpha and HIV-2 Infection](#)
- ▶ [Uncoating and Nuclear Entry](#)
- ▶ [Virus-Host Evolution and Positive Selection](#)

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Tropism and Properties of the HIV-1 Envelope Glycoprotein in Transmission

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Definition

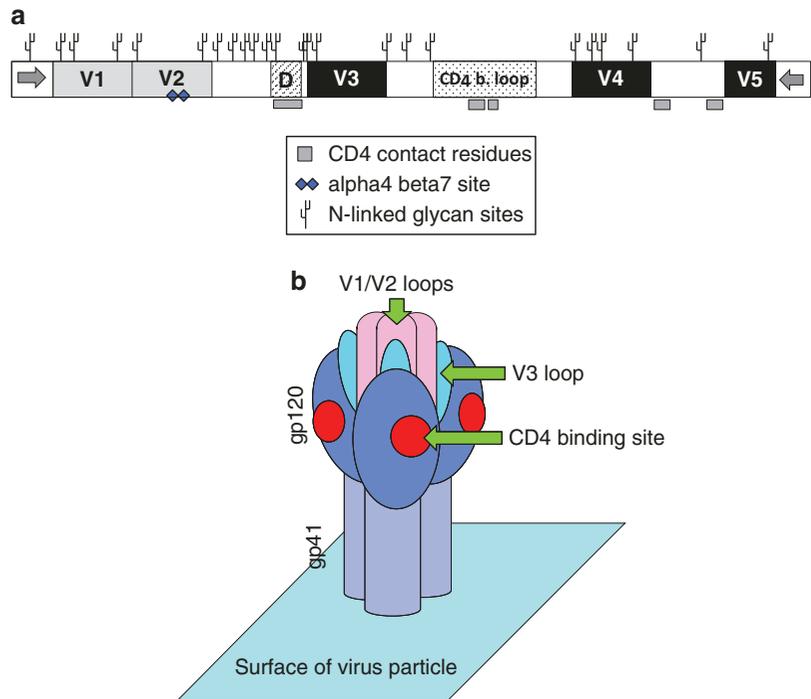
The envelope glycoprotein (Env) spikes on transmitting HIV-1 virus particles mediate infection of the first cells in the new host. These Envs must elude host defenses including innate inhibitors and sometimes neutralizing antibodies. They may also direct mechanisms to penetrate epithelial cell barriers to facilitate mucosal transmission.

Prophylactic vaccines that elicit neutralizing antibodies and microbicides need to be designed and optimized to target transmitting viruses. However, it is as yet unclear whether Envs of viruses that successfully transmit carry specific properties or vulnerabilities that can be targeted by such strategies. What we do know is that viruses that use the coreceptor, CCR5, are preferentially transmitted and that early data suggest these viruses primarily infect CD4+ T-cells over macrophages or dendritic cells.

Some transmitted viruses carry Envs with fewer surface sugars and shorter variable loops that usually protect against neutralizing antibodies. However, it is not known whether the structure of these Envs confers vulnerability to neutralizing antibodies that can be induced by vaccines or to inhibitors that may be used as prophylaxis. The challenge is to exploit our burgeoning knowledge of transmitted, founder

Tropism and Properties of the HIV-1 Envelope Glycoprotein in Transmission, Fig. 1

The HIV-1 envelope glycoprotein. (a) A linear representation of surface gp120 showing the variable loops, N-linked glycan sites, the $\alpha 4\beta 7$ integrin binding site in V2, and CD4 contact sites including loop D and the CD4 binding loop. (b) Schematic diagram of the Env trimer



viruses to identify their Achilles' heels in order to design more effective vaccines and prophylactic microbicides.

Background

A comprehensive understanding of the mechanisms of HIV-1 transmission and establishment of infection in a new host is important for the development of effective vaccines and microbicides.

Vaccines that aim to elicit protective antibodies will need to target sites on the envelope glycoproteins (Envs) present on the surface of virus particles (Kwong et al. 1998). Inhibitors that block Env functions are candidates for inclusion in prophylactic microbicides. Further development of both strategies would be enhanced by specifically targeting the properties of the transmitting HIV-1. It is clear that HIV-1 variants that use the coreceptor, CCR5, to infect cells are usually transmitted regardless of how transmission occurs. However, it is unclear whether transmitted/founder (T/F) viruses carry specific properties that confer an advantage during transmission or whether transfer of virions into the

new host is a chance event involving viral variants that happen to be replicating at donor sites of transmission. Several characteristics associated with T/F viruses have been reported but are unlikely to represent the complete picture. Here, the current knowledge of the different routes and mechanisms of transmission is reviewed. Focusing on the HIV-1 Env spike (Fig. 1), the properties associated with transmission, the origins of the transmitting virus in the donor, and the early cell targets for infection in the recipient are discussed.

Themes and Questions About HIV-1 Transmission

HIV-1 is transmitted via sexual contact, from mother to child and via blood contact (Table 1). However, whichever route is used, similar questions arise. Where is the transmitted virus (es) coming from? Is it cell-free or carried in by infected cells? Which and where are the first target cells in the new host during transmission? How does the transmitting virus avoid host innate inhibitory factors, breach protective epithelial cell

Tropism and Properties of the HIV-1 Envelope Glycoprotein in Transmission, Table 1 HIV-1 routes of transmission

Route of infection		Target tissue	Predominant vehicle	Epithelial cell barrier
Male to female		Vagina, cervix	Semen	Stratified in vaginal and ectocervix, single columnar in endocervix
Male to male		Rectum	Semen	Single columnar
		Penis	Feces, rectal secretions	Stratified
Female to male		Penis	Cervical and vaginal secretions	Stratified
Intravenous drug use			Blood	None
Mother to child	In utero	Placenta	Unknown	Syncytiotrophoblast and cytotrophoblast layers
	Intrapartum	Oropharynx, intestine	Maternal blood and cervicovaginal secretions	Stratified in oropharynx, single columnar in intestine
	Breast-feeding	Oropharynx, intestine	Breast milk	Stratified in oropharynx, single columnar in intestine

barriers, and access target cells? What is the receptor use and cell tropism of T/F viruses? If CD4+ T-cells are major early targets for infection, then what are the roles of other immune cells that express CD4 and CCR5 including dendritic cells and macrophages? Does transmitting virus need to target activated CD4+ T-cells for successful transmission or do resting CD4+ T-cells contribute? What are the soluble factors at transmission sites that protect or enhance Env-mediated infection? Do transmission routes or mechanisms vary depending on HIV-1 clade?

It is clear that R5 variants are predominantly transmitted regardless of route, while early data indicates that they are mainly non-macrophage-tropic (non-mac-tropic). Several studies suggest that transmitted viruses carry a heightened replicative fitness. However, it is unclear how this property influences the structure and function of the Env trimer or whether it provides vulnerability that can be specifically targeted in the design of vaccines and microbicides.

Routes of Transmission, Numbers of Viruses Transmitted, and Identification of Transmitted/Founder Viruses

Most people infected with HIV-1 reside in resource-limited settings where infection is

mainly spread via heterosexual contact or from infected mothers to their children (MTCT) (Table 1). Sexual transmission and MTCT occur more readily from individuals who have high viral loads in their plasma, particularly during the acute and later stages of disease (Powers et al. 2008, 2011). Infection of genital tissues by other sexually transmitted pathogens and the resulting inflammatory responses greatly increases the chances of HIV transmission (Powers et al. 2008).

The different routes of transmission vary in the source of virus in the donor and target tissues in the recipient. However, it is unclear whether this affects the composition of HIV-1 variants that reach sites of transmission in the donor and/or the phenotypes of variants transferred into the new host. It has also been unclear whether the initial cell type infected in the new host varies depending on route.

In recent years, extensive sequencing of plasma samples within days or weeks of infection has allowed characterization of viral variants that establish infection. Such sequencing yields information on the number of distinct variants transmitted, while consensus sequences have been deduced to be representative of the virus that was transmitted, i.e., the T/F virus (Keele et al. 2008). A single virus variant is transmitted in 64–90% of heterosexual transmission cases, in approximately 60% of men who have sex with

men, but only in 40% of infections through injection drug use. Generally, single or a few viral variants have been detected in intrapartum-infected infants, while more variants have been detected in utero-infected infants. The transmitted variants that establish infection in the new host are frequently derived from a minor variant present in the donor. Is this chance or selection?

The HIV-1 Envelope Glycoprotein Spike and Cell Tropism

HIV-1 particles bind the receptor CD4 and coreceptor proteins on the surface of immune cells to trigger entry into cells (Clapham and McKnight 2002). Virus strains that use CCR5 as a coreceptor have been designated as R5, those that use CXCR4 are termed X4, and those that can use both coreceptors as R5X4. While HIV-1 R5 viruses are usually transmitted, CXCR4-using variants generally emerge late in disease in up to 50% of individuals infected with clade B HIV-1, although this happens less frequently in subjects infected with clade C HIV-1, the predominant type of HIV-1 in Africa and globally. The designation R5 has often been used interchangeably with the terms mac-tropism or M-tropism, while X4 viruses are assumed to be T-tropic, infecting mainly T-cells. However, this interpretation is an oversimplification (Goodenow and Collman 2006). R5 viruses are generally able to infect CD4+ T-cells. However, they vary extensively in macrophage infection, with highly mac-tropic variants present in the brain and non-mac-tropic R5 viruses predominant in immune tissue (Duenas-Decamp et al. 2010). CXCR4-using variants do target CD4+ T-cells when they emerge. However, highly mac-tropic X4 virus isolates have also been described including CNS-derived ones.

Macrophages express much lower amounts of CD4 compared to T-cells. An important determinant of macrophage infectivity for R5 viruses is an Env adaptation to use these low amounts of CD4 on the cell surface for infection (Duenas-Decamp et al. 2010). Current data suggest that successfully transmitting viruses are predominantly non-mac-tropic R5, although little is

known about the factors during transmission that confer this selection.

How Does HIV Penetrate Epithelial Cell Barriers at the Mucosa?

Except for infection via the blood, HIV must penetrate protective epithelial cell layers at the mucosa. These epithelial cells do not express CD4 and are not usually susceptible to HIV infection. Stratified epithelia line the vagina, while a single layer of columnar epithelial cells protects the endocervix and intestine. Syncytiotrophoblast and cytotrophoblast cell layers line the fetal side of the placenta. Epithelial cells at these different sites form a system of tight junctions that effectively eliminate passive diffusion of donor virions or infected cells through the epithelial barriers to reach susceptible cells underneath. Mucosal epithelial cells in the vagina, endocervix, and rectum express SDF-1 (CXCL12), which binds and downregulates CXCR4 from cells in the proximity (Agace et al. 2000), helping to explain why CXCR4-using variants are infrequently transmitted.

Several mechanisms have been proposed for HIV-1 penetration of epithelial cell layers. First, damage and breaches in the system of tight gap junctions caused by other infectious agents and resulting inflammation will allow virus or infected cells to enter. Further, inflammation may induce CD4+ T-cells to penetrate the stratified epithelia of the vagina, ectocervix, and foreskin from the underlying submucosa to facilitate their infection by transmitting virions. Second, maturing dendritic cells including LC and other DCs within or close to epithelial layer(s) can trap virus particles before migrating into the submucosa or to lymph nodes and transferring virus to susceptible T-cells (Hladik and McElrath 2008). A third potential mechanism involves transcytosis where virus particles are delivered to the submucosal sites via a vesicular route through the cytoplasm of epithelial cells. This process is initiated by virion attachment to cells via Env interactions with cell surface receptors including galactoside ceramide and heparin sulfate proteoglycans. Whether T/F viruses

confer more efficient transcytosis has not yet been reported. The extent each of these different mechanisms contributes to transmission is not resolved.

The Role of Virus Transfer via Cell: Cell Contact During Transmission

The relative contributions of cell-free and cell-associated virus to transmission are unclear. However, it is strongly believed that virus transfer via cell-to-cell contact plays an important role during different stages of transmission. There are several mechanisms that confer infection via cell-to-cell transfer that could be relevant for transmission. However, transfer across synapses that form between cell contacts is likely to be the most significant (Chen et al. 2007; Geijtenbeek et al. 2000).

HIV-1 infection of CD4⁺ T-cells can be mediated via immunological or virological synapses. DCs present antigen to T-cells by forming immunological synapses. HIV exploits this system. Maturing DCs capture virus particles via interactions between GM3 glycosphingolipids assembled on particles and SIGLEC-1 on the surface of DCs. -C-type lectins expressed on DCs including DC-SIGN also augment attachment by binding Env on virions. However, these receptors are downregulated on DC maturation, while SIGLEC-1 is upregulated. Immature DCs may degrade captured virions via a lysosomal route, with trans-infection occurring predominantly from mature DCs. Captured virions are transported into a surface-accessible compartment that releases them into synapses as they form at T-cell contacts.

Virological synapses occur between infected and uninfected T-cells and direct a polarized burst of virus budding into the intercellular space. They are believed to support substantial levels of HIV replication particularly in immune tissue where cell-to-cell contact is extensive. T-cell-to-T-cell synapses are initiated by interactions between Env on the infected cell and CD4 on the target cell and are supported by further interactions involving integrins. It is possible that infected cells from the HIV-1⁺ donor form synapses with cells in the HIV-negative recipient at sites of transmission resulting in bursts of virus

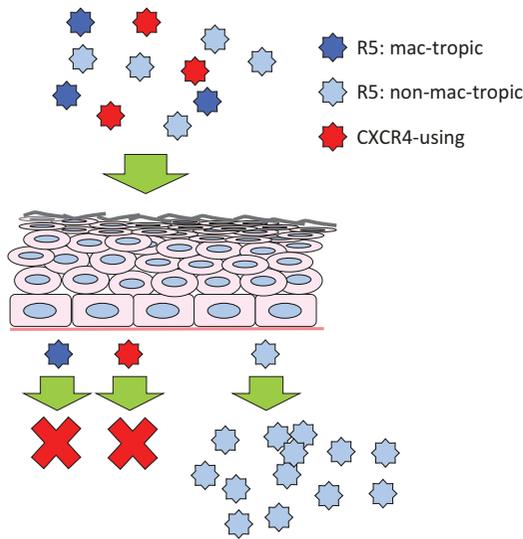
production that facilitate transmission. Regardless, synaptic transfer of virions is likely to be an important mode of virus expansion after infection.

It is not clear whether T/F HIV variants confer more efficient infection via synapses compared to non-transmitted HIV. One study reported T/F virus particles had nearly twice as much Env and bound more efficiently to DCs. However, if the primary mode of virion capture by DCs results from interactions between SIGLEC-1 and host moieties on virus particles, then capture may be primarily Env independent.

Finally, non-mac-tropic R5 viruses that require high levels of CD4 for infection may preferentially infect CD4⁺ T-cells via synapses. Infection by these T/F viruses will be greatly enhanced by the high levels of both CD4 and CCR5 that become concentrated on the target cell side of the synapse.

The Properties of Transmitting Viruses

Different studies have sought to identify properties of T/F viruses that are unique or different from those of viruses in the donor (transmitter) or from later stages of disease. These studies included investigation of T/F viruses deduced from newly infected female subjects, from heterosexually or homosexually infected males, and from neonates (Kishko et al. 2011). They have been augmented by the study of HIV-1 variants derived from early infection and by investigating Env genes as well as complete viruses. Transmitted HIV-1 variants are predominantly R5 that require high levels of CD4 for infection and do not confer efficient infection of macrophages. Most sexually transmitted T/F Envs also differ from about half of Envs derived from the chronic stage in how they use CCR5. About 50% of chronic stage R5 Envs confer some ability to exploit CCR5 occupied by the CCR5 antagonist, maraviroc, while few T/F Envs do this. The significance of this observation for transmission is unclear. However, it suggests that HIV-1 in late disease carry Env that can exploit different levels or conformations of CCR5 for infection, while T/F viruses usually cannot.



Tropism and Properties of the HIV-1 Envelope Glycoprotein in Transmission, Fig. 2 The transmission bottleneck: chance or selective transfer? Mac-tropic and non-mac-tropic R5 viruses as well as CXCR4-using viruses have been detected in semen, yet current data suggests that non-mac-tropic R5 variants are preferentially transmitted in sexual transmission. Is this because they have an overall predominance over other variants at sites of transmission or do they carry characteristics that favor transmission and replication in the new host? Transmitted variants have been reported to have fewer protective glycans and shorter variable loops and to replicate faster. Do these properties confer more efficient transmission (not shown)?

Several studies have reported that T/F Envs carry fewer protective sugars and have shorter variable loops on gp120 (Fig. 1) compared to Envs after seroconversion. These observations were made for T/F Env in sexual and mother-to-child transmission. However, some studies failed to confirm this, with sexually transmitted clade B Envs being least clear. Fewer glycans and shorter variable loops may increase the exposure of Env sites that interact with CD4 and enhance replication efficiency. T/F viruses were reported to be 1.7-fold more infectious compared to chronic stage viruses. Shorter variable loops and fewer glycans may increase sensitivity to neutralizing antibodies and could represent a potential vulnerability to vaccines and inhibitors. Neutralizing antibodies are absent during the acute phase, and these conditions should favor neutralization

sensitive strains, particularly if they confer an increased replication capacity that promotes transmission. Moreover, for viruses transmitted sexually, there is evidence that T/F viruses may be modestly more sensitive to different neutralizing antibodies, including pooled HIV-1+ plasma and Mabs that target the CD4 binding site on gp120 or sites on gp41. However, infant T/F Env and Env from breast milk of transmitting mothers did not differ in a range of properties including DC-mediated trans-infection, CCR5-use, infectivity and sensitivity to neutralizing antibodies, and inhibition by breast milk (Fouda et al. 2013). Nevertheless, at least for sexually transmitted Envs, current data is consistent with the selection of HIV-1 variants that carry increased replication capacity and may be more neutralization sensitive during and following transmission.

The Role of Neutralizing Antibodies in Transmission

Sexually transmitted virus usually enters an individual who has no immunity to HIV. However, this is not always the case and examples of superinfection have been documented. It is not clear whether the presence of immunity including neutralizing antibodies impacts on the efficiency of superinfection. Estimates of superinfection frequency vary from zero to levels similar to the infection rate for seronegative individuals. Some studies have suggested a level of protection. For example, among female sex workers, the incidence of superinfection was estimated at 4.7% compared to 7.8% for infection of seronegative women, although this difference was not statistically significant (Chohan et al. 2010). There is no data yet to suggest that superinfecting viruses are inherently more resistant to neutralizing antibodies. However, no studies have yet deduced T/F sequences and Env for superinfections.

Mother-to-child HIV-1 transmission usually occurs in the context of maternal antibodies that are passively transferred to the fetus over the last trimester of pregnancy. Maternal IgG is transferred through the placenta beginning around the 20th week of gestation. Secretory IgA, IgM, and

IgG are also present in colostrum and breast milk. These antibodies do not cross the infant's intestinal epithelium in substantial quantities. However, they may help protect against infection or penetration of the intestine. For example, purified dimeric IgA and IgM prepared from pooled HIV-1+ sera were shown to inhibit HIV-1 transcytosis *in vitro* using epithelial cell lines.

Placental-transferred neutralizing antibodies can reach high levels in the infant and potentially confer protection from HIV infection during later stages of pregnancy, delivery, and breast-feeding. Nonetheless, the role of maternal and infant neutralizing antibodies in transmission is controversial. Several studies of MTCT have indicated that mothers who do not transmit HIV carry higher levels of autologous neutralizing antibodies in plasma, while other studies did not support this (Fouda et al. 2013).

Many studies have tested whether the levels of heterologous neutralizing antibodies in the mother protected against MTCT, with several showing a positive correlation. Most studies on neutralization sensitivity have exploited maternal plasma or serum. Fewer have investigated neutralizing antibodies in breast milk or in secretions from other mucosal sites. However, Fouda et al. reported that plasma-derived antibodies in breast milk confer most of neutralizing activity present, albeit at levels that are likely too low to be protective (Fouda et al. 2011). Antibodies that conferred antibody-dependent cell-mediated cytotoxicity (ADCC) are present in breast milk and have been associated with reduced MTCT from mothers with high viral loads.

In summary, current data indicates that maternal antibodies transferred across the placenta *in utero* confer some level of protection in MTCT so that more neutralization-resistant viruses may carry an advantage for transmission to the neonate or infant.

Inhibitory and Enhancing Factors at Sites of Transmission

Cervical secretions, breast milk, and semen have been reported to inhibit HIV-1 infection, while semen also contains enhancing activity.

Numerous factors have now been described as either inhibiting or enhancing infection. These include semen-derived enhancer of virus infection (SEVI) and other amyloid peptides in semen that enhance HIV-1 infection by over 100-fold by increasing virion attachment to cells (Munch et al. 2007). A number of cationic peptides that are inhibitory have also been described. Human α -defensins HD5 and HD6 (small cysteine-rich cationic proteins) are present in vaginal, cervical lavage (where they are increased in the presence of STIs), in the intestine, and sometimes in breast milk. HD5 was reported to inhibit HIV-1 infection by blocking entry via an interaction with a site on gp120 that overlapped the CD4 and coreceptor binding sites. Human neutrophil defensins (HNPs 1–3 and LL-37) are also present in vaginal fluids and inhibit HIV-1 infection. HNP-1 was shown to bind both Env and CD4 and to block HIV-1 entry at several stages as well as inhibiting at later stages of replication. Whether SEVI or α -defensins have distinct effects on T/F Envs has not yet been extensively studied.

Breast milk also contains inhibitory activity effective against HIV-1 *in vitro*. This activity correlated with the level of sialyl-Lewis^x motifs, tetrasaccharide carbohydrates, usually attached to O-glycans on mucin including MUC1 and other glycoproteins or glycolipids. MUC1, MUC6, bile salt-stimulated lipase (BSSL), and Lewis X (LeX)-containing glycoproteins were each shown to block trans-infection from DCs to T-cells and sometimes cell-free infection. The mechanism of inhibition for sialyl-Lewis^x containing factors has not been reported although Env is a likely target. Finally half of breast milk samples contained factors that enhanced R5 virus infection mediated via cell-to-cell contact.

Source of HIV-1 at Sites of Transmission and Initial Cells Targeted in the New Host

If transmitting R5 viruses are predominantly non-mac-tropic and require high levels of CD4 for infection, it would implicate CD4+ T-cells that (express high CD4) as the source of HIV at

donor sites of transmission and as initial targets in the new host. Infection via synapses (where CD4 is concentrated) is also likely to play an important role. However, it may rule out macrophages and DC populations (which express low CD4) as viral sources in the donor and as early targets for infection. The predominance of T/F viruses and Env identified as non-mac-tropic is intriguing, since mac-tropic R5 variants and CXCR4-using viruses both confer efficient infection of CD4⁺ T-cells *in vitro*. The role of CD4⁺ T-cells as initial targets is supported by experiments where cervical explant tissues were tested for infection *in vitro* and by vaginal SIV inoculation of rhesus macaques. In the SIV model, CD4⁺ T-cells that included minimally activated (but not truly resting) as well as activated and proliferating T-cells are targeted (Haase 2010). It is possible that immunological synapses formed between LC/DCs and T-cells may act to simultaneously transfer activation signals and captured virions to T-cells.

Sources of HIV for Sexual Transmission

Our knowledge of HIV-1 variants and their properties at sites of transmission is incomplete. However, it is clear that R5 viruses predominate over CXCR4-using variants at least in semen and breast milk. "Opportunity" therefore partly explains why R5 viruses are preferentially transmitted in sexual and MTC transmission.

Semen mediates HIV-1 transmission via vaginal and rectal routes. HIV-1 is shed intermittently into semen and is present as cell-free and cell-associated virus comprising both T-cells and cells of macrophage lineage (Le Tortorec and Dejudic-Rainsford 2010). Spermatozoa themselves are not infected and not thought to play a significant role in capturing and delivering viral particles (Le Tortorec and Dejudic-Rainsford 2010).

Virus in semen is derived from both genital tract tissues and blood. Cell-free virus in semen is also sometimes genetically distinct from proviral DNA implicating a distinct tissue source. The tissue origins of HIV-1 tropism variants in semen have not been precisely established. The seminal vesicles and prostate, which contribute up to 90% of seminal plasma, are likely sources. Seminal

vesicles contain susceptible CD163⁺ macrophages, while the prostate contains infected T-cells. The testes and epididymis may be less significant contributors since vasectomy does not greatly affect semen viral loads, although the testes contain infected T-cells.

Several studies indicate that HIV-1 R5 variants are predominant in semen consistent with their preferential transmission over CXCR4-using viruses. However, CXCR4-using variants do reach the semen and are sometimes detected at lower levels. Full-length R5 Env sequences PCR-amplified from either proviral DNA or vRNA in semen from later stages of infection varied extensively in macrophage infectivity, although most were non-mac-tropic. No studies on the tropism of HIV-1 Env present in semen have been reported for the acute phase of infection when a substantial number of transmissions occur.

Cervical and vaginal secretions of HIV⁺ women contain sCD25 and sCD14, soluble markers for lymphocytes, and macrophages, respectively. Generally, inflammation and the presence of STIs are associated with increased virus shedding into transmission tissues and fluids of HIV⁺ subjects. R5 variants are predominantly detected in cervicovaginal secretions. However, there are no reports that assessed whether mac-tropic or non-mac-tropic HIV-1 R5 is present.

Initial Target Cells in Sexual Transmission

In the vagina, ectocervix, and particularly the transitional zone, there are microenvironments that are rich in immune cells including CD4⁺ T-cells. These foci of immune cells are concentrated close to the surface. Similarly, the rectal canal may be an area of high susceptibility mediating a higher rate of infection compared to the vaginal route (Powers et al. 2008). Submucosal DCs are enriched in the rectal canal and may extend dendrites into the lumen for initial capture of virions. High concentrations of highly permissive $\alpha 4\beta 7 + CD4^+$ T-cells (Arthos et al. 2008) are also present in the lamina propria of the rectum with their frequency correlating with susceptibility to rectal SIV infection.

Submucosal macrophages in the intestine are not susceptible to HIV due to reduced CD4 and

CCR5 expression as well as post-entry restrictions further highlighting T-cells as initial targets for infection although those in the anal canal express higher levels of CCR5 and may be more susceptible. In contrast, vaginal/cervical macrophages are susceptible to mac-tropic R5 viruses at least *in vitro*.

Contact with cervical and vaginal secretions or rectal material mediates infection of the penis. Circumcision reduces the risk of infection in men by >50%, indicating that the foreskin is an important site for the infection. Men with the largest foreskin surface area are the most susceptible. However, the tip of the urethra has also been implicated as a site for infection. The inner foreskin is more susceptible to infection containing a higher density of potential target cells compared to the outer foreskin with LCs found within the stratified epithelium, while CD4+ T-cells, macrophages, and DCs usually reside in the submucosa, although as discussed above, inflammation can lead to the recruitment of CD4+ T-cells into the stratified epithelium.

Mother-to-Child Transmission

MTCT can occur *in utero*, at the time of delivery, and during breast-feeding (Table 1).

Transmission *in utero* across the placenta is inefficient with most infections during pregnancy occurring late on when the placenta begins to separate from the uterus, and there is potential exposure to maternal blood. HIV-1 clade C viruses were reported to transmit *in utero* more efficiently compared to clades A and D, although whether this is due to Env is not known. On the maternal side of the placenta, the decidua basalis usually contains only low levels of T-cells along with macrophages, both of which have been shown by immunohistochemistry to be occasionally infected *in vivo*. However, while decidua basalis macrophages are permissive, the T-cells are poorly permissive to HIV-1 R5 infection *in vitro*.

Precisely how HIV-1 traverses the syncytiotrophoblast and cytotrophoblast layers and enters infant tissue beneath is not understood. These outer cell layers are CD4 negative and not easily infected. Few T-cells are usually present within the placental villi on the fetal side of the

trophoblast layers, although Hofbauer cells (macrophage lineage) have been shown to be occasionally infected using immunohistochemistry. Inflammation of the placenta increases the risk of transmission and may encourage the infiltration of CD4+ T-cells and other immune cells into maternal and fetal sites. The tropism and properties of virus transmitted via this route are not known.

During delivery, infants are exposed to amniotic fluid, genital tract secretions, and maternal blood, which are likely swallowed before infecting fetal cells. Amniotic fluid only occasionally carries HIV-1, leaving genital secretions and blood contamination as likely viral sources. Both cell-free and cell-associated viruses have been implicated in transmission during delivery.

Both R5 and X4 viruses have been detected in breast milk. Mastitis (even subclinical) increases viral load. Moreover, both cell-free and cell-associated viruses have been implicated in transmission via breast milk. Variants detected in breast milk are frequently distinct from plasma virus. However, breast tissue does not form a truly independent compartment for HIV replication.

Infants are infected *intrapartum* and *postpartum* after swallowing infected maternal fluid and breast milk. It is unclear where the initial infection event occurs with the oral cavity, the tonsils, and the upper gastrointestinal tract as possible entry sites. Higher LPS levels in the plasma of infants (a measure of intestinal permeability) were recently reported to be a predictor of infection via breast milk. Alternatively, virions may penetrate the intestinal epithelial layer via transcytosis through enterocytes or M cells.

Conclusion

The various routes of mucosal HIV-1 transmission usually require the virus to (1) evade innate inhibitory factors and sometimes neutralizing antibodies and (2) penetrate epithelial cell barriers to access susceptible host cells. The different routes into the new host may exert distinct selection pressures on viruses (and their Env) that have the potential to transmit. Current data indicates that R5 HIV-1 variants that are non-macrophage-

tropic are preferentially transmitted targeting CD4+ T-cells in or close to the mucosa of the new host. At least for some clades, Envs of transmitted variant Env have shorter variable loops and fewer glycans. Such variants may replicate faster but also offer potential vulnerabilities to neutralizing antibodies or inhibitors. Our knowledge of the properties of T/F viruses for different routes of transmission is growing. The challenge is to understand how T/F Env properties impact on Env structure and to exploit this information to design more effective vaccines and microbicides.

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HIV-related immune compromise, compounded by immune immaturity in the very young (Perez-Velez and Marais 2012).

There are specific management challenges in HIV-associated tuberculosis, including selection and timing of antiretroviral therapy (ART) and recognition and management of shared drug toxicities and pharmacokinetic interactions. Immune reconstitution inflammatory syndrome (IRIS) is a common complication during treatment of HIV-associated tuberculosis and results in substantial morbidity. The problems of limited drug options and prolonged duration of therapy for drug-resistant tuberculosis are further exacerbated in HIV-infected patients because of overlapping toxicity with ART.

For the future, key challenges in HIV-associated tuberculosis include development of sensitive and low-cost point of care diagnostic tests to hasten appropriate initiation of anti-tuberculosis therapy and widespread implementation of existing evidence-based prevention strategies supported by the discovery of a more effective tuberculosis vaccine that can be used in HIV-infected people. New antituberculosis drugs are now in advanced clinical development and show potential for shortening the duration of therapy and improving cure rates in drug-resistant tuberculosis, but there remain concerns about toxicity and pharmacokinetic interactions with ART. This chapter provides an up to date overview of these issues with an emphasis on high burden and resource poor settings.

Tuberculosis and HIV

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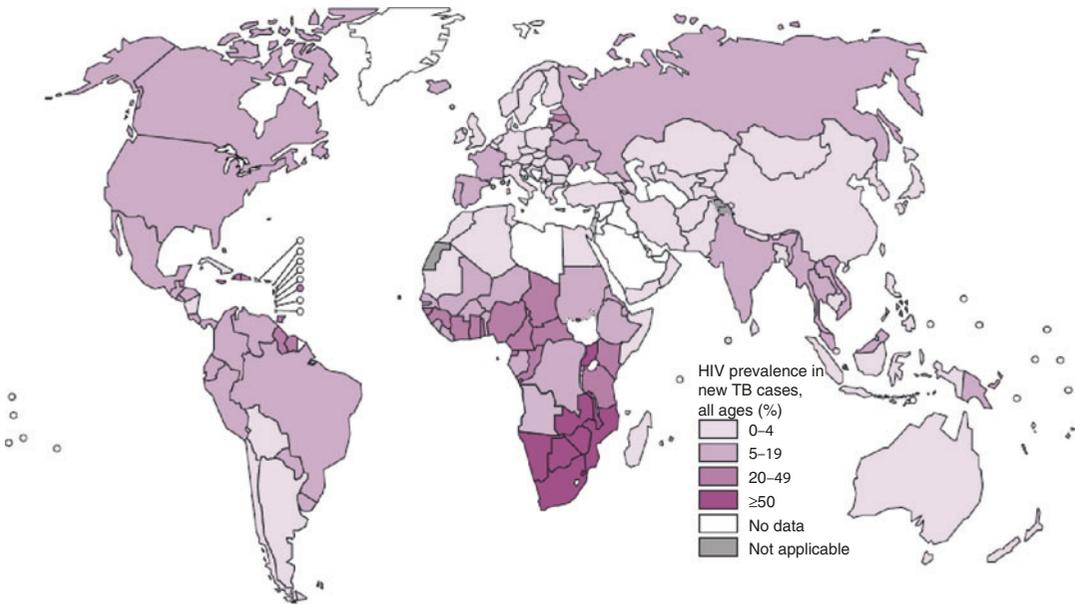
Introduction

HIV is a major driver of the global tuberculosis epidemic. The immune dysfunction associated with HIV increases susceptibility to tuberculosis, influences its clinical presentation, and alters the performance of diagnostic tests. Extrapulmonary tuberculosis is more common in HIV, and rapid progression of disease and a predilection for the central nervous system both contribute to the higher overall mortality in coinfecting patients.

Children also suffer a high burden of tuberculosis disease in areas where *M. tuberculosis* transmission is poorly controlled (Graham et al. 2014). HIV-infected children are especially vulnerable due to (1) increased tuberculosis exposure in HIV-affected households and (2) increased risk of disease progression following infection due to

Epidemiology

A third of the world's population have been infected with *M. tuberculosis* and of these around nine million people developed active tuberculosis disease in 2013 (WHO Global Tuberculosis Report 2014a). After HIV itself, tuberculosis is the second most common infectious cause of death in adults globally, and with 13% of all new tuberculosis cases occurring in HIV-infected people, tuberculosis is the most important opportunistic infection in HIV-infected people worldwide. Tuberculosis has a disproportionate impact on developing nations,



Tuberculosis and HIV, Fig. 1 Estimated HIV prevalence in new and relapse TB cases, 2013 (WHO global report 2014a)

with 22 of these countries accounting for 82% of all incident tuberculosis cases globally. Average incidence rates in high-burden countries are around 150–300 cases per 100,000 population per annum, while in Southern Africa rates are above 500 per 100,000. Sub-Saharan Africa experiences the greatest burden of HIV-associated tuberculosis, where around 80% of the global cases and deaths occur, and in Southern Africa over 50% of new tuberculosis cases are HIV-infected (Fig. 1). The HIV epidemic has profoundly influenced the epidemiology of tuberculosis in the region: in South Africa, Lesotho, and Swaziland, three countries with the highest per capita HIV prevalence in the world, active tuberculosis develops in ~1 person in every 100 per year, the majority of whom are HIV coinfectd.

Although global tuberculosis mortality has been declining over the past decade, an estimated 1.5 million people died from the disease in 2013, with a quarter of these deaths occurring in HIV-infected people; in Africa up to 40% of all AIDS deaths are from tuberculosis. These outcomes are unacceptable in the context of effective antituberculosis therapy that consistently results in treatment success in over 80% of drug susceptible cases in

functional tuberculosis programs, as well as improving ART coverage in notified HIV-infected tuberculosis patients, and fall short of Millennium Development Goal targets. Parts of the explanation for this are the worse treatment outcomes and higher mortality in HIV-associated tuberculosis. Globally the mortality during tuberculosis treatment is threefold higher amongst those coinfectd with HIV, and even in Africa where overall mortality from tuberculosis is higher, HIV-infected tuberculosis patients are twice as likely to die as HIV-negative patients within tuberculosis programs.

The burgeoning drug-resistant tuberculosis (Box 1) epidemic represents a major threat to global tuberculosis control. In 2013, 3% of new cases and 20% of retreatment episodes were estimated to have multidrug-resistant (MDR) tuberculosis, with almost half a million cases occurring worldwide. In MDR tuberculosis programmes, fewer than half of patients initiating therapy are successfully treated, largely because of the higher mortality associated with MDR tuberculosis and frequent disengagement from care due to drug toxicity and extended treatment durations. High rates of loss to follow-up contribute to the widening gap between detection and

treatment, and together with inadequate coverage of diagnostics for drug-resistant tuberculosis and long duration required for culture-based confirmation, results in only around 10% of global cases of MDR tuberculosis achieving cure.

Box 1 Definitions of drug-resistant TB

Multidrug resistant (MDR)	Resistance to rifampicin plus isoniazid
Pre-extensively drug resistant (pre-XDR)	MDR plus resistance to either a fluoroquinolone or an injectable agent ^a
Extensively drug resistant (XDR)	MDR plus resistance to both a fluoroquinolone and an injectable agent ^a

^aAmikacin, kanamycin, or capreomycin

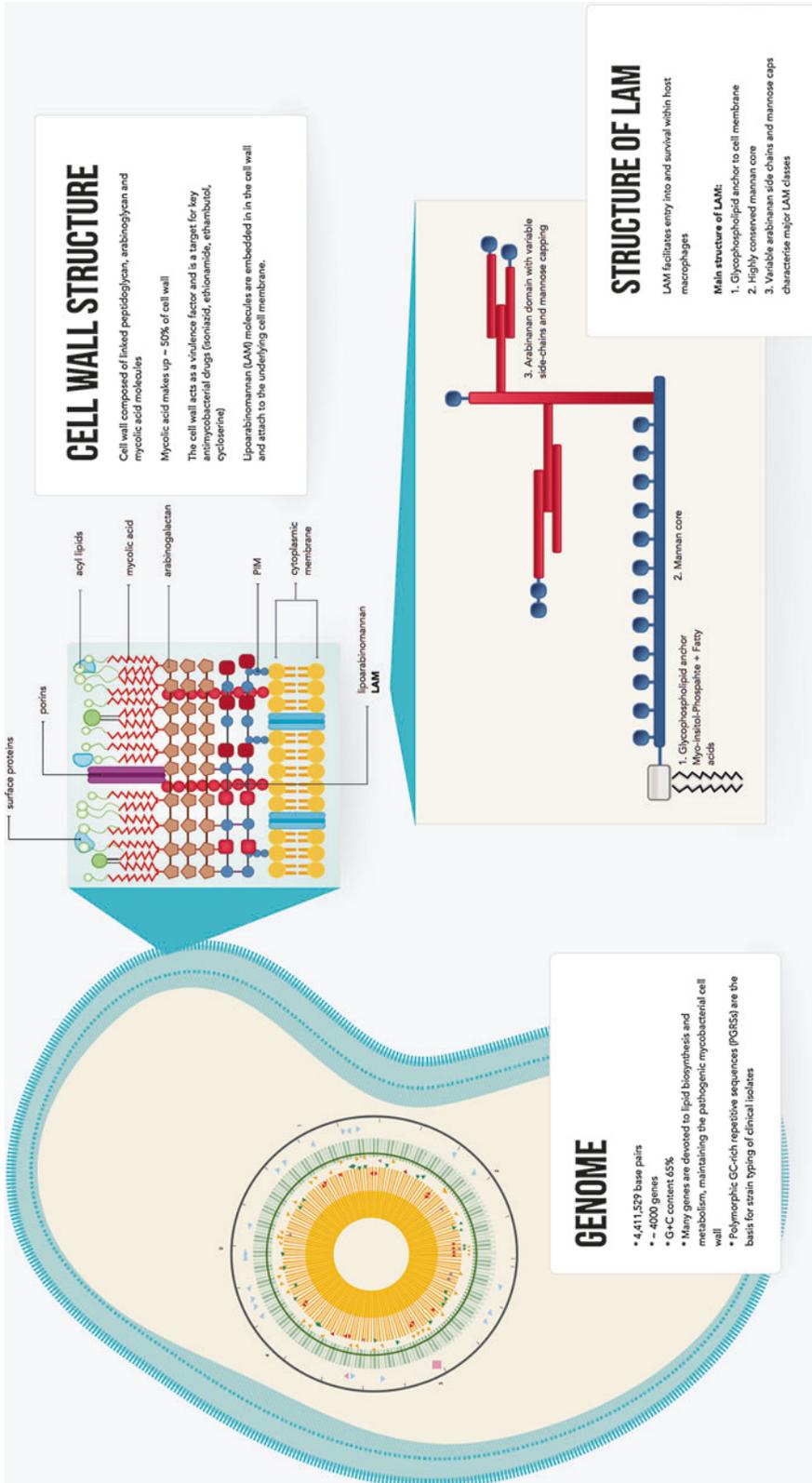
Almost a third of all cases with MDR tuberculosis have additional resistance to fluoroquinolones or injectable agents or both, a consequence of widespread misuse of anti-tuberculosis agents, difficulties in retaining patients in care and transmission of resistant tuberculosis. Less than a quarter of patients successfully complete treatment for extensively drug resistant (XDR) tuberculosis, with an average global mortality of 35%. In South Africa, the mortality and treatment failure rate for XDR tuberculosis is even higher, influenced by the subgroup of HIV-infected patients not yet on ART (Pietersen et al. 2014). The greatest burden of MDR tuberculosis is in the Russian Federation, India, and China, where HIV prevalence is increasing, but sub-Saharan Africa represents 15% of global cases, and therefore a substantial number of patients will be coinfecting with HIV. Similarly to drug sensitive tuberculosis, patients with HIV-associated drug-resistant tuberculosis have worse outcomes than HIV-uninfected patients, although this difference is mitigated by ART.

Microbiology

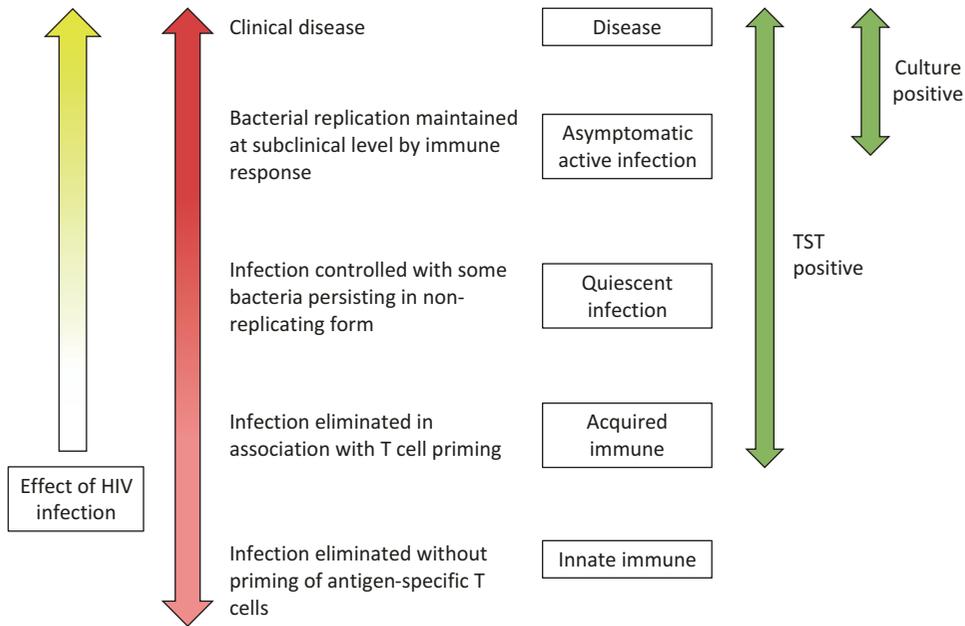
The causative organisms of tuberculosis belong to a family of seven species of mycobacteria in the

M. tuberculosis complex. *M. tuberculosis* is by far the most important cause of tuberculosis in humans. The other species are predominantly zoonoses, but *M. bovis* does uncommonly cause human disease through animal contact or from drinking unpasteurized milk. Humans are the only natural reservoir for *M. tuberculosis*, and transmission takes place exclusively from symptomatic individuals with pulmonary disease via expectoration of small respiratory droplet nuclei containing *M. tuberculosis* bacilli.

M. tuberculosis has a number of unique biological characteristics that directly impact on the pathophysiology and diagnosis of tuberculosis. It is an aerobic nonspore-forming Gram-positive bacillus surrounded by a cell wall of mycolic acids and lipopolysaccharides (Fig. 2). This waxy lipid layer is responsible for the characteristic phenomenon of “acid-fast” staining where aniline dyes such as carbol fuchsin are not removed by the addition of an acid alcohol decoloriser and retained within the cell wall, facilitating smear microscopy, the most common diagnostic test in use in developing countries. Mycolic acids are also implicated in the characteristic serpentine cording pattern (where *M. tuberculosis* bacilli are seen to clump together in parallel under light microscopy) and are involved in eliciting granuloma formation in host tissue. Lipoarabinomannan (LAM) antigens embedded in the cell wall and attached to the underlying plasma membrane act as major virulence factors by inhibiting crucial innate immune responses, thus enhancing survival of *M. tuberculosis* in the human host. The detection of LAM antigen in urine is being exploited as a promising diagnostic test for tuberculosis in patients with advanced HIV (discussed below). *M. tuberculosis* is a slow-growing organism with a generation time of around 24 h in vitro, resulting in prolonged time to recovery from laboratory culture media, a feature that impacts greatly on the ability to diagnose and control this disease in high-burden settings. The genome is large, containing around 4000 genes housed within a circular chromosome made up of 4.4 million base pairs, a large proportion of which are devoted to the production of enzymes involved in lipogenesis and lipolysis



Tuberculosis and HIV, Fig. 2 Structure of *M tuberculosis*



Tuberculosis and HIV, Fig. 3 Spectrum of infection (Adapted from: Barry et al. (2009))

required for the maintenance of its pathogenic and complex cell wall (Cole et al. 1998).

Latent TB Infection

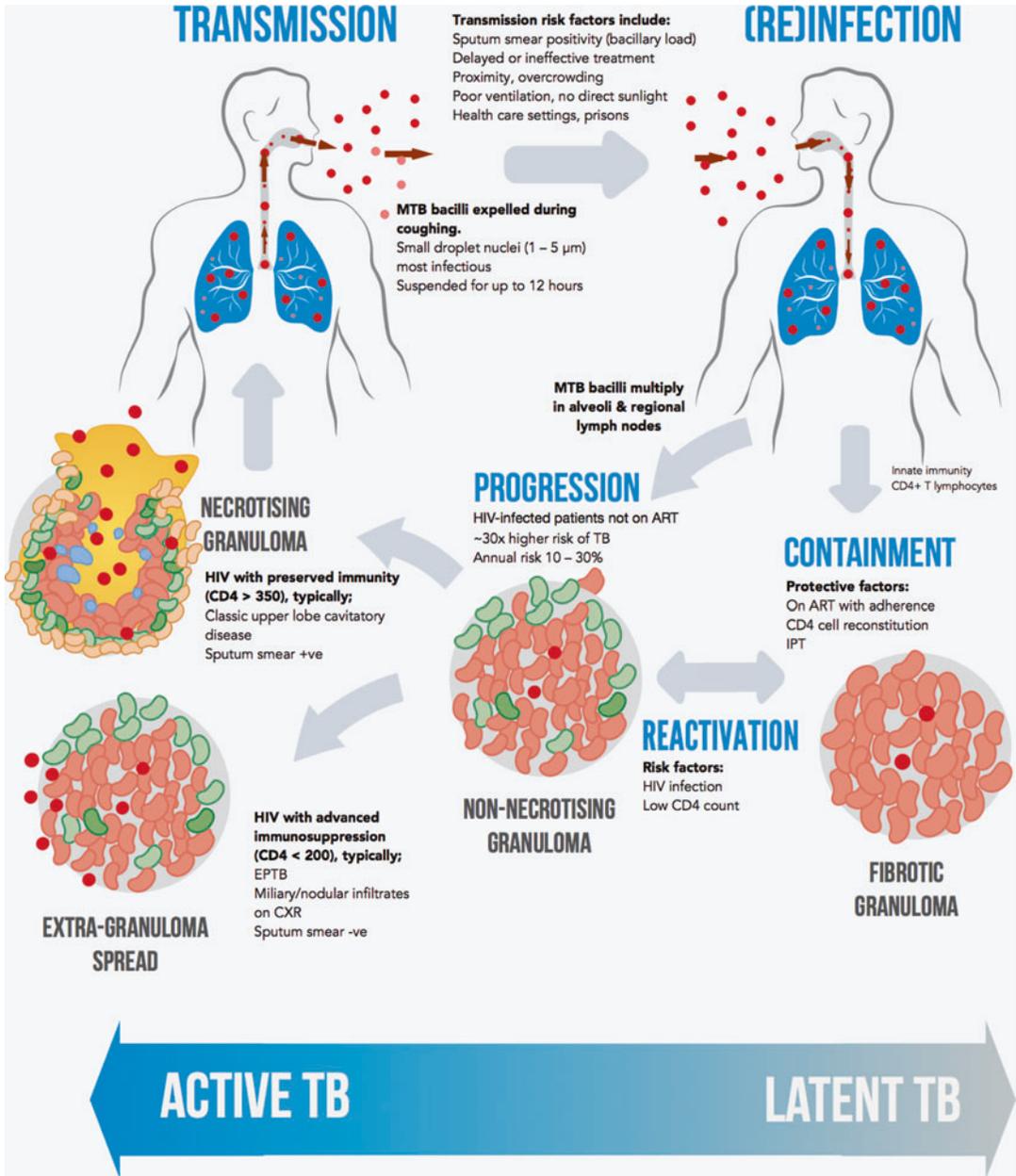
As in all infectious diseases, the clinical manifestations of tuberculosis result from a dynamic interaction between the host immune response and virulence factors of the organism. Perhaps the key reason for the success of *M. tuberculosis* to persist and spread globally is its ability to become metabolically inactive and enter a state of dormancy in host tissues, setting up chronic subclinical latent infection (LTBI). The organism is held in check by a competent cell-mediated immune response but may later reactivate when immunity is perturbed. During treatment, slowly dividing subpopulations enable *M. tuberculosis* to evade the action of many antimicrobial agents (i.e., phenotypic insensitivity) requiring prolonged treatment regimens to achieve sterilization.

The development of specific cell-mediated immunity against *M. tuberculosis* coincides with tuberculin skin test (TST) positivity, a delayed hypersensitivity reaction to the subcutaneous

injection of purified *M. tuberculosis* antigens; in asymptomatic people, a positive skin test signifies LTBI. Because of immune dysfunction, patients with HIV have reduced overall positivity rates and less vigorous responses to TST; the size of induration indicating a positive TST is lowered to ≥ 5 mm in HIV-infected individuals (from ≥ 10 mm in immunocompetent adults) to account for this. Although tuberculosis has traditionally been understood as a bimodal disease with patients having either latent or active infection depending on the absence or presence of clinical symptoms, it is increasingly being recognized that latent tuberculosis is part of a spectrum that spans from the possible elimination of *M. tuberculosis* by the innate immune system, through immune containment, to asymptomatic yet culture-positive disease, through to active and symptomatic disease (Fig. 3).

Pathophysiological Interactions Between HIV and TB

Because of the centrality of CD4 lymphocytes in granuloma formation and controlling *M.*



Tuberculosis and HIV, Fig. 4 Transmission and pathophysiology of HIV-associated tuberculosis

tuberculosis infection, it is predictable that HIV infection, the hallmark of which is CD4 cell depletion and dysfunction, has profound effects on the incidence, course, and clinical presentation of tuberculosis. HIV-infected individuals have a ~30-fold higher risk of developing tuberculosis than the immunocompetent population, due to

both reactivation of LTBI and progression from infection or reinfection to disease (Fig. 4). The risk of developing tuberculosis doubles within the first year after HIV-seroconversion and progressively increases with declining CD4 counts (Lawn et al. 2009). But even when on effective ART and with CD4 counts in the normal range, HIV-

infected people have a roughly fourfold higher risk of tuberculosis compared to HIV-uninfected people in high-burden communities, suggesting incomplete restoration of tuberculosis-specific immune function.

HIV-infected individuals with low CD4 counts are unable to mount an adequate inflammatory response, thereby limiting tissue damage but allowing unrestrained bacillary replication and spread. Rather than causing localized apical cavitary lung disease, tuberculosis becomes disseminated resulting in extrapulmonary organ involvement, intra-thoracic adenopathy, and military infiltration of the lung parenchyma. In addition to its impact on the clinical manifestations, HIV infection causes a more rapid progression of tuberculosis from the onset of symptoms to severe disease. Many patients with low CD4 counts who are sick enough to be hospitalized with HIV-associated tuberculosis report that they have been ill for less than 4 weeks.

Active tuberculosis also has detrimental effects on HIV infection, including significant increases in HIV viral load and a higher risk of progression to AIDS and death. There are a number of potential mechanistic explanations for this, including the increased expression of cell surface coreceptors CCR5 and CXCR4 required for HIV entry and enhanced transcription of HIV-associated proteins and thus viral replication because of upregulation of NF- κ B in HIV-infected monocytes.

Clinical and Radiological Manifestations

Because the pathology of *tuberculosis* is largely determined by host immune response, the clinical presentation depends on the degree of immunocompromise. HIV-infected patients with preserved CD4 cell counts, particularly >350 cells/ μ L, are therefore more likely to manifest similar symptoms and signs to those who are HIV-uninfected: a chronic cough that may be associated with hemoptysis, accompanied by weight loss, night sweats, and fever. The chest X-ray may demonstrate upper lobe cavitation that is also associated with high bacillary load in sputum and greater chance of diagnosis by smear microscopy.

By contrast, the clinical presentation of tuberculosis in HIV-coinfected patients with CD4 counts <200 cells/ μ L and advanced immunosuppression is often nonspecific, with atypical pulmonary manifestations, frequent extrapulmonary involvement, and a higher prevalence of smear-negative disease. Although respiratory symptoms may be completely absent, the majority of HIV-infected patients with disseminated disease will have pulmonary involvement as evidenced by positive sputum cultures. The mean CD4 count at ART initiation in sub-Saharan Africa remains below 200 cells/ μ L, but with improved access to ART and higher CD4 count thresholds for treatment initiation in high-burden settings, the clinical presentation of HIV-associated tuberculosis may begin to shift towards the typical syndrome seen in immunocompetent individuals.

Establishing a diagnosis of tuberculosis can be particularly problematic in HIV-infected children, since the TST is less sensitive than in HIV-uninfected children, failure to thrive is a typical feature of both tuberculosis and HIV, and chronic pulmonary symptoms may be due to other HIV-related conditions such as gastroesophageal reflux and bronchiectasis. Chest X-ray interpretation is complicated by HIV-related comorbidity such as recurrent bacterial pneumonia, lymphocytic interstitial pneumonitis (LIP), pulmonary Kaposi sarcoma and atypical tuberculosis presentations with disease phenotypes reflecting poor organism containment (Marais et al. 2007).

Extrapulmonary tuberculosis associated with HIV may manifest either as a systemic illness or with involvement of a particular organ system dominating. The former syndrome is often referred to as “disseminated tuberculosis,” where patients may present with weight loss, prolonged fevers, pallor of mucous membranes, lymphadenopathy, and/or abdominal tenderness. In contrast to the chronic indolent course of pulmonary disease in immunocompetent people, disseminated tuberculosis can progress rapidly in patients with advanced HIV, necessitating more urgent diagnosis and treatment initiation. Mild hepatosplenomegaly can occur from infiltration by tuberculous granulomas, and this is exploited diagnostically with the use of ultrasonography to

demonstrate microabscesses in the spleen. Large tender hepatomegaly occurs mainly, but not exclusively, in the context of unmasking or paradoxical tuberculosis IRIS, where an exaggerated immune reaction to *M. tuberculosis* antigen causes widespread expansion of intrahepatic granulomas. Chest radiography may be completely normal in up to 30% despite a positive sputum *M. tuberculosis* culture or reveal subtle nodular infiltrates, miliary patterns or consolidation, with or without intrathoracic lymphadenopathy or pleural effusions. Anaemia is common and results from a number of factors including iron malutilization from chronic inflammation, nutritional deficiencies, bone marrow suppression from HIV itself, and infiltration by tuberculosis. Bone marrow dysfunction may lead to pancytopenia. Renal impairment frequently accompanies disseminated tuberculosis in HIV-infected patients, and although often multifactorial, in certain cases it may result from direct involvement of the renal parenchyma by tuberculosis. This is the basis for the urine LAM antigen test, which, as discussed below, performs much better in patients with very low CD4 counts where renal tuberculosis is more likely. Although often accompanied by organ dysfunction, disseminated tuberculosis may have no localizing features, and in high-burden settings, tuberculosis is a common cause of pyrexia of unknown origin in hospitalized patients with HIV.

The most important discreet sites of extrapulmonary involvement in HIV are lymph nodes, pleura, the central nervous system (CNS), and pericardium, but virtually any organ system can be affected including the skeletal system, genitourinary tract, and skin.

Tuberculous Adenitis

Extrathoracic lymph nodes are affected in around a fifth of patients with HIV-associated tuberculosis, commonly in the cervical and axillary regions. Involved nodes are typically soft, tender, and fixed to underlying tissue. Groups of nodes can become matted together and coalesce to form massive swellings which may develop fluctuant areas and open onto overlying skin as chronic discharging sinus tracts. Lymphoma is the most important alternative diagnosis to consider in

HIV-infected patients with adenopathy and constitutional symptoms and should be excluded with an excisional lymph node biopsy, particularly in those not improving on empiric antituberculosis therapy. Other causes of adenopathy in HIV include Kaposi sarcoma, multicentric Castleman's disease, and infection due to cryptococcus or dimorphic fungi.

Pleural tuberculosis

Pleural effusions are more common in HIV-associated tuberculosis and occur more frequently in those with CD4 cell counts < 200 cells/microL. In high-burden settings, the finding of a unilateral lymphocytic pleural effusion in a young person has a very high positive predictive value for tuberculosis and can be used as a basis for empiric antituberculosis therapy. Symptoms include pleuritic chest pain and dry cough, which may progress to cause dyspnoea as the effusion enlarges; the indolent nature of the disease can allow effusions to become massive, occasionally leading to respiratory compromise and requiring urgent intercostal needle drainage. Simple pleural effusions may become complicated to form empyema, either due to superadded bacterial infection or from tuberculosis itself. Other causes of pleural effusion in HIV include parapneumonic effusion (due to bacterial organisms), Kaposi sarcoma, cryptococcosis, and primary effusion lymphoma.

CNS Tuberculosis

CNS disease is the most devastating form of tuberculosis – it is difficult to diagnose, requires prolonged therapy, and leaves a large proportion of patients disabled or dead. The three clinical categories of CNS tuberculosis, meningitis, intracranial tuberculoma, and spinal arachnoiditis, occur more commonly in HIV-infected patients and have been reported in up to a quarter of extrapulmonary tuberculosis presentations.

In sub-Saharan Africa, tuberculosis causes over a third of all cases of meningitis in HIV-infected adults (Veltman et al. 2014). The overall inpatient mortality from tuberculous meningitis (TBM) is up to 60%, but those with HIV coinfection have higher rates of death at 6–9 months after starting antituberculosis therapy compared to

HIV-uninfected patients (up to 67 versus 30%). Factors consistently associated with reduced survival in HIV include prolonged duration of symptoms, reduced level of consciousness, and lower CD4 cell counts. The presentation of HIV-associated TBM is similar to that in HIV-uninfected patients and has a broad clinical spectrum, ranging from a subacute illness with constitutional symptoms and chronic headache to sudden onset of severe meningitis progressing rapidly to coma. Stroke, cranial neuropathies, and hydrocephalus are important complications of TBM. Evidence of extracranial tuberculosis is found in about half of patients with HIV coinfection and TBM. Cerebrospinal fluid (CSF) analysis typically shows a lymphocytic pleocytosis with low glucose and raised protein, but this pattern is extremely variable, and up to 5% of patients with culture-confirmed TBM have a completely normal initial CSF examination. In a randomized controlled trial, the addition of corticosteroids to anti-tuberculosis therapy resulted in a 31% reduction in mortality in patients with TBM, including those with less severe presentations. This survival benefit was not statistically significant in the smaller HIV-infected subgroup, but the finding was similar in terms of direction of effect (Thwaites et al. 2004), and on the basis of this, corticosteroids are recommended for all patients with TBM. The most important differential diagnosis is cryptococcal meningitis, the commonest cause of meningitis in HIV-infected patients in developing countries and clinically indistinguishable from TBM. Cryptococcal meningitis should therefore be excluded in all patients with suspected TBM by performing a cryptococcal antigen test and fungal culture on the CSF. Other aetiologies that may mimic TBM include bacterial meningitis, neurosyphilis, and encephalitis caused by cytomegalovirus (CMV) or herpes simplex virus (HSV), particularly at CD4 counts <50 cells/ μ L.

Cerebral tuberculomas may occur with or without concomitant meningitis, and present with headache, focal neurological deficits, seizures, or reduced levels of consciousness. On contrast computed tomography (CT) imaging the lesions show variable central density surrounded by peripheral ring enhancement and

edema, often with mass effect. The number and distribution of the lesions is not specific, and CT cannot reliably distinguish tuberculomas from other causes of mass lesions in HIV, especially toxoplasmosis.

Spinal tuberculous arachnoiditis typically presents with mixed upper and lower motor neuron signs of the legs and sphincter dysfunction. The CSF may show the typical changes of TBM but can be normal, and the diagnosis is usually made with magnetic resonance imaging (MRI) demonstrating nodular arachnoiditis in the appropriate clinical context. Infections that may mimic this disease in HIV-infected patients include cytomegalovirus (CMV) polyradiculopathy and neurosyphilis.

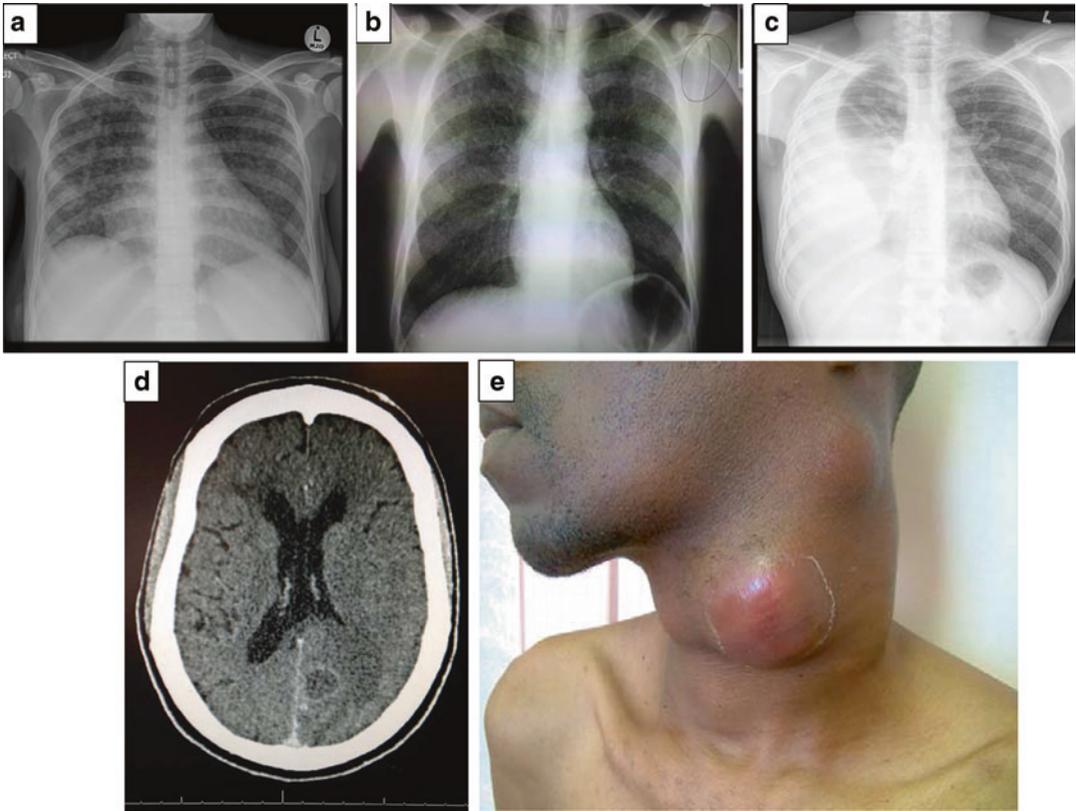
Tuberculous Pericarditis

The incidence of tuberculous pericarditis has risen dramatically in HIV-endemic regions. In developing countries, tuberculosis is responsible for over 50% of cases of pericarditis, and up to 75% of patients with large pericardial effusions in sub-Saharan Africa are HIV-infected, with tuberculosis the cause in most. Disseminated disease is more common in HIV-infected patients with tuberculous pericarditis, who present more frequently with myopericarditis, dyspnoea, and hemodynamic instability and have higher 6-month mortality (40%) than those who are HIV-uninfected. Corticosteroids are not recommended for tuberculous pericarditis in HIV as they have no significant impact on a composite outcome of death, tamponade, and constriction, and are associated with an increased risk of HIV-associated cancers. Other possible causes of pericardial effusion in HIV are pyogenic (may be as coinfection with tuberculosis), cryptococcosis, Kaposi sarcoma, and lymphoma.

Representative clinical and radiological images are presented in Figs. 5 and 6.

Diagnosis

Clinicians should have an especially high index of suspicion for tuberculosis in any HIV-infected

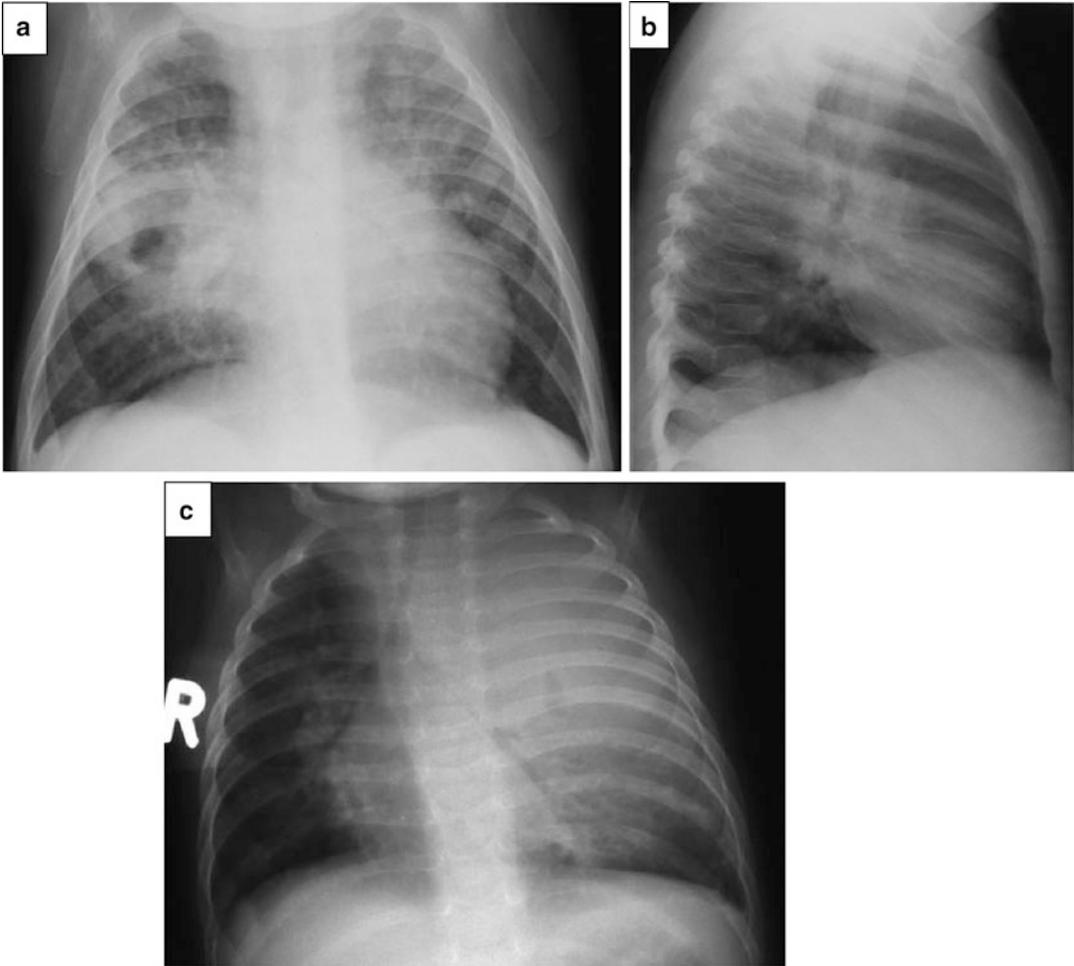


Tuberculosis and HIV, Fig. 5 Representative images of the clinical and radiological spectrum of HIV-associated TB in adult patients. Clockwise from top left: (a) Chest radiograph showing nodular parenchymal infiltrates, pericardial effusion, and mediastinal adenopathy; (b) Chest radiograph showing a diffuse miliary infiltrate with hilar and mediastinal adenopathy; (c) Chest radiograph showing a large right pleural effusion

with nodular parenchymal infiltrates; (d) Contrast-enhanced CT scan showing a ring enhancing mass lesion with central hyperdensity in the posterior fossa, surrounded by edema and causing generalized brain swelling; (e) massive cervical adenopathy with cold abscess formation (reproduced from Meintjes et al, *Lancet Infect Dis.* 2008 Aug;8(8):516-23 with permission from Elsevier)

patient with rapid loss of weight and a reduced level of function in the absence of chronic diarrhoeal illness as an explanation. The decision to start empiric antituberculosis therapy depends mainly on the severity of the clinical presentation and access to diagnostic tests, with a lower threshold in ill patients or those with a rapidly deteriorating condition. Potential pitfalls of empiric treatment include missing another serious opportunistic disease or drug-resistant tuberculosis and exposing patients unnecessarily to prolonged courses of antituberculosis therapy. Therefore, although the clinical pretest probability of tuberculosis in endemic regions may be high, it is

important to send clinical samples to the laboratory to confirm the diagnosis and obtain drug susceptibility data. Establishing microbiological confirmation of disease is particularly problematic in HIV-infected children who are unable to expectorate. However the principles of case detection and management are similar to those in adults, with collection of alternative respiratory specimens such as induced sputum, gastric aspirates or stool in children who are too young to expectorate, and nonrespiratory specimens such as fine needle aspiration biopsy (FNAB) in children with peripheral lymph node masses (Siberry et al. 2013; Marais et al. 2011).



Tuberculosis and HIV, Fig. 6 Representative images of the clinical and radiological spectrum of HIV-associated tuberculosis in paediatric patients. Clockwise from top left: (a) Chest radiograph showing a complicated Ghon focus. This reflects poor disease containment at the point of entry – infants and severely immune compromised individuals are particularly vulnerable; (b) Lateral Chest radiograph showing hilar adenopathy. The dense circular ring around the hilum (so-called doughnut sign) is indicative of perihilar lymph

node involvement; (c) Chest radiograph showing caseating (expansile) pneumonia. Tuberculous lymph nodes cause airway narrowing (almost pathognomonic), since unlike other mass lesions it traps and compresses the airway. Lymph nodes can also “rupture” (with sinus formation) into the airway which may result in aspiration of caseous material and development of dense caseating pneumonia. Tuberculosis causes radiological appearances that are often more severe than the clinical signs and symptoms

T

Commonly used diagnostic tests are discussed below and their key performance characteristics are summarized in Table 1.

Growth-Based Detection

The reference standard for diagnosing tuberculosis is a positive culture of *M. tuberculosis* on a clinical specimen. Culture remains the most

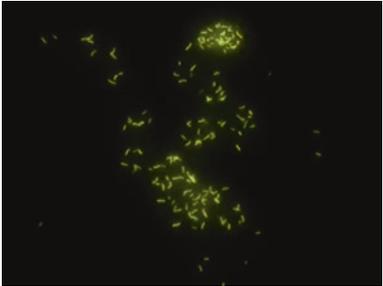
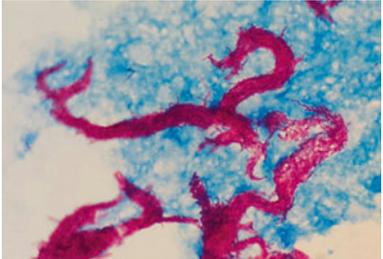
sensitive method for diagnosis (with an ability to detect 10 bacteria per mL of sputum) and allows for subsequent speciation, drug susceptibility testing, and monitoring of microbiological response to therapy. Automated liquid culture systems such as the mycobacterial growth indicator tube (MGIT; Becton Dickinson Diagnostic Instruments Systems) have largely replaced

Tuberculosis and HIV, Table 1 Key performance characteristics of diagnostic tests for tuberculosis when performed in HIV-infected patients

	Limit of detection (bacilli per ml of sputum)	Sensitivity (on sputum unless other specified)	Comments
<p>Solid culture</p>  <p><i>M. tuberculosis</i> colonies on Löwenstein-Jensen (LJ) medium</p>	10–100	Reference test	Reference test Can be used for speciation and drug susceptibility testing Used to monitor response to therapy 2–6 weeks for result (median time to detection ~ 24 days)
<p>Liquid culture</p>  <p>BACTEC MGIT system [Becton Dickinson, Sparks, MD]</p>	10–100	Slightly higher sensitivity than solid culture media	Reference test Automated system More rapid results than solid culture media Higher contamination rates Can be used for speciation and drug susceptibility testing Used to monitor response to therapy 1–6 weeks for result (median time to detection ~15 days)
<p>Smear microscopy</p>  <p>Acid-fast bacilli (ZN stain) seen on a direct clinical specimen</p>	≥10,000	30–40% Higher sensitivity with fluorescence microscopy Yield can be increased by collecting two early morning specimens	Most widely used test in developing countries Cannot distinguish nontuberculous mycobacteria from <i>M. tuberculosis</i> Identifies the most infectious patients Used to monitor response to therapy

(continued)

Tuberculosis and HIV, Table 1 (continued)

	Limit of detection (bacilli per ml of sputum)	Sensitivity (on sputum unless other specified)	Comments
 <p data-bbox="141 620 524 672">Fluorescence microscopy (auramine stain) on a direct clinical specimen</p>  <p data-bbox="141 977 524 1029">Cording of acid-fast bacilli (ZN stain) on light microscopy taken from MGIT culture</p>			
<p data-bbox="141 1058 314 1087">Xpert[®] MTB/RIF</p> 	<p data-bbox="553 1058 623 1087">10–100</p>	<p data-bbox="755 1058 919 1248">Single specimen: Overall 79–84% Smear-negative disease 68% (incremental yield from 2 to 3 specimens)</p>	<p data-bbox="977 1058 1184 1329">Rapid turnaround time Detects rifampicin resistance Cannot be used to monitor response to therapy Positive results are not reliable after a recent episode of tuberculosis treatment</p>

(continued)

Tuberculosis and HIV, Table 1 (continued)

	Limit of detection (bacilli per ml of sputum)	Sensitivity (on sputum unless other specified)	Comments
			
<p>Determine[®] TB-LAM Ag</p> 	<p>Detects <i>M. tuberculosis</i> LAM antigen in unprocessed urine</p>	<p>Active case finding in ART clinic 67% (at CD4 <50) HIV-infected inpatients 40–70% (depending on CD4 count)</p>	<p>High specificity Point of care test, yields results in ≤25 min Best performance in CD4 counts <50 Very poor sensitivity with CD4 >150</p>

Images courtesy of Chad Center, National Health Laboratory Services, Groote Schuur Hospital

conventional solid media culture because of simpler laboratory workflows, higher sensitivity, and earlier detection times. However, the median time to detection still ranges between 11 and 15 days, and therefore cultures cannot be used to guide initial treatment decisions in HIV-infected patients with severe tuberculosis. A drawback of liquid culture systems is the high rate of contamination, causing 5–10% of tests to fail.

The yield of culture in extrapulmonary specimens varies depending on the clinical site and degree of immunosuppression. The sensitivity when performed on CSF and pleural fluid is relatively low compared with sputum. Growth of *M. tuberculosis* from a clinical site represents viable

replicating bacilli and should be interpreted as indicating active disease and a need for anti-tuberculosis therapy. However, there are a substantial proportion of patients with so-called culture-negative tuberculosis who will respond to treatment; these patients should be evaluated according to the WHO 2007 algorithm (WHO 2007).

Smear Microscopy

Sputum smear microscopy, traditionally performed with Ziehl-Neelsen (ZN) staining, is a rapid, specific method of detecting infectious patients with tuberculosis and for monitoring response to therapy. Microscopy requires at least

10,000 organisms per mL of sputum, and therefore will detect fewer cases in HIV-infected patients who frequently have sputum paucibacillary or extrapulmonary disease (Reid and Shah 2009). In some settings, over two-thirds of HIV-infected patients with active tuberculosis requiring therapy may be missed by sputum smear microscopy. Although sputum induction with nebulized hypertonic saline potentially identifies approximately 25% additional cases in symptomatic patients who are smear-negative or unable to spontaneously produce sputum, a negative smear never excludes the diagnosis of tuberculosis in HIV-coinfected patients. Smear microscopy has a poor yield when performed on CSF and pleural fluid but is a useful investigation for needle biopsy material from lymph nodes.

Molecular Methods

With the limitations of delayed time to detection for culture and poor sensitivity of smear, more rapid and reliable diagnostics are needed. Xpert[®] MTB/Rif (Cepheid, Sunnyvale, CA, USA) is a closed real time polymerase chain reaction (PCR) assay that amplifies an 81 base-pair region of the *M. tuberculosis* RNA polymerase β subunit (*rpoB*) gene. This target is specific for *M. tuberculosis* complex and is the site of mutations that underlie rifampicin resistance in the majority of MDR tuberculosis strains, therefore allowing rapid detection of rifampicin resistant tuberculosis directly from clinical specimens. The cartridge-based system minimizes contamination, requires minimal technical skills, and, importantly, is able to produce a result within 2 h.

Compared with culture as the reference test for confirmed tuberculosis, the overall sensitivity of Xpert is 88%, and slightly lower in HIV at 79%. As an add-on test after a negative smear microscopy result, the sensitivity drops to 68% (Steingart et al. 2014). Therefore, a third of smear-negative culture-confirmed tuberculosis cases may still be missed by Xpert testing. The diagnostic yield of sputum Xpert has been shown to be poor in hospitalized acutely ill HIV-infected inpatients, identifying only 28% of confirmed tuberculosis cases in one study from a high-burden setting. The major reason for this is such

Tuberculosis and HIV, Table 2 Performance of Xpert for the diagnosis of extrapulmonary tuberculosis on selected specimens (compared against culture) (WHO 2013)

	Lymph node	CSF	Pleural fluid
Sensitivity	84.9 %	79.5% relative to culture-positive TBM (50% relative to clinical reference standard)	43.7%
Specificity	92.5%	98.6%	98.1%

patients have difficulty producing a sputum specimen for testing.

Because PCR is able to detect DNA from both live and dead organisms, molecular-based diagnostics may test positive despite being culture-negative. There is evidence that around a quarter of patients successfully treated will remain Xpert positive at 6 months. Thus, Xpert should not be used to monitor response to therapy and cannot be used alone to confirm a recurrent episode of tuberculosis after a recent treatment episode (the precise duration has not been defined but we suggest confirming with culture if treated for tuberculosis within the last year).

Xpert is being increasingly used for the diagnosis of tuberculosis from extrapulmonary specimens. It performs especially well on lymph node aspirates and other tissue samples and is a useful rule-in test for TBM using CSF; the WHO recommends its use on these specimens as a replacement for smear microscopy in the appropriate clinical setting (WHO 2013). Clinicians should, however, be aware that Xpert still lacks sensitivity to exclude TBM in patients in whom the diagnosis is clinically suspected, and empiric therapy should not be withheld because of a negative CSF result. Xpert performs poorly on blood and pleural fluid and is not recommended for use on these specimens (Table 2). An additional limitation of Xpert is that it is unable to differentiate *M bovis* and *M bovis*-associated Bacille Calmette-Guérin (BCG) disease from tuberculosis. This is especially relevant in children with BCG-induced axillary adenitis, which is often misdiagnosed as tuberculosis (Hesseling et al. 2006). There is emerging

evidence that the assay has good performance characteristics on urine when used in patients with low CD4 counts and disseminated tuberculosis.

Other molecular diagnostic tests are available, including line probe assays (LPA) that use reverse hybridization technology to speciate and detect drug resistance mutations in mycobacteria. Unlike Xpert, LPA assays are prone to contamination and perform unreliably on direct clinical specimens. The value of LPA is its ability to detect resistance to other antituberculosis drugs in addition to rifampicin as well as in detecting dual strain infection. Its use on microscopy-positive specimens or cultured isolates has reduced time to initiation of drug-resistant tuberculosis therapy.

Urinary Lipoarabinomannan (LAM)

Detection of LAM in the urine represents renal tract involvement with tuberculosis and is therefore found more commonly in patients with more severe immune compromise and disseminated disease (Lawn 2012). The test is available as an immunochromatographic lateral flow assay (Determine[®] TB-LAM Ag [urine LAM], Alere, Waltham, MA, USA) that can be used at point of care by adding unprocessed urine onto the test strip.

When used for screening outpatients entering an ART program in a high-burden setting, the overall sensitivity of the urine LAM for diagnosing culture-confirmed tuberculosis is less than 30%, but it detects up to two-thirds of cases in the subgroup with CD4 counts <50 cells/ μ L. The specificity is excellent, exceeding 98%, and the positive predictive value is over 90% in patients with advanced HIV.

Urine LAM can provide a rapid diagnosis of tuberculosis in hospitalized patients. In unselected HIV-infected medical admissions with a high prevalence of extrapulmonary tuberculosis, urine LAM detects 40% of confirmed cases. When combined with urine Xpert, testing the overall sensitivity may rise to 70%, and for those with CD4 counts <100 cells/ μ L, the combined urine tests have the ability to diagnose tuberculosis in 85% of cases. In HIV-infected inpatients with suspected tuberculosis in another high-burden setting, urine LAM detected two thirds of confirmed

cases overall and half of cases with negative sputum smear microscopy. Unfortunately yields in children have been disappointing.

Histopathology

Pathological examination of biopsied tissue is frequently performed in addition to microbiological and molecular testing. This often provides important supportive diagnostic information and can be used as a basis for empiric antituberculosis therapy while awaiting culture results or if culture is not possible. The characteristic pathological lesion of tuberculosis infection is the granuloma, but these may be poorly formed, lacking the caseating centre, in HIV-infected patients with advanced immunosuppression. Other conditions associated with HIV, such as deep fungal infection and lymphoma, may also lead to granuloma formation, limiting the specificity of the finding. ZN (or equivalent) stains can be performed in tissue samples but do not distinguish tuberculosis from infection with nontuberculous mycobacteria. However, the finding of granulomas and acid fast bacilli within tissue macrophages is strongly supportive of tuberculosis in high-burden settings.

Management

Antituberculosis Therapy

The antimicrobial therapy for HIV-associated drug sensitive tuberculosis is the same as in HIV-uninfected patients. WHO recommends a standard regimen of 2 months of rifampicin, isoniazid, ethambutol, and pyrazinamide followed by 4 months of rifampicin and isoniazid, preferably in fixed dose combination tablets and dosed according to weight. Therapy should be administered 7 days a week throughout the course. Generally the duration of therapy for extrapulmonary tuberculosis is the same as for pulmonary tuberculosis but is extended for neurological and bone involvement. The treatment of MDR tuberculosis is more toxic and, because no drug in the standard WHO second line regimen is as effective in terms of sterilizing activity as the rifamycins, it requires an extended course. At least 2 new bactericidal agents (preferably a fluoroquinolone plus an

injectable) should be included in a regimen for any isolate with rifampicin resistance, together with 2–3 other antituberculosis drugs to which the isolate is likely to be susceptible. Treatment should be continued for at least 18 months following conversion of sputum cultures to negative. Constructing treatment regimens for pre-XDR and XDR-TB is complex, particularly in HIV-infected patients requiring ART, and should be done in consultation with an expert.

In general, TB treatment recommendations in HIV-infected children are similar to HIV-uninfected children, but given an increased risk of disease relapse in children with significant immune compromise, treatment for drug-sensitive TB may be prolonged from 6 to 9 months (Siberry et al. 2013).

Adjunctive Therapy

Unless contraindicated because of previous intolerance, all HIV-infected patients with tuberculosis should receive co-trimoxazole preventive therapy and pyridoxine. Co-trimoxazole prophylaxis prevents bacterial infections, diarrhoea, malaria, and pneumocystis pneumonia and has been shown to reduce mortality by over 40% in patients with HIV-associated tuberculosis. In developed countries co-trimoxazole can be safely stopped when the CD4 count is above 200 cells/ μ L, but long term provision is recommended in sub-Saharan Africa because of ongoing protection against malaria and bacterial infection (Church et al. 2015). The recommendation for pyridoxine supplementation is based on the observation that sensory polyneuropathy is more common in patients with HIV and tuberculosis, possibly because of vitamin B6 deficiency caused by isoniazid therapy.

Antiretroviral Therapy

Observational studies demonstrated a reduction in mortality risk of over 65% in patients with HIV-associated tuberculosis who are receiving concurrent ART, and a randomized controlled trial performed in South Africa showed improved survival for patients with CD4 counts \leq 500 cells/ μ L who started ART during antituberculosis therapy (Abdool Karim et al. 2010). WHO

recommends that all HIV-infected patients with active tuberculosis be initiated on ART regardless of CD4 count.

ART initiation in patients with tuberculosis is, however, accompanied by potential complications, including the risks of IRIS and overlapping drug toxicities, which are more likely to occur if ART is started early after the initiation of antituberculosis therapy. Clinical trials have demonstrated a survival benefit for patients with CD4 counts $<$ 50 cells/ μ L starting ART within 2 weeks of antituberculosis therapy compared to deferring to around 8 weeks; this should be done in all patients with CD4 count $<$ 50 cells/ μ L unless there is TBM or concomitant cryptococcal meningitis. In contrast, those with higher CD4 counts do not have an increased risk of death or AIDS-defining events if ART is delayed up to 8 weeks. This provides an important opportunity to establish patients on antituberculosis therapy and a window to offer adequate counseling for lifelong ART. Those with CD4 counts $>$ 220 cells/ μ L can safely delay ART until after completing the full course of antituberculosis therapy, but this may not be advisable on a programmatic level because of the potential for loss to follow up with such delays. Unless there is another compelling indication to initiate ART urgently, such as an additional AIDS-defining disease, patients with CD4 counts $>$ 50 cells/ μ L should therefore have linkage to ART services and start ART within 8 weeks of antituberculosis treatment. In TBM, although a clinical trial showed no mortality difference between early versus deferred ART, it has been recommended that ART be delayed for 4–8 weeks regardless of CD4 count to reduce risk of neurological tuberculosis-IRIS.

The WHO-recommended ART regimen for patients with tuberculosis is tenofovir, lamivudine/emtricitabine, and efavirenz. These drugs are available in fixed-dose combination tablets and do not require dose adjustment when given with antituberculosis therapy. Despite an FDA recommendation to use higher doses of efavirenz during treatment with rifampicin, there is no evidence that efavirenz concentrations are lower or virological outcomes worse in patients being treated for tuberculosis and with standard

efavirenz doses, and the practice of increasing efavirenz to 800 mg daily is not advised.

Nevirapine based ART is inferior to efavirenz based regimens in patients with tuberculosis, with fewer patients achieving HIV-viral suppression. Nevirapine results in higher rates of cutaneous drug reactions and liver injury and undergoes significant enzyme induction by rifampicin, with the potential for subtherapeutic concentrations. If nevirapine is prescribed for patients on rifamycin-containing antituberculosis therapy, the starting dose should be 200 mg twice daily (i.e., the lead-in dose of 200 mg daily is omitted).

Drug Toxicity and Drug-drug Interactions

There are multiple potential drug-drug interactions and shared toxicities between ART and anti-tuberculosis therapy, especially when treating drug-resistant tuberculosis or using second-line ART. Tables 3 and 4 list the most clinically significant drug-drug interactions and toxicities of commonly used agents with advice on coadministration. Significant drug-drug interactions between rifampicin and protease inhibitors used as first-line treatment in young children is problematic, with a need to “superboost” standard lopinavir/ritonavir combinations by adding additional ritonavir to overcome the inducing effect of rifampicin.

Cutaneous drug reactions (CDRs) are common in HIV-associated tuberculosis and have a wide spectrum of clinical presentations. These range from mild morbiliform rashes to Steven-Johnson syndrome and toxic epidermal necrolysis or systemic involvement with eosinophilia and hepatitis. Mild rash can be treated symptomatically with continuation of all drugs and close clinical observation. Patients with severe CDRs need to immediately stop all potentially offending agents and be admitted to hospital. Co-trimoxazole is an important cause of adverse drug reactions in HIV and should be stopped in all cases of suspected CDR. In general, non-nucleotide reverse transcriptase inhibitors (NNRTIs) should be avoided once implicated in a severe CDR, but it is often possible to successfully rechallenge antituberculosis drugs. Expert advice should be sought in all cases.

Derangement of liver functions occurs in up to 30% of patients receiving antituberculosis therapy or first-line ART, but only the minority represents significant drug-induced liver injury (DILI). The clinical spectrum ranges from mild transient asymptomatic transaminitis (termed “hepatic adaptation”) to severe hepatitis and liver failure. Mild asymptomatic liver enzyme elevations are benign and do not warrant a switch in therapy. Examples include mild derangement of canalicular liver enzymes in HIV-infected patients that is usually multifactorial and without clinical significance, and isolated hyperbilirubinaemia in patients on rifampicin or atazanavir. It is important to exclude other potential causes of liver injury such as other hepatotoxic agents (e.g., co-trimoxazole and fluconazole), hepatitis viruses, and toxins. Table 5 lists common indications for changing regimens in CDRs and DILI.

Immune Reconstitution Inflammatory Syndrome

IRIS results from rapid restoration of pathogen-specific immunity after the initiation of ART. The resulting inflammatory reaction leads to either a clinical deterioration of tuberculosis for which the patient is on treatment (paradoxical TB-IRIS) or the atypical inflammatory presentation of active tuberculosis during early ART where the diagnosis was missed prior to ART (unmasking TB-IRIS) (Meintjes et al. 2008).

Paradoxical TB-IRIS is common, occurring in up to 54% of patients starting ART while on antituberculosis treatment. Risk factors include lower baseline CD4 count, shorter interval to starting ART, disseminated tuberculosis or extrapulmonary tuberculosis, and a rapid rise in CD4 count. Symptoms usually occur in the first few weeks (median 10–14 days) and up to 3 months after ART initiation. Clinical manifestations may include new constitutional, respiratory, or abdominal symptoms or new or worsening focal lesions such as enlarging lymph nodes, tuberculous abscesses, or pleural effusion. There may also be worsening radiological features of tuberculosis such as a new chest X-ray infiltrate or enlarging

Tuberculosis and HIV, Table 3 Common drug interactions between antituberculosis therapy and ART in adults^a

Anti-tuberculosis therapy	Antiretroviral	Interaction	Comments
Rifampicin-based	Efavirenz	Efavirenz concentrations not significantly reduced	No dose adjustment required
	Nevirapine	Reduced concentrations of nevirapine	Preferably use efavirenz Omit 200 mg daily lead-in dose and start with 200 mg BD
	Rilpivirine	Reduced concentrations of rilpivirine	Do not coadminister
	Etravirine	Reduced concentrations of etravirine	Do not coadminister
	Lopinavir/ ritonavir	Markedly reduced concentrations of lopinavir	Requires double dose with 4 tablets (800/200 mg) BD in adults Increase the dose gradually: 3 tabs BD for a week then 4 tabs BD for duration of rifampicin use Reduce dose back to 2 tabs BD (400/100 mg) 1–2 weeks after rifampicin stopped
	Atazanavir/ ritonavir	Markedly reduced concentrations of atazanavir	Do not coadminister
	Darunavir/ritonavir	Markedly reduced concentrations of darunavir	Do not coadminister
	Raltegravir	Reduced concentrations of raltegravir	A recent clinical trial suggests that dose increase is not required
	Dolutegravir	Reduced concentrations of dolutegravir	Requires increased frequency of dolutegravir dosing with 50 mg BD
Rifabutin-based	Efavirenz	Reduced concentration of rifabutin	Requires increased dose of rifabutin 450 mg daily
	Nevirapine	Non-significant increase in rifabutin	No dose adjustment required
	Rilpivirine	Reduced concentrations of rilpivirine	Avoid if possible, otherwise requires increased dose of rilpivirine 50 mg daily
	Etravirine	Reduced concentrations of etravirine	Do not coadminister if used together with a boosted protease inhibitor
	All ritonavir-boosted protease inhibitors	Increased concentrations of rifabutin	Requires reduced dose of rifabutin 150 mg alternate days or 150 mg daily (guidelines vary) Standard doses of ritonavir-boosted lopinavir, atazanavir, and darunavir
	Raltegravir	No significant interactions	No dose adjustment required
	Dolutegravir	No significant interactions	No dose adjustment required

^aGuidelines for dosing and coadministration of rifamycins and ART in children are different. Expert consultation should be sought

tuberculoma on brain imaging. IRIS is a diagnosis of exclusion, and other potential causes of clinical deterioration in patients with tuberculosis need to be investigated, such as poor treatment adherence, poor drug absorption, drug toxicity, a new

opportunistic infection, and especially drug-resistant tuberculosis.

Non-neurological TB-IRIS is usually a self-limiting condition with a low mortality but is associated with significant morbidity and

Tuberculosis and HIV, Table 4 Important shared toxicities of ART and antituberculosis treatment and other drugs used in HIV-infected patients

Adverse effect	Antituberculosis drug	Antiretroviral	Other
Gastrointestinal disturbance	All antituberculosis drugs especially ethionamide and PAS	AZT, PIs, ddi	Macrolides
Liver injury	Rif, INH, PZA, quinolones, ethionamide, PAS	NNRTIs, PIs	Co-trimoxazole, azole antifungals, anticonvulsants
Nephrotoxicity	Injectables, Rif	TDF ^a	Amphotericin B
Drug rash	All antituberculosis drugs	NNRTIs (NVP > EFV), DRV, RAL	Co-trimoxazole
Peripheral neuropathy	INH, terizidone ^b , ethionamide, linezolid	d4T, ddi	
Neuropsychiatric	INH (especially high dose), terizidone, ethionamide	EFV	

AZT zidovudine, d4T stavudine, ddi didanosine, EFV efavirenz, TDF tenofovir, PIs protease inhibitors, DRV darunavir, RAL raltegravir, NNRTIs non-nucleoside reverse transcriptase inhibitors, Rif rifampicin, INH isoniazid, PZA pyrazinamide, PAS para-amino salicylic acid

^aAvoid TDF if using an injectable agent for a prolonged period

^bTerizidone side effects also apply to cycloserine

Tuberculosis and HIV, Table 5 Indications for stopping suspected culprit drugs in cutaneous drug reactions and drug-induced liver injury

CDR	DILI
Systemic symptoms	Symptomatic ^a with ALT > 120 IU/l
Mucosal involvement	Asymptomatic with ALT > 200 IU/l
Severe skin involvement	Any transaminitis with bilirubin > 40 µmol/l
Deranged liver enzymes	Prolonged INR or encephalopathy

ALT alanine transaminase, CDR cutaneous drug reaction, DILI drug induced liver injury

^aNausea, vomiting, abdominal pain, jaundice

frequently results in hospital admission (25% of cases). In children who received a BCG vaccination at birth, axillary BCG adenitis (ipsilateral to the site of vaccination) is common following ART initiation in infancy but rarely requires medical intervention. Patients with TB-IRIS should almost always be continued on ART. Those with paradoxical TB-IRIS should be continued on anti-tuberculosis therapy and those with unmasking tuberculosis should be initiated on anti-tuberculosis treatment. The addition of corticosteroids is strongly recommended for patients with neurological involvement and for any life-threatening manifestation (e.g., massive

mediastinal adenopathy causing airway compression or rapidly enlarging pericardial effusion (together with pericardiocentesis)). Also, corticosteroids (starting at a dose of 1.5 mg/kg/day of prednisone) were shown in a clinical trial to provide more rapid symptomatic relief and reduce hospitalization in patients with IRIS that was not immediately life-threatening (Meintjes et al. 2010). Corticosteroids should only be prescribed when the diagnosis of paradoxical TB-IRIS is certain. Patients with mild symptoms can be offered symptomatic treatment alone. Therapeutic needle aspiration of fluctuant lymph nodes, symptomatic abscesses, and rapidly enlarging effusions is an important component of the management of IRIS. Guidelines for corticosteroid prescription in HIV-associated tuberculosis, including paradoxical TB-IRIS are listed in Table 6.

Prevention

Although ART has a major impact on reducing incidence of TB (~65% risk reduction), it is often initiated at low CD4 counts in high tuberculosis-burden areas and does not mitigate the heightened risk of tuberculosis associated with HIV completely. Reactivation of LTBI and transmission of active tuberculosis will therefore

Tuberculosis and HIV, Table 6 Use of corticosteroids in HIV-associated TB

Indication	Drug and dose
TBM or tuberculoma	Dexamethasone (or equivalent steroid) 0.4 mg/kg/day, reduce by 0.1 mg/kg every week until week 4 Thereafter 4 mg daily, tapering by 1 mg per week (total duration 6–8 weeks)
Paradoxical TB-IRIS with significant symptoms	Prednisone 1.5 mg/kg/day for 2 weeks Then 0.75 mg/kg/day for 2 weeks Some patients relapse on stopping steroids after 4 weeks and require steroids for longer duration and in such cases steroids should be restarted at 1.5 mg/kg/day and then weaned according to symptom control
Pericardial effusion	Corticosteroids not indicated

persist at high levels in areas of high HIV and tuberculosis prevalence despite expanding ART programs unless additional public health interventions are implemented. As a response to this, WHO launched the “3I’s” policy, a package of interventions for tuberculosis control that in addition to earlier ART initiation (regardless of CD4 count) includes intensive case finding, infection control, and isoniazid preventive therapy (IPT).

Universal early ART is standard of care in children with HIV infection, irrespective of CD4 count or clinical staging, and offers good protection against tuberculosis disease (Violari et al. 2008). However, as in adults, tuberculosis vulnerability remains elevated and WHO guidelines advise that all HIV-infected children in close contact with an infectious tuberculosis case, irrespective of age, should receive a course of preventive chemotherapy once active tuberculosis has been excluded; using a symptom-based approach (WHO 2014b). Reexposure to an infectious case justifies repeat preventive therapy, since past therapy provides no protection against reinfection. In sub-Saharan Africa, many women in their reproductive years are dually infected with tuberculosis and HIV. Active tuberculosis case finding among HIV-infected pregnant women

presents an important opportunity to decrease the perinatal risks for mother and baby. If a baby is born to a HIV-infected mother with active tuberculosis, it is essential to ensure that the mother is on antituberculosis and HIV treatment, while the baby is assessed for tuberculosis, HIV, and other congenital infections. In the absence of tuberculosis disease, the baby should receive preventive therapy if the mother remains symptomatic or has been on antituberculosis treatment for less than 2 months.

IPT is also a safe and effective measure to reduce tuberculosis in HIV-infected adults and has an additive effect when given together with ART. Studies in the pre-ART era demonstrated that IPT reduced the incidence of tuberculosis by 33% overall, with greater benefit (60%) and a reduction in mortality in those that are TST positive (Akolo et al. 2010). In Botswana, a recent randomised controlled trial showed a 74% reduced risk of tuberculosis in TST positive HIV-infected adults who received 36 months versus 6 months of IPT (Samandari et al. 2011). Clinical trial data from South Africa also suggest that long term (up to 6 years) IPT is without major complications and effective in ART-naïve patients with positive tuberculin skin tests (Martinson et al. 2011).

An ideal setting to upscale IPT is in ART clinics, where patients are already established in care and are more likely to adhere to IPT. In a clinical trial conducted in a high-burden area, 12 months of IPT reduced the incidence of tuberculosis by almost 40% when given together with ART. Importantly, benefit was also seen in the subgroup of TST-negative patients, thus removing the requirement for TST testing prior to IPT in this setting (Rangaka et al. 2014). Together, these findings have led to the WHO recommendation to provide IPT to all HIV-infected people in whom active tuberculosis is not suspected.

The two main exclusion criteria for IPT are medical contraindications to isoniazid (such as hypersensitivity to the drug, peripheral neuropathy, severe liver disease, or alcohol abuse) and evidence of active tuberculosis disease. Although the rates of isoniazid monoresistance in people who develop tuberculosis while on IPT are similar to controls,

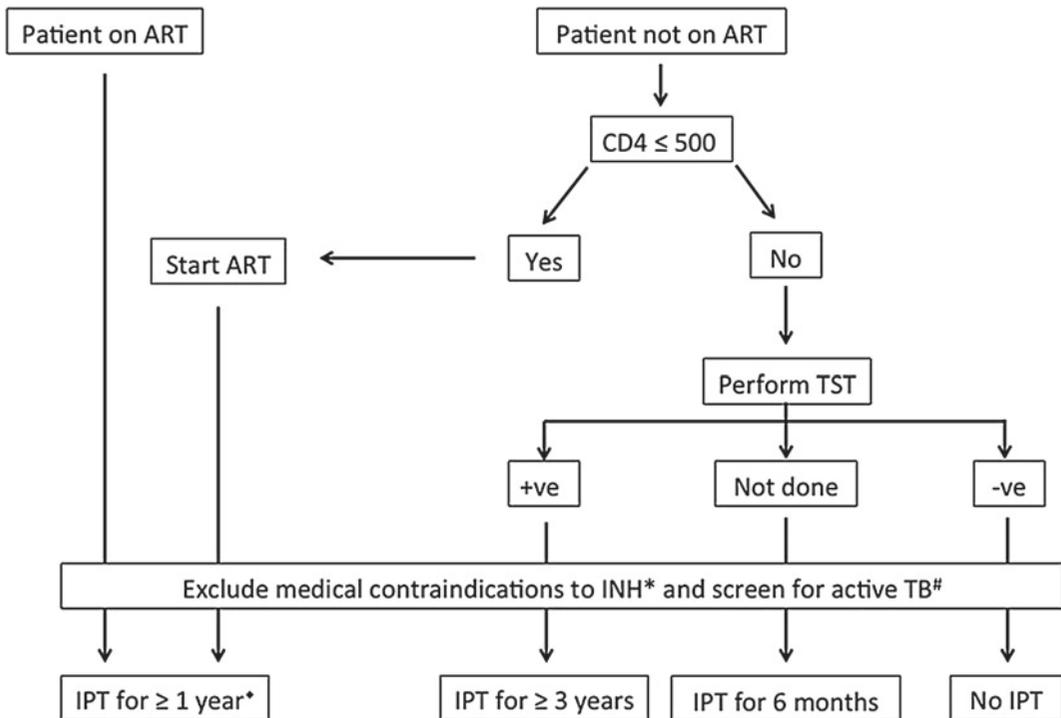


Figure based on South African National Department of Health Guidelines and evidence from clinical trials

INH isoniazid, TST tuberculin skin test

*Hypersensitivity or intolerance to isoniazid, peripheral neuropathy, pre-existing liver disease or alcohol abuse

#WHO symptom screen: any cough, fever, night sweats or weight loss. If any symptom present defer IPT until confirmed culture-negative and symptoms have resolved

*If TST -ve or not done continue for 1 year; if TST+ve continue for ≥ 3 years

Tuberculosis and HIV, Fig. 7 Approach to prescribing IPT

the potential for this complication is greater if patients with active tuberculosis are inadvertently placed on isoniazid monotherapy. WHO recommends using a 4-symptom screen for active case finding prior to initiating IPT, and any adult patient with current cough, fever, night sweats, or weight loss should not receive IPT until active tuberculosis is ruled out by a negative sputum culture and the symptoms have resolved. An approach to IPT in adults that synthesizes the evidence from different studies is presented in Fig. 7.

Despite the strong evidence base for efficacy and safety, large scale uptake of IPT amongst HIV-infected adults has been slow. The reasons

for this are complex but include toxicity associated with isoniazid, the long duration of therapy, and adherence concerns. Clinical trials have shown equivalent effectiveness, reduced toxicity, and higher completion rates with 3 months of isoniazid combined with a rifamycin compared to the longer courses of isoniazid monotherapy. However, WHO has not endorsed any other regimen besides isoniazid monotherapy for the prevention of tuberculosis, and further study is needed to evaluate the durability of short-course combination preventative therapy, particularly in HIV-infected patients in high tuberculosis-burden settings.

New Developments and Research Priorities

The collision of the HIV and tuberculosis epidemics has created a resurgence of tuberculosis as a global health crisis, and improved control of tuberculosis is an international public health priority. Global funding for tuberculosis research, however, needs to increase substantially if game-changing breakthroughs in tuberculosis diagnosis, treatment, and prevention are to be made. Although provision of ART and IPT will likely reduce tuberculosis incidence in high-HIV prevalence areas, this protection is incomplete and will not lead to achievement of the Stop TB Partnership and WHO goal of a tuberculosis-free world by the year 2050 without the discovery of novel preventive strategies.

Diagnosis. Early detection and treatment are important aspects of tuberculosis infection control and could help in reducing transmission in the community. Although Xpert has been a major advance in early tuberculosis diagnostics, it still lacks sensitivity compared with culture, especially in HIV-infected patients, and has not been shown to reduce mortality when used in clinics. The WHO symptom screen has a lower sensitivity when used for active case finding in HIV-infected patients on ART, and a better tool is needed to facilitate the scale-up of IPT in this high-risk population. Thus, the development of rapid low-cost point of care tests with a similar sensitivity to culture is a key research priority.

Treatment. A key obstacle to tuberculosis control is the reliance on prolonged and poorly tolerated treatment regimens, particularly for drug-resistant tuberculosis. This leads to suboptimal levels of adherence (which promotes further drug resistance) and treatment failures favoring ongoing transmission. Recent trials of treatment shortening regimens (to 4 months) for drug-sensitive tuberculosis have been disappointing. However, the drug development pipeline has yielded a number of new agents, some with novel mechanisms of action against *M. tuberculosis* and with promising results in clinical trials.

Bedaquiline is a diarylquinoline drug that inhibits mycobacterial ATP synthase and

demonstrates potent bactericidal activity against replicating and nonreplicating bacilli. In phase II clinical trials and in a larger open label study of patients with MDR-tuberculosis, including a number with HIV coinfection, the addition of bedaquiline to MDR regimens led to higher rates of culture conversion in a significantly shorter time. An important consideration with bedaquiline is that it prolongs the QT interval, potentially predisposing to tachyarrhythmias and requiring ECG monitoring. Bedaquiline cannot be coadministered with efavirenz due to its inducing effect on efavirenz metabolism; concomitant use with protease inhibitors increases bedaquiline concentrations two- to threefold, raising concerns about toxicity. Further study is needed to confirm the efficacy and assess safety of this promising new drug in HIV-coinfecting patients but results from patients in bedaquiline access programs are encouraging.

Other agents that have shown promise in clinical trials include linezolid, an oxazolidinone antibiotic that has been repurposed as an anti-tuberculosis agent; delamanid, a nitroimidazooxazole derivative that inhibits mycolic acid synthesis and demonstrates similar early bactericidal activity (EBA) to rifampicin; and pretomanid (PA824), a nitroimidazole that kills rapidly dividing and dormant bacilli with EBA comparable to isoniazid, suggesting a potential use in treatment shortening.

Prevention. The ultimate long-term intervention for the control of epidemic infectious diseases is effective vaccination. WHO recommends universal neonatal BCG vaccination in areas with moderate to high prevalence of tuberculosis. The efficacy rate has a wide range, from 0% to 80% in clinical trials; this variable protection against pulmonary disease is influenced by age of administration, geographic location, and environmental exposure to nontuberculous mycobacteria. Vaccination is most effective in mycobacteria-naïve newborns and infants in whom it reduces the risk of tuberculosis by about 80%, particularly meningitis and disseminated disease, and also results in a mortality benefit. Besides this incomplete protection to *M. tuberculosis* infection, the main drawback of BCG is that protective immunity is

lost in early adulthood when risk of HIV acquisition and tuberculosis disease is highest. The vaccine also performs less well in infants in sub-Saharan Africa where more frequent environmental mycobacterial exposures may “mask” and/or “block” immune responses to *M. bovis* antigens contained in the BCG vaccine. Furthermore, BCG vaccination is contraindicated in HIV-infected infants in whom it may cause disseminated BCG disease (Hesseling et al. 2006). WHO emphasizes that all pregnant women in tuberculosis- endemic settings should be tested for HIV infection and receive optimal care to prevent vertical HIV transmission. When a baby born to a mother with HIV-associated tuberculosis receives antituberculosis treatment or preventive therapy, a decision on BCG vaccination can be postponed until both the HIV and tuberculosis infection status is known.

These significant limitations have given impetus to the development of new vaccine strategies, but unfortunately, clinical trials of candidate vaccines have been disappointing to date. A recent phase 2 study of a promising Vaccinia Ankara virus-vectored vaccine termed MVA85A failed to demonstrate efficacy against tuberculosis disease or *M. tuberculosis* infection in HIV-uninfected infants primed with BCG in a high-burden setting, despite being immunogenic in animal studies and healthy adults. This vaccine also failed to show protection in HIV-infected adults. Understanding correlates of protection and identifying surrogate endpoints of protective immunity against active disease remain challenges in tuberculosis vaccine development and are important areas of ongoing research that could potentially accelerate vaccine development.

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Uncoating and Nuclear Entry

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Definition

Uncoating is defined as disassembly of the viral capsid and release of the viral ribonucleoprotein complex (vRNP), including the viral genome and its associated proteins, following fusion of HIV-1 with target cells. By contrast to most retroviruses, HIV-1 and other lentiviruses are capable of infecting nondividing cells. Entry into the nucleus of nondividing cells requires facilitated transport of the reverse transcribed viral genome across the nuclear membrane through nuclear pore complexes (NPC).

Introduction

Following budding from an infected cell, HIV-1 particles undergo a proteolytic maturation process that results in formation of the mature viral capsid, a distinct feature of the infectious virion. The viral capsid, consisting of a regular hexagonal array of capsid protein (CA) subunits in the shape of a cone, plays a critical role following entry into target cells. This overview describes the current

state of knowledge of the viral capsid and its functions in the early phase of HIV-1 infection, including the mechanism of uncoating and the interaction of CA with host factors that are necessary for nuclear entry and integration.

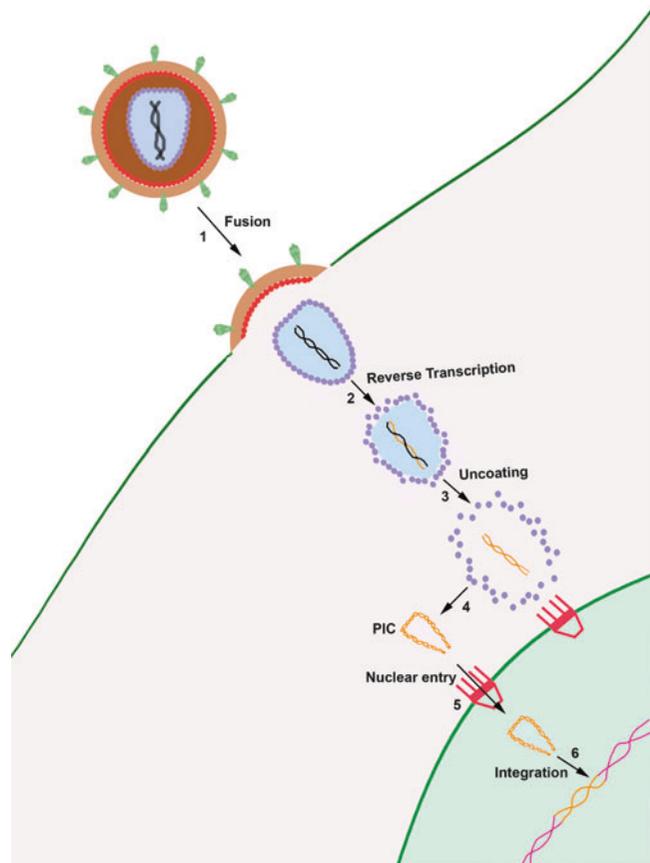
The HIV-1 life cycle can be divided into early and late phases. The early phase includes processes occurring before integration of viral DNA into the host genome. The late phase is comprised of post-integration processes including transcription, mRNA export, translation, particle assembly, budding from the host cell membrane, and maturation. The early phase (Fig. 1) begins with the fusion of the viral membrane with the cell membrane, enabled by binding of viral glycoproteins gp120 and gp41 to the host cell receptor CD4. Besides CD4, a coreceptor, CCR5 or CXCR4, is also required for fusion (► [HIV Life Cycle: Overview](#)). Upon fusion, the viral core is released into the cytoplasm where it undergoes reverse transcription and uncoating. Reverse transcription begins with the formation of a reverse-transcription complex (RTC) which subsequently converts into a high molecular weight DNA-protein complex termed the pre-integration complex (PIC), which is then transported into the nucleus where it integrates into the host chromosomal DNA.

The mature HIV-1 capsid is a conical structure comprised of ~1,500 CA subunits arranged as a fullerene cone (reviewed in Ganser-Pornillos et al. 2012; Fig. 2). The capsid is composed of ~250 CA hexamers and 12 pentamers, with 7

Uncoating and Nuclear Entry,

Fig. 1 Schematic of the early phase of HIV-1 infection.

(1) An HIV-1 particle fuses with the host cell membrane and releases its core into the cytoplasm. (2) While traversing the cytoplasm, the core undergoes reverse transcription and uncoating. (3) Uncoating occurs in the cytoplasm, but the temporal and spatial details of this step are not clear. (4) Upon completion of reverse transcription, the preintegration complex (PIC) is formed. (5) The PIC is imported to the nucleus through the nuclear pore complex. (6) In the nucleus, the viral DNA is integrated into the host chromosome.



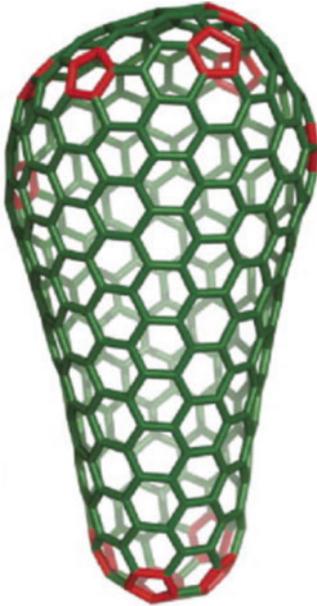
pentamers at the broad end and 5 at the narrow end. CA contains distinct amino-terminal and carboxy-terminal domains (NTD and CTD, respectively) joined by a flexible hinge. CA-NTD forms hexameric and pentameric rings that are connected by CTD-CTD intersubunit contacts.

The HIV-1 capsid is a metastable structure, and its main function is to properly deliver the viral genome and associated proteins into the cell. The architecture and stability of the capsid is important for HIV-1 infectivity: mutations in CA that alter the morphology of the capsid also compromise infectivity (reviewed in Ganser-Pornillos et al. 2012). Similarly, mutations in CA that alter the intrinsic stability of the capsid also impair viral infectivity (Forshey et al. 2002). While the structure of the HIV-1 capsid and its interactions with host proteins have received much attention in

recent years, the mechanism of uncoating, including temporal and spatial aspects, is poorly understood. Therefore, uncoating is a relatively new and active area of HIV-1 research.

Assays of HIV-1 Uncoating

Uncoating is challenging to study, owing to the difficulty of analyzing the process as it occurs in the target cell. Various approaches have been employed to study uncoating, including biochemical, imaging, and cell fractionation. One approach involved isolating reverse-transcription complexes (RTCs) from HIV-1 infected cells by sedimentation ultracentrifugation and probed for co-sedimented CA (reviewed in Arhel 2010). In another approach, CA was immunoprecipitated from HIV-1-infected cell extracts and assayed



Uncoating and Nuclear Entry, Fig. 2 Geometry of the HIV-1 capsid. The capsid is modeled as a cone consisting of a hexameric lattice with five pentamers at the narrow end and five at the broad end. The pentamers allow closure of the fullerene cone. The viral genome and associated proteins reside within the structure in mature HIV-1 particles (Reprinted from *Current Opinion in Structural Biology*, Vol. 18, B. K. Ganser-Pornillos, M. Yeager, and W. I. Sundquist, “The Structural Biology of HIV Assembly” pages 203–217 (2008), with permission from Elsevier)

for the levels of the associated viral genome by PCR. Only small quantities of CA were found associated with RTCs recovered from the cytoplasm of infected cells, which suggested that uncoating occurs within a few hours following cell entry.

A quantitative biochemical approach that has been employed involves isolation of purified cores from concentrated HIV-1 virions by detergent treatment of virions followed by sucrose gradient ultracentrifugation (Aiken 2009). Cores isolated from wild-type HIV-1 virions in this manner contain 10–15% of the CA present in the original virions. Incubation of these cores at 37 °C promotes their uncoating in a time-dependent manner. This cell-free assay permits analysis of the effects of CA mutations and

putative host factors on capsid stability. A variation of this approach allows study of uncoating in infected cells (Stremlau et al. 2006). This method, known as the “fate-of-capsid” assay, involves ultracentrifugation of infected cell lysates through a sucrose cushion and determination of the quantity of pelletable CA as a percentage of total CA within the cell. This assay is useful for studying the effects of host factors on uncoating as well as the uncoating of viral mutants in target cells.

Imaging-based assays to study uncoating in the cells have also been developed. A fluorescence microscopy-based assay that detects association of capsid with viral components following entry into cells has been described (Hulme et al. 2011). Results from this assay suggest that uncoating occurs relatively rapidly (within 1–2 h) after fusion. Similar to the fate-of-capsid assay, the imaging assay is also useful to study effects of CA mutations on uncoating in HIV-1-infected cells. A similar approach employs scanning electron microscopy in combination with immunolabeling of CA to visualize intracellular viral complexes (reviewed in Arhel 2010). In one study, intact cores were observed at the periphery of the nuclear membrane several hours after infection (Ref?), suggesting that uncoating occurs after docking to the nuclear pore, presumably following completion of reverse transcription.

Collectively, these assays have initiated an understanding of the process of uncoating and its importance in HIV-1 infection. However, there remains no clear consensus on the timing or location of uncoating within the cell. Biochemical assays are inherently limited due to the obvious lack of the complete repertoire of cellular components; by contrast, imaging-based assays must be interpreted carefully owing to the uncertainty of whether the virion being imaged represents an infectious event. The fate-of-capsid assay, which has proven useful for studying capsid-targeting inhibitors of infection including TRIM5 α , has yet to be fully validated as a bona fide uncoating assay and often exhibits a poor signal-to-noise ratio. Because each assay has its limitations, uncoating is currently best studied through a combination of approaches.

Uncoating and Reverse Transcription

CA mutations that alter intrinsic capsid stability and reduce viral infectivity frequently result in impaired reverse transcription in target cells (Forshey et al. 2002), suggesting a functional connection between reverse transcription and uncoating. A recent study has revealed a role for reverse transcription in promoting HIV-1 uncoating (Hulme et al. 2011). Using fluorescent-image-based and restriction-based assays, it was observed that pharmacological inhibition of reverse transcription slowed the rate of HIV-1 uncoating. Molecular events occurring in the core during the process of reverse transcription may apply pressure on the capsid and thereby promote uncoating. Interestingly, another study also demonstrated that reverse transcription is necessary for uncoating (reviewed in Arhel 2010). In HIV-1-infected cells treated with a reverse transcriptase inhibitor, imaging by scanning electron microscopy revealed intact HIV-1 capsids at the nuclear membrane and nuclear pore. Taken together, these studies provide compelling evidence that reverse transcription induces HIV-1 uncoating.

Modulation of Uncoating by Host Factors

The tripartite motif 5- α protein (TRIM5 α) is a cytoplasmic host factor that restricts retrovirus infection in a species-specific manner. TRIM5 α was originally discovered as a host factor that inhibits infection of old-world monkey cells by HIV-1 (Stremlau et al. 2006). TRIM5 α restricts a broad range of retroviruses, including HIV-1, HIV-2, simian immunodeficiency viruses (SIVs) from several primate species, N-tropic murine leukemia virus (N-MLV), and equine infectious anemia virus (EIAV) (reviewed in Luban 2007). Analysis of incoming HIV-1 particles by the fate-of-capsid assay has indicated that TRIM5 α results in accelerated uncoating (Stremlau et al. 2006). In addition, TRIM5 α also alters the normal passage of viral DNA to the nucleus. Human and new-world monkey TRIM5 α associate less

efficiently with the HIV-1 capsid and therefore do not restrict infection (Stremlau et al. 2006). In some monkey cells, TRIMCyp, a fusion protein derived from the TRIM5 α gene, also restricts HIV-1 by inducing premature uncoating of the capsid. These findings reinforce the conclusion that premature uncoating is detrimental to infection.

Cyclophilin A (CypA) is an abundant cytoplasmic host protein. It binds to the HIV-1 capsid and facilitates replication in a cell-type-dependent manner (reviewed in Luban 2007). Besides HIV-1, CypA interacts with a minority of lentiviral capsids, including SIVcpz and FIV. CypA appears to act at an early stage of HIV-1 infection before reverse transcription. Several lines of evidence suggest that CypA modulates HIV-1 uncoating. Depending on the target cell type, CypA may either stimulate or inhibit uncoating (Li et al. 2009). In addition, CypA also promotes restriction of HIV-1 by TRIM5 α in rhesus macaques and African green monkeys by an unknown mechanism (reviewed in Luban 2007).

Uncoating and Integration

In addition to reverse transcription, there is evidence for a link between uncoating and proviral integration. HIV-1 CA mutants T54A/N57A and Q63A/Q67A are impaired for infection yet are competent for reverse transcription and nuclear entry. In the case of Q63A/Q67A, PICs recovered from the cytoplasm contained more CA than the wild-type PICs and were impaired for integration *in vitro* (Dismuke and Aiken 2006). These mutants exhibit altered capsid stability *in vitro* and uncoating defects in target cells, suggesting that completion of uncoating is necessary for integration. The association of CA with PICs in the nucleus can also be inferred from artificial restriction factors generated by fusion of CypA with the mouse restriction factor Fv-1 or TRIM proteins. These engineered proteins recognize CA via CypA and restrict HIV-1 at a post-nuclear entry step. Moreover, the small-molecule Coumermycin-A1 inhibits HIV-1 infection at a step between nuclear

entry and integration, and mutation A105S in CA renders the virus resistant to the inhibitor (reviewed in Fassati 2012). Another mutation N74D in CA alters the integration pattern of HIV-1 to genomic regions sparse in transcription units, indicating that CA controls integration site preferences (Ocwieja et al. 2011). Collectively, these findings suggest that uncoating influences HIV-1 integration.

Small-Molecule Modulators of HIV-1 Uncoating

Highly active antiretroviral therapies (HAART) against HIV-1 have proven very effective in reducing viral loads and delaying progression to AIDS. However, emerging viral resistance against drugs even in the HAART era has pressed the need for novel classes of HIV-1 inhibitors. Since the viral CA protein plays important roles in both early and late stages of HIV-1 infection, it represents an attractive target for antiretroviral therapy.

As mentioned previously, HIV-1 uncoating is a finely tuned process, and the proper stability of the capsid during early post-entry stage is critical for efficient infection. PF-3450074 (PF74) is a member of a novel class of small-molecule compounds identified in a high-throughput screen for inhibitors of HIV-1 replication (Blair et al. 2010). Mechanistic studies of PF74 action demonstrated that the compound induces premature uncoating of the capsid prior to completion of reverse transcription. Serial passage of the virus in cells led to emergence of a resistant mutant with five point mutations in CA. The mutations inhibited binding of PF74 to HIV-1 particles. Interestingly, PF74 also affected a late stage of the virus life cycle, resulting in the production of particles with morphologically aberrant cores. The crystal structure of HIV-1 CA-NTD protein in complex with PF74 demonstrated that the compound binds a pocket on CA that had not been previously identified as a small-molecule binding site.

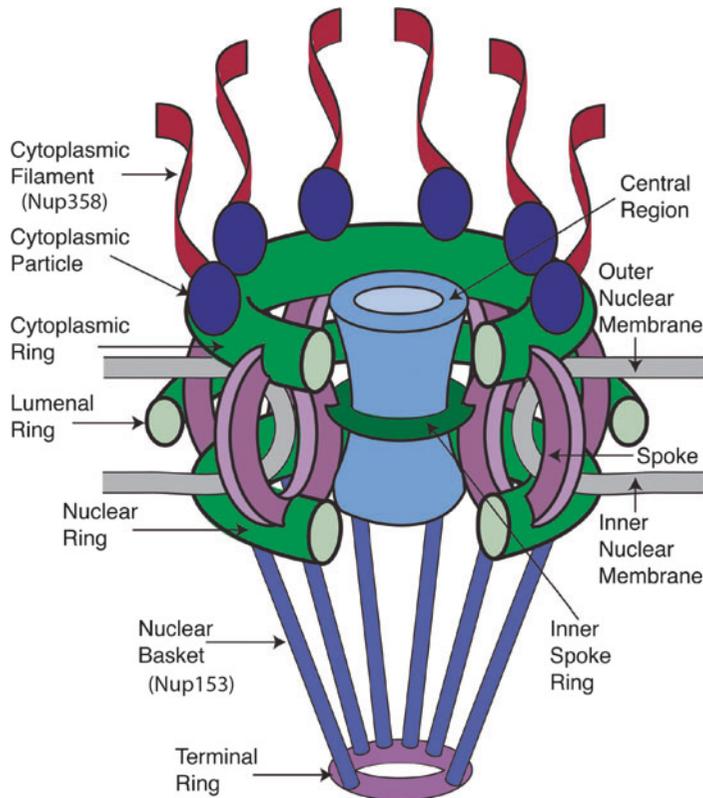
Another small-molecule compound, I-XW-053, was also identified as a specific inhibitor of HIV-1 replication (Kortagere et al. 2012). The compound bound CA protein in the NTD. Mutations I37A and R173A in CA caused pronounced decrease in

compound binding. Functionally, I-XW-053 inhibited HIV-1 reverse transcription, indirectly pointing to modulation of uncoating. Collectively, these studies underscore the potential of CA as a therapeutic target.

Uncoating and Nuclear Entry

A distinguishing feature of lentiviruses is their ability to infect nondividing cells such as macrophages and microglia. To infect a nondividing cell, the viral genome must traverse the intact nuclear membrane. Embedded in the nuclear membrane are nuclear pore complexes (NPC). NPCs are composed of ~30 different proteins known as nucleoporins (Nups), each in multiple copies per NPC (Fig. 3). In the center of the NPC, perpendicular to the nuclear membrane, is a central channel, called the nuclear pore, through which molecules can pass. Nuclear pores have a maximum diameter of 90 nm, a depth of 180 nm, and a mass of 125 MDa. The nuclear pore allows diffusion of ions and molecules smaller than 9 nm across the nuclear envelope and facilitated transport of molecules up to 39 nm in diameter. HIV-1 PICs are too large (~56 nm) to pass through the nuclear pore by passive diffusion and must therefore be actively transported through it in nondividing cells. Accordingly, several viral and cellular factors appear to control nuclear entry of the HIV-1 PIC.

Several viral proteins such as MA, Vpr, and IN were originally identified as components containing putative nuclear localization signals (NLS) and were therefore suggested to mediate the entry of HIV-1 into the nucleus. However, it now appears that none of these viral proteins is necessary for infection of nondividing cells. Genetic studies of chimeric HIV-1 viruses containing portions of the murine leukemia virus (MLV), which cannot infect nondividing cells, indicated that CA is the key (Yamashita and Emerman 2004). How does CA confer HIV-1 with the ability to traverse the nuclear pore? One potential explanation is that the uncoating is different between the two viruses. HIV-1 sheds its capsid quite early after infection, as evidenced by



Uncoating and Nuclear Entry, Fig. 3 Schematic of the nuclear pore complex. Embedded in the nuclear membrane, the NPC consists of a series of concentric rings surrounding a central channel with limited diameter. Cytoplasmic filaments containing Nup358/RanBP2 extend into the cytoplasm, while the Nup153-containing nuclear basket projects into the nucleoplasm. Other Nups project into

the pore, creating a hydrophobic channel through which proteins and RNA must pass (Reprinted from *Developmental Cell*, Vol. 4, M. Sundtharalingam and S. Wentz, "Peering through the Pore: Nuclear Pore Complex Structure, Assembly, and Function" pages 775–789 (2011), with permission from Cell Press)

the low abundance of CA associated with the RTC and PIC, morphological analysis of the RTC by electron microscopy, and ability of RNA aptamers and specific nucleoporins to interact with the incoming viral RNA genome. By contrast, MLV retains the capsid shell at least until nuclear entry, as evident by the higher amount of RTC-associated CA and electron microscopic examination of MLV-infected cells. Moreover, a CA mutant exhibiting higher levels of PIC-associated CA was impaired for nuclear entry (Dismuke and Aiken 2006). Thus, the rate and extent of uncoating may be key to HIV-1 nuclear entry in nondividing cells.

Host Cell Factors Influencing HIV-1 Nuclear Entry

Several genome-wide siRNA screens have identified host proteins that are important for early stages of HIV-1 infection. One widely studied factor is transportin 3 (TNPO3/TRN-SR2). TNPO3 is a member of the importin β superfamily of proteins, whose physiological function in the cells is translocation of proteins from the cytoplasm into the nucleus. Importins bind to their cargo in the cytoplasm and traverse through the nuclear membrane. Inside the nucleus, binding of RanGTP to the importin: cargo complex causes a

conformational change in the importin leading to release of cargo from the complex (reviewed in Fassati 2012). It was initially thought that TNPO3 facilitates nuclear import of HIV-1 via binding to IN. However, it is now apparent that CA is a major viral determinant of the TNPO3 dependence of HIV-1 infection and that TNPO3 does not facilitate nuclear entry. Moreover, TNPO3 was also shown to bind directly to the viral capsid and is necessary for efficient integration. Depletion of TNPO3 in target cells also alters the integration site preference of HIV-1; in TNPO3-depleted cells, HIV-1 predominantly integrated in gene-sparse regions versus normal cells where integration was observed in gene-dense regions (Ocwieja et al. 2011). Additionally, the CA mutant N74D, which does not depend on TNPO3 for infection, also favors integration into gene-sparse regions (Schaller et al. 2011). Collectively, these findings provide strong evidence for requirement of TNPO3 in a post-nuclear entry step in HIV-1 infection.

TNPO3 also promotes nuclear entry of the SR-rich proteins. Cleavage and polyadenylation specificity factor 6 (CPSF6) is an SR-rich protein which could be a potential candidate for TNPO3-mediated nuclear import. An artificially truncated form of this protein, CPSF6-358, reduces HIV infectivity at the level of nuclear import. Notably, CPSF6-358 is predominantly localized in the cytoplasm presumably because it cannot bind TNPO3; however, it can bind to capsid-like CA complexes *in vitro*. Thus, TNPO3 may act by sequestering endogenous CPSF6, which could otherwise delay HIV-1 uncoating, thereby inhibiting nuclear entry and/or integration.

Several nucleoporins were also identified as HIV-1 dependency factors in genome-wide siRNA screens. Nup153 is important for HIV-1 infection, and the viral determinant was mapped to CA (reviewed in Fassati 2012). Interestingly, the N74D mutation in CA reduced the dependence of HIV-1 infection on Nup153. Thus, Nup153 dependence of infection is genetically linked to the viral capsid. Depletion of Nup153 in target cells caused moderate reduction in HIV-1 nuclear entry but a pronounced reduction in integration.

Nup358/RanBP2 is also important for HIV-1 infection. This large protein contains a filamentous phenylalanine/glycine (FG)-rich domain that protrudes on the cytoplasmic side of the NPC. It also contains a cyclophilin-like domain on its C-terminus. HIV-1 CA binds directly to the Cyp-domain of Nup358/RanBP2, and depletion of Nup358/RanBP2 results in reduction in nuclear import. Thus, HIV-1 may engage the NPC via Nup358/RanBP2. This implies that some CA remains bound to the PIC prior to docking to the NPC. The ability to use Nup358/RanBP2 is dependent on CA-CypA interaction. In addition to nuclear import, depletion of Nup358/RanBP2 reduced integration of HIV-1 in gene-dense regions, and this may influence transcription of the integrated viral genome (Ocwieja et al. 2011).

Conclusion

Uncoating is a critical step in HIV-1 life cycle, but an understanding of the mechanism and timing of uncoating is incomplete. Through the development and application of biochemical, cell fractionation, and cell imaging approaches, it has become apparent that capsid stability is very important for efficient HIV-1 infection. Mutations in CA that modulate capsid stability affect reverse transcription, nuclear entry, and integration efficiency and targeting. Host restriction factors perturb uncoating and inhibit infection, while other host proteins promote HIV-1 infection by modulating proper uncoating and facilitating nuclear entry of the PIC. A more complete understanding of HIV-1 uncoating will facilitate the development of inhibitors targeting this key step in HIV-1 infection.

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Update on HIV-1 and HIV-2 Dual Infection

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Definition

Dual HIV-1 and HIV-2 infection is the coexistence of the two viruses in an individual. It is most frequent in West Africa where HIV-2 is endemic. Dual HIV infection can be proven only by the presence of both HIV-1 and HIV-2 DNA or RNA using type-specific PCR for the isolation of both viruses from the same individual. However, HIV-2 RNA may not be detectable and therefore cannot be used as a diagnostic test. In addition, diagnosis based on rapid HIV testing, often used in West Africa, is often unreliable.

Introduction

Although human immunodeficiency virus type 1 (HIV-1) infection is responsible for most of the global AIDS pandemic, HIV type 2 (HIV-2) is an important cause of disease in a number of regions of the world. However, based on this origin (Kanki et al. 1987), HIV-2 epidemic has remained essentially confined to West Africa with a limited spread to other regions (Kanki et al. 1987). In West Africa, 9–16% of

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HIV-infected patients are estimated to be dually infected with both HIV-1 and HIV-2 (Chang et al. 2002; Rouet et al. 2004). Although dual seropositivity is common in the West African region, the true rate of dual infection remains unclear. Being infected by one type of HIV does not protect against the other type, and transmission may occur in patients already infected (e.g., superinfected). This entry provides information on the epidemiology, diagnosis, and clinical manifestations of dual HIV seropositivity, presenting the treatment options for these patients.

Epidemiology

Coinfection with both HIV-1 and HIV-2 occurs in countries where both viruses circulate, e.g., the West African region. The distribution of these two viral infections is both heterogeneous and unstable; however, HIV-1 and HIV-2 overlap in West Africa. In this region, the prevalence of dual infection with the two viruses has been ranged between 1% and 15% (Rouet et al. 2004; Holmgren et al. 2003; van der Loeff et al. 2006; van Tienen et al. 2011). It is not clear whether social factors, biological factors, or both account for the less worldwide spread of HIV-2.

In mid-1999, a study in Ghana, which included 854 outpatients, reported that HIV-1 infection alone accounted for 84.5% of cases, compared with 9.5% and 6.0% for HIV-2 and dual HIV-1/HIV-2 infection, respectively (Chang et al. 2002). A study in Guinea-Bissau, a country where HIV-2 infection is endemic, reported that in 2006, among the 2,548 people tested for HIV, 106 (4.2%) were HIV-1 infected, 100 (3.9%) HIV-2, and 12 participants (0.5%) positive for both viruses, thus indicating that dual HIV infection accounted for 10% of HIV-positive patients (da Silva et al. 2008). The incidence of dual HIV-1/HIV-2 seropositivity has also been estimated in Gambia. Indeed, in 1996, 654 participants (279 men and 375 women) were negative for dual infection and were then reexamined in 2006, which contributed to a total of 6,222 person-years. Four female participants had

become HIV-1/HIV-2 seropositive, which corresponded to an incidence of HIV-1/HIV-2 reaction of 0.06 per 100 person-years (da Silva et al. 2008). Another study at the sexually transmitted infections research clinic of the Medical Research Council (MRC) laboratories near the capital of Banjul in Gambia reported that the prevalence of dual HIV-1/ HIV-2 reaction varied between 0.8% (1988–1991) and 1.2% (1998–2000) over a 16-year observation period, and thus, the prevalence of dual HIV reaction neither increased nor decreased during this period. Dual HIV infection accounted for 4–6% of all HIV infections during the study period in these countries (van der Loeff et al. 2006).

Transmission and Disease Progression

HIV-1 and HIV-2 share the same transmission route via sexual contact, perinatal, or parental transmission. However, the frequency of transmission is less than that of HIV-1, which is most likely due to a very low viral load in many asymptomatic individuals (Kanki et al. 1994). HIV-2 is thought to have a protective effect against HIV-1 superinfection or limited HIV progression (Otten et al. 2004).

Several important issues raised about the impact of the dual-infection single host, for example, what is the potential for HIV-1/HIV-2 recombinant formation, are there synergistic or inhibitory mechanisms that result in distinct viral replication dynamics in dual-infected individuals compared to that of HIV-1 or HIV-2 in mono-infected individuals, and what factors should be considered when choosing antiretroviral regimens in HIV-1/HIV-2 dual-infected individuals? In nonhuman primates, HIV-2 was reported to have a protective effect against SHIV superinfection or HIV progression (Otten et al. 2004).

In the 1990s, there was controversy over whether infection by HIV-2 would protect against HIV-1 and whether the HIV-1 epidemic would, therefore, slow down in the regions affected by HIV-2 (Travers et al. 1995; Wiktor et al. 1999). Unfortunately, several subsequent studies did not find this protective effect

(Schim van der Loeff et al. 2001). It is important to distinguish coinfection from superinfection as well as reinfection with HIV-1 or HIV-2. One case of asymptomatic superinfection with HIV-1 subtype AG was reported in a woman infected with HIV-2 who did not have cross-neutralizing autologous HIV-1 antibodies before and soon after HIV-1 superinfection. This evidence supports a mechanism other than cross-neutralizing antibodies for the mild course of HIV-1 infection in this woman (Gunthard et al. 2009). In fact, superinfection is defined as the reinfection of an individual who already has an established infection with a heterologous HIV-1 or HIV-2 strain. Reports in West Africa noted that dual HIV reaction was more common in older women (Holmgren et al. 2003) and the clinical manifestations of dual HIV infection are similar to those of HIV-1.

Finally, infection with both HIV-1 and HIV-2 generally carries the same prognosis and a similar mortality rate as HIV-1, except one study in rural community in Gambia which reported lower mortality among HIV-2-infected patients (van Tienen et al. 2011). Two studies in Guinea-Bissau found conflicting results: (1) among 285 HIV-2- and 53 dual-infected patients, there found no difference in mortality with follow-up to 19 years in rural Caio (van Tienen et al. 2011), and (2) among 223 patients that included 32 dually infected (14.3%), it was suggested that being infected with HIV-1 and HIV-2 is associated to a slower rate of disease progression particularly in patients in whom HIV-2 infection preceded HIV-1 infection. These observations in a cohort with a long follow-up (approximately 20 years) showed that HIV-2 has an inhibitory effect of the rate of HIV-1 disease progression (Esbjornsson et al. 2012). In this latter study, the rate in CD4+ T-cell percentage was similar with 1 infection only and dual infection, with an average decline of 1.2% per year ($P = 0.36$, with the use of a mixed model with interaction term removed). However, the CD4+ T-cell percentage was significantly higher in participants with dual infection (31.3%) than in those with HIV-1 infection only (23.3%) ($P < 0.001$) (Esbjornsson et al. 2012).

Screening and Diagnosis of Dual Infection with HIV-1 and HIV-2

The detection of HIV-2 infection is based on the demonstration of virus-specific antibodies using enzyme-linked immunosorbent assay-based techniques. In suspected HIV-1/HIV-2 cases, dual seroreactivity of both HIV-1 and HIV-2 alone is not sufficient for diagnosis. Dual infection can be proven only by the presence of both HIV-1 and HIV-2 DNA or RNA using specific PCR for the isolation of both viruses from the same individual (Peeters et al. 1992). However, HIV-2 RNA may be negative and, therefore, cannot be used to rule out dual infection. HIV-2 proviral DNA may be low or repeatedly negative in certain asymptomatic individuals, thereby confounding the diagnostic confirmation (Damond et al. 1998).

Serology based on specific synthetic peptides has proven to be useful for diagnosing dual infection. In West Africa, rapid HIV assays are being used for the diagnosis of dual reactivity. The serological diagnosis of dual infection is based on similar high levels of antibodies binding to the immunodominant epitope of gp41 as well as the V3 region of both HIV-1 and HIV-2.

In West Africa, current serological tests for the diagnosis of HIV-2 include the following, and two consecutive rapid tests are recommended by the national HIV program:

- For screening purposes: Determine (Abbott Diagnostic Division, Hoofddorp, Pays-Bas, Netherlands), ELISA (Murex Diagnostics Limited, Dartford, United Kingdom), and Murex ICE VIH 1.0.2 (Abbott, Murex Diagnostics Limited, Dartford, United Kingdom).
- In case of positivity with screening tests: Genie II HIV-1/2 (Bio-Rad Laboratories, Marnes-la-Coquette, France), ImmunoComb HIV 1&2 BiSpot II (Orgenics Ltd., Yavne, Israel), Bioline HIV-1/2 (Standard Diagnostics, Inc., Republic of Korea), or western blot analysis.
- Final confirmation is performed using Pepti-LAV 1–2 (Bio-Rad Laboratories), HIV-1 and HIV-2 western blot analyses, and HIV-1- and HIV-2-type-specific DNA PCR and is dependent on the type of tests used in the laboratory.

Update on HIV-1 and HIV-2 Dual Infection, Table 1 Serological and viral characteristics of 35 women diagnosed on-site with dual HIV-1 and HIV-2

HIV test used	HIV-2 n (%)	HIV-1 n (%)	HIV-1 and 2 n (%)	Negative n (%)
Pepti-LAV assay	19 (54.3)	2 (5.7)	14 (40.0)	–
Western blot for HIV-1 and HIV-2	17 (48.6)	3 (8.6)	15 (42.8)	–
Homemade EIAs (gp41/36 and V3)	20 (57.2)	6 (17.1)	9 (25.7)	–
HIV-1 and HIV-2 proviral DNA detected by real-time PCR assays	17 (48.6)	6 (17.1)	9 (25.7)	3 (8.6)

infection using the Genie II assay (Adapted from Rouet et al., Abidjan, Côte d'Ivoire (Rouet et al. 2004))

In the final meeting report of the Réseau Africain des praticiens assurant la prise en charge médicale des personnes vivant avec le VIH/SIDA (African Network of Practitioners on AIDS (ANEPA)) (RESAPSI) and the International epidemiological database to evaluate AIDS (IeDEA), workshops on HIV-2, it was stated that 30–70% of HIV-1 + HIV-2 seroreactive patients are misdiagnosed, and the use of rapid testing is not recommend for diagnosis due to the unreliability of these methods (RESAPSI et al. 2011; RESAPSI et al. 2009). For example, in 2004, a study in Abidjan assessed dual reactivity in specimens from HIV-infected patients using the Genie II assay. Rouet et al. (Table 1) demonstrated that the rates of concordance between real-time PCR and serological assays were 25.7%, 82.9%, 74.3%, and 80.0% for the Genie II, Pepti-LAV, WB, and custom ELISA assays, respectively (Rouet et al. 2004).

A cohort of 47 HIV-1 and HIV-2 dually seropositive individuals from Senegal, West Africa, was screened for the presence of HIV-1 and HIV-2 gag and env PBMC viral DNA sequences using PCR. Of the 47 dual HIV-1/HIV-2 seropositive individuals that were tested, 19 (40.4%) had infection with both HIV-1 and HIV-2, which was confirmed by genetic sequence analysis, whereas HIV-1 or HIV-2 was confirmed in 17 (36.2%) and 9 (19.1%) of cases, respectively (Gottlieb et al. 2008a). Due to the lack of commercially available tests, or assays approved by the Food and Drug Administration (FDA), HIV-2 RNA levels generally cannot be monitored in patients that have started ART. Certain HIV-1 RNA assays, such as the Roche AMPLICOR HIV-1 Monitor and NucliSENS EasyQ, may detect HIV-2 RNA. However, these commercial HIV-1

assays are not approved for the quantification of HIV-2 RNA. A report has also shown that ImmunoComb had a high degree of concordance with PCR testing and provided confirmation of HIV-1/HIV-2 dual infection (Walther-Jallow et al. 1999). Recently, a highly sensitive HIV-2 VL assay that is suitable for clinical and research use was developed (Chang et al. 2012).

Clinical Manifestations

In the pre-ART era, studies showed that people reactive to both HIV-1 and HIV-2 as well as those who were infected to HIV-2 had a frequency of AIDS-associated symptoms and signs similar to that in HIV-1-infected patients (Whittle et al. 1992). In the IeDEA West Africa Collaboration Cohort, dual-infected patients tend to present at a more advanced stage of the disease than those with only HIV-2 (44% vs. 36%) (Drylewicz et al. 2010). Preliminary data showed that patients on ART that were infected with both HIV-1 and HIV-2 were younger (40 years at the initiation of ART) and were more likely to be men (46%) than those infected with HIV-1 (37%) (Drylewicz et al. 2010) and HIV-2 mono-infection (Holmgren et al. 2003; Schim van der Loeff et al. 2002).

Antiretroviral Therapy for HIV-1/HIV-2 Dual Infection

There has been minimal experience regarding the use of antiretroviral therapy for HIV-2 infection as well as HIV-1 and HIV-2 coinfection. In coinfecting patients, HIV-1 typically outcompetes the lower replicative capacity of the HIV-2 virus

(Koblavi-Deme et al. 2004). This assumption is supported by frequent reports of low viral loads in the majority of studies that compared HIV-2-infected and HIV-1-infected persons (Koblavi-Deme et al. 2004). These findings suggested that in vivo immunodeficiency must be driven mainly by HIV-1 in coinfecting persons. Accordingly, the treatment of HIV-1 infections may be considered a priority in coinfecting persons.

When to Start Treatment

The lack of randomized treatment studies in dually reactive patients makes it difficult to determine when therapy should be initiated (Gottlieb et al. 2008b). The treatment of dually infected individuals should be performed using an HIV-2 active regimen to ensure effective treatment of both viruses. Given that the HIV-2 plasma viral load is usually undetectable or low in dual infections, both viruses should be treated and monitored. WHO guidelines for middle- and low-income resource countries recommended to start ART for HIV-1 + HIV-2 as the same threshold than HIV-1 below 350 cells/mL (WHO 2010). The results obtained with the HIV-2 cohort ANRS C05 showing lesser immunological response to ART in HIV-2 infected patients compared with those only infected with HIV-1 (Drylewicz et al. 2008). These findings have not been documented in HIV-1-/HIV-2-infected patients. So in France, the threshold to initiate ART in dual infection is similar for HIV-2 mono-infections (<500 cells/mm³). In developed countries where viral load measurement is available for the two viruses, HIV-2 viral load (>100 copies) should be a criteria to start above 500 CD4 cells/. (Ref)

Clinical Studies

Clinical studies of HIV-2 infection have been limited to a small number of case series and cohort reports. Based on a case series study at Bichat Claude Bernard Hospital, Landman et al. (2009) reported that 36 patients were identified as being dual seropositive for HIV-1 and HIV-2. This diagnosis was confirmed by specific env (V3 and gp36) peptide serology and HIV-1 and HIV-2 western blot analyses. Among these 36 patients, 17 (10 women) with regular clinical,

immunological, and virological follow-up for at least 1 year were included in the retrospective observational study. Eleven patients received 1 to 7 different antiretroviral regimens. At the time of the first-line regimen, the median CD4 cell count was 130 cells/mm³ (16–290), the median HIV-1 viral load was 4.6 log copies/ml (2.8–5.8), and HIV-2 viral loads were detectable in two patients (4.47 and 4.74 log copies/ml). After a median follow-up of 2.6 years (0.3–7.6), the median CD4 cell count was 338 cells/mm³ (102–654), and plasma HIV-1 and HIV-2 RNA were undetectable in 10 of 11 cases. The ongoing treatment included lopinavir/ritonavir in nine cases and two or three nucleoside reverse transcriptase inhibitors. Detectable HIV-2 plasma RNA was observed in two of the 11 patients during their follow-up. Jallow et al. reported that in Gambia, eight dually infected individuals were successfully treated with AZT/3TC/LPV/r, and a complete suppression of both viruses for more than 3 years was achieved (Jallow et al. 2009). In the IeDEA West Africa collaboration, which studied changes in CD4 levels over a 12-month period in patients taking ART, the observed baseline median CD4 counts were marginally comparable in the three groups: 155 cells/μL in HIV-1-positive patients ($n = 9,842$), 148 cells/μL in HIV-2-positive patients ($n = 270$), and 152 cells/μL in dually positive patients ($n = 321$) ($P = 0.52$). Among patients treated with a PI-containing regimen, the CD4 response did not differ significantly between HIV-1-positive, HIV-2-positive, and dually reactive patients before ($P = 0.73$) and after 3 months of treatments ($P = 0.37$). The estimated CD4 counts at 12 months were similar in HIV-2-positive (278 cells/μL, CI = 248; 307, $P = 0.22$) and dually positive (271 cells/μL, CI = 238; 303, $p = 0.25$) patients compared to HIV-1-positive patients (303 cells/μL, CI = 292; 315) (Drylewicz et al. 2010).

Options for ART Regimen Sequencing in HIV-1/HIV-2 Dual Infection

Given the different susceptibilities of HIV-2 strains to protease inhibitors as well as the more

Update on HIV-1 and HIV-2 Dual Infection, Table 2 Preferred and alternative first-line and second-line regimens in HIV-1 and HIV-2 dually reactive patients

	First line	Second line
Preferred regimen	2 NRTIs + boosted HIV-2 active PI	2 NRTIs + boosted PI ± INI
	Zidovudine (ZDV) + lamivudine (3TC) or tenofovir (TDF) + 3TC or emtricitabine (FTC)	TDF + 3TC/FTC if ZDV + 3TC first line ZDV + 3TC if TDF + 3TC/FTC first line
	+ <i>Lopinavir/r or saquinavir/r or darunavir/r</i>	+ <i>Darunavir/r + raltegravir or elvitegravir/c</i> <i>if lopinavir in first line</i>
Alternative regimen	3 NRTIs	2 NRTIs + boosted PI ± INI
	TDF + 3TC or FTC + ZDV if CD4 > 200 cells/mm	TDF + 3TC/FTC + <i>Saquinavir/r or lopinavir/r or darunavir/r</i> <i>Or boosted PI + raltegravir or elvitegravir/c</i>
	2 NRTIs + 1 INI	2 NRTI + PI/r
	ZDV + 3TC or TDF + 3TC or FTC + <i>raltegravir or elvitegravir/cobicistat</i>	TDF + 3TC/FTC if ZDV + 3TC first line ZDV + 3TC if TDF + 3TC/FTC first line + <i>lopinavir/r or saquinavir/r</i>

NRTI nucleos(t)ide reverse transcriptase inhibitor, *PI* protease inhibitor, *PI/r* protease inhibitor boosted with ritonavir, *INI* integrase strand transfer inhibitor

limited options for the treatment of HIV-2 infection, there is a need for caution prior to initiating antiretroviral therapy in patients with both HIV-1 and HIV-2 infection. In the case of dual infection, antiviral drugs active against both viruses should be given, and both plasma HIV-1 and HIV-2 RNA levels should be periodically measured. The recommended regimen should be similar to the regimen recommended for HIV-2 patients. Treatment-naïve, coinfecting patients should be treated with ritonavir-boosted protease inhibitors plus 2 NRTIs. If treatment fails, the resistance patterns of both viruses should be evaluated. Some protease inhibitors such as nelfinavir, atazanavir, or fosamprenavir are less effective against HIV-2 and may be used as part of a regimen for HIV-1-/HIV-2-reactive patients if adequate treatment for HIV-2 is also included.

The preferred option in first line should be 2 NRTI plus lopinavir/r particularly in resource-limited settings, ideally TDF/FTC/LPV/r. Lopinavir resistance in HIV-2 occurs with the V47A mutant, and evidence suggests V47A retains susceptibility to saquinavir making it an attractive choice in second line. The issue there is the risk of HIV-1 PI cross-resistance to saquinavir. Thus, the recommendation in case of dual infection if genotype test is not available is to use

darunavir/r as a second-line PI ideally with an integrase inhibitor. Initiating second-line therapy following a confirmed failure with a boosted PI must be considered as a salvage therapy based on genotype test. In fact, using a PI-sparing first-line regimen (e.g., INI based) in dually infected patients may allow them a potent second-line regimen based on a boosted PI plus 2 NRTI and a chance at an effective third-line regimen that is determined by genotyping.

In this case, the sequencing regimen could be based in the first line on 3 NRTI or 2 NRTI plus an integrase inhibitor (e.g., raltegravir) (Table 2). The advantages of a raltegravir- or elvitegravir-based are that HIV dually infected patients may spare PI regimens in first line and represent an alternative in case of tuberculosis with rifampin-based regimen. Currently, rifabutin, a safe and effective alternative to rifampicin, is not available and affordable in resource-limited settings. To date tuberculosis programs provide only rifampicin in most resource-limited settings (RLS). A less than optimal regimen in dually infected patients with TB coinfection in RLS is a 3 NRTI-regimen; however, failure rates in mono-HIV-1 infection are high, and in HIV-2, 3 NRTIs are less potent below 200 CD4 cells/mm³ and the higher failure rate reported comparing to PIs boosting regimen

(Benard et al. 2011). The main limitation of an INI regimen is the cost 7–10 as high as 3 NRTI regimens. Despite limited cases, most of the data of the efficacy of integrase inhibitors are in vitro study. Genetic barrier is low, and the risk of rapid resistance emergence is a key issue (Roquebert et al. 2008). The advantages are to reduce of pill burden, with a possibility of one pill once a day if TDF/FTC/EVG/c is used, a strong option in first line for HIV dually infected patients.

The second-line treatment regimens are the same for those with HIV-1 infection. Landman and colleagues reported good viral response to raltegravir combined with an active protease inhibitor (e.g., darunavir) (Landman et al. 2009).

Monitoring of Treatment Responses and HIV-2 Drug Resistance

Immunological and virological follow-up of both HIV-1 and HIV-2 in dually seropositive patients is recommended. In the case of dual infection, both plasma HIV-1 and HIV-2 RNA levels should be periodically measured because the replication of both viruses can occur before ART treatment is initiated. A French cohort reported HIV-2 protease resistance mutations in two cases, which emphasizes the importance of selecting drugs that are active toward both viruses (Landman et al. 2009). These two patients had received atazanavir/ritonavir and did not respond to the subsequent lopinavir/ritonavir regimen. These findings are consistent with the several studies that report reduced HIV-2 sensitivity to several protease inhibitors, which include atazanavir/ritonavir, fosamprenavir/ritonavir, and tipranavir/ritonavir.

Conclusion

The diagnosis of dual HIV infection remains difficult without the measurement of both viruses by PCR and the appropriate detection algorithms when using a serological testing Tchounga et al. 2014. Immunological and virological follow-up of both HIV-1 and HIV-2 in dually seropositive patients is recommended.

HIV dual infection may not be a static condition. Levels of HIV-2 may decrease with disease progression or sequester in tissue reservoirs. The choice of ART treatment should consider the efficacy of drugs toward both viruses. A limited number of studies are available on dual HIV infection, and we encourage a large prospective cohort to better describe their experience regarding the management of both the HIV-1 and HIV-2 viruses in coinfecting individuals Landman et al. (2009).

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Use of Technology and Social Networking in HIV Prevention

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Definition: Technology and Social Networking

In this discussion, the term *technology* is used to specifically refer to various devices used to access the Internet, including both traditional desktop and laptop computers, and more recent mobile technologies, such as tablets and smartphones. The term *social networking websites* (web 2.0) is used to refer to Internet applications in which the content of a website is mostly driven by the users of the site (Rietmeijer and McFarlane 2009). These technologies are widely used for social communication, enabling them to be used for HIV prevention among both at-risk populations and the general public.

Background

The proliferation of new technologies and social networking websites creates new and exciting opportunities for HIV prevention. Used by more than 273 million people in the United States and over two billion people worldwide, the Internet has undoubtedly changed how its users access information and communicate. Social networking websites (e.g., Facebook, Twitter, Google+, YouTube) are increasingly popular, with 68% of Internet users accessing such websites at least once per month (eMarketer 2013). Similarly, increasing numbers of people from diverse social groups (e.g., ages, genders, and races/ethnicities) are now owners of mobile devices such as smartphones and tablets, connecting billions of users to the Internet.

These trends allow the general public to easily retrieve and share health information from across the globe. Population-based studies have found

that 59% of US adults have consulted the Internet for health-related information in the past year (Pew Internet and American Life Project 2013). Additionally, 15% of all adults who access social networking websites have used them to seek out health-related information (Pew Internet and American Life Project 2013). Altogether, these technologies and websites represent important opportunities to enhance targeted recruitment for HIV prevention and interventions among Internet users.

Technology/Virtual Space as a New Sex Venue

In the past decade, the Internet has been increasingly utilized as a means to seek romantic and sex partners (Chiasson et al. 2006; Liao et al. 2006), replacing other traditional venues for meeting partners such as neighborhoods, friends, and the workplace (Ross 2005; Rosenfeld and Thomas 2012). Indeed, a large US study (Cooper et al. 2002) found that 9.8% of the people using the Internet for online sexual activities were also using it to find people to date. Potential partners can be found through numerous online venues, such as chat rooms, discussion forums, instant messaging systems, web-based personal advertisements, websites soliciting sex (Liao et al. 2006), and dating websites, mobile device applications (i.e., apps), and social networking websites.

The Internet facilitates communication of sexual desires as well as increased numbers of sexual and romantic in-person meetings (Ross 2005) due to its accessibility, affordability, and anonymity (Cooper 1998). This facilitating effect of the Internet on sexual behavior applies to both heterosexual and nonheterosexual individuals (Rosenfeld and Thomas 2012). Gender differences have been noted in individuals' preferred online sexual activity. For example, men most frequently use the Internet to view sexually explicit material (i.e., pornography), whereas women often use the Internet to stay in contact with existing sex or love partners (Cooper et al. 2002).

The Internet is especially useful as a sex venue when partners are particularly hard to find.

Individuals who face a smaller market for meeting potential partners, such as gay men (including men who have sex with men, or MSM), lesbians, bisexuals, and middle-aged or older heterosexuals, are especially likely to use the Internet for this purpose (Ross 2005; Rosenfeld and Thomas 2012). These groups may prefer online sex venues rather than offline locations because of their heightened perceived privacy and safety as well as their anonymity, accessibility, and affordability (Cooper 1998). As such, same-sex couples have a remarkably higher rate of meeting through the Internet (>60% compared to 22% for heterosexual adults) (Rosenfeld and Thomas 2012).

Although gay men, bisexuals, and other MSM represent approximately 2% of the US population, they are one of the populations most severely affected by HIV (Centers for Disease Control and Prevention 2013). Compared to other groups, MSM utilize the Internet as a primary means for sexual and romantic partners. Chiasson et al. (2006) noted in their review article that MSM who use the Internet to find sex partners are younger, are more likely to frequent public and commercial sex environments, do not identify as gay, report sex with women, and are more likely to have a previous sexually transmitted infection (STI) compared to MSM who do not use the Internet to find sex partners. More recently, researchers have begun to explore heterosexuals' use of the Internet for seeking sexual partners. Available evidence suggests that heterosexuals' use of the Internet to meet partners for both short- and long-term romantic and sexual relationships is increasing rapidly (Cooper 1998; Rosenfeld and Thomas 2012). Thus, further investigation of the Internet as a mediator of sexual (risk) behaviors among heterosexuals is needed to elucidate the processes and mechanisms unique to this community.

Technology/Virtual Space as a New STI/HIV Risk Venue: The Contribution of Technology to HIV/AIDS Risk

The Internet is already being conceptualized as a risk environment that can facilitate the spread of

HIV and other STIs. Research supports the notion that people who use the Internet for sexual purposes experience a sense of disinhibition due to the vast variety of sexual interests and activities available to them, such as viewing pornography, engaging in cybersex, and seeking sex partners online. Moreover, it has been proposed that the disinhibition experienced online might expedite and carry over into offline sexual behavior. Conversely, it has been suggested that the distance afforded by the Internet allows people to reassess sexual boundaries and to experiment with one's sexuality, which may have a positive influence on one's sexual experience and well-being (Ross 2005).

Existing empirical evidence supports the conceptualization of the Internet as a fairly new HIV/STI risk venue. People who seek sex on the Internet are more likely to have concomitant risk factors for HIV/STI than people who do not seek sex on the Internet (McFarlane et al. 2000). In fact, some scholars further argue that for populations with levels of education and income sufficient to support computer use, the Internet has become an efficient facilitator of behavior and practices that have been taking place for many years among certain high-risk individuals (Toomey and Rothenberg 2000).

Most of the research conducted on Internet-mediated sexual behavior, so far, has focused on MSM (Chiasson et al. 2006). In their meta-analytic review article of online sex-seeking MSM, Liau et al. (2006) reported that 40% of MSM use the Internet to look for sex partners, and those who seek sexual partnerships online have riskier sexual behavior profiles than their counterparts who do not use the Internet to meet partners. Serosorting tendencies (i.e., condomless anal intercourse (CAI) between men of the same serostatus) are more prevalent among MSM who look for partners online versus MSM who do not. Furthermore, HIV-positive men who meet sexual partners through the Internet are more likely to report CAI with other HIV-positive men (Liau et al. 2006). HIV-negative men with online partners are also more likely to report having HIV-positive partners and more likely to report CAI with serodiscordant partners. It is hence plausible that the Internet may be creating a network of

high-risk men, facilitating transmission of HIV and other STIs among its sex-seeking users, who may also spread infection to other partners met offline (Liau et al. 2006).

Technology/Virtual Space as a New Prevention Venue

The Internet is rapidly becoming an important site for HIV prevention and other STIs due to its wide outreach and availability as well as its affordability and perceived privacy. Additionally, the Internet has numerous advantages relevant to HIV prevention efforts, including interactivity and multimedia features, the ability to deliver individualized content (Noar and Willoughby 2012), the ability to reach diverse and geographically dispersed populations (Chiasson et al. 2006), the automated delivery of interventions, and rapidly-attained large sample sizes (Birnbaum 2004) which can result in lower program costs (Rietmeijer and McFarlane 2009). There are three categories of existing HIV/STI prevention interventions on the Internet: (1) enhancing STIs and HIV testing, (2) increasing partner notification and treatment, and (3) encouraging behavior change for the prevention of HIV and other STIs (Rietmeijer and Shamos 2007).

Social networking websites are also increasingly used for the purpose of HIV prevention. Social networking websites serve HIV prevention efforts in three main areas: (1) as sources of publicly available information, (2) as intervention venues, and (3) as recruitment tools (Young 2012). The potential of utilizing social networking websites for HIV prevention purposes is enormous. 13% of US adults have gone online to find others who might have health concerns similar to theirs. Seven percent of US adults have gotten any health information on these websites (Pew Internet and American Life Project 2013). Social networking websites are an exciting prevention platform given their potential to reach vulnerable populations such as adolescents and young adults. Indeed, adolescents are gradually spending large amounts of time on these

websites (Pew Internet and American Life Project 2013).

There is growing evidence that the use of different technologies and Internet applications leads to improved health and health care. Numerous studies have demonstrated the effectiveness of computer- and Internet-based programs in promoting HIV/STI awareness and safer sex knowledge, attitudes, self-efficacy, and other theoretical mediators of safer sex. More importantly, such interventions have been found to increase reported safer sexual behaviors, most notably condom use, among its participants (Noar and Willoughby 2012).

Recent evidence suggests that MSM who frequent a variety of (offline) venues can also be reached on the Internet (Groves et al. 2013), thus potentially broadening the impact of prevention messages delivered in virtual environments. In fact, many MSM access HIV prevention information online. Some scholars suggest that men who seek sex with men on the Internet may be particularly in need of Internet-based HIV prevention because they tend to be more educated, insured, and less likely to be exposed to offline public health messages (Bull et al. 2001; Chiasson et al. 2006). Other unreachable populations, such as men resistant to in-person prevention efforts and high-risk individuals, like HIV-positive men who use the Internet for sex and are engaged in high-risk practices, may also be successfully targeted through the Internet (Chiasson et al. 2006).

Although not yet well studied, mobile devices are another useful tool for HIV prevention dissemination. Young adults are much more likely than older adults to have a smartphone and to use it to look for health information. Nearly 10% of US adults who have mobile phones – and more than half of American adults currently own one (Pew Internet and American Life Project 2013) – have registered to use a mobile health app (Pew Internet and American Life Project 2013). Smartphone applications designed for the dissemination of health-related information, social networking, and romantic and sexual partnering are an understudied platform for HIV prevention that may attract various high-risk and vulnerable populations such as MSM, Latinos,

and African Americans. In fact, it was found that Latinos and African Americans are more likely than Whites to own a smartphone and that African Americans are also more likely than Whites to connect to the Internet using their smartphone (Pew Internet and American Life Project 2013). Smartphone applications can also be used for seeking sex partners. For example, Grindr is a GPS-based application designed for social networking as well as romantic and sexual partnering among MSM and is extremely popular with millions of users worldwide. These features make Grindr and similar mobile devices apps potentially useful for different types of HIV prevention among MSM and possibly other populations.

Suggested Guidelines for Designing Online Prevention Intervention

When designing an online prevention intervention program/application, several issues should be taken into consideration. First, technologies are constantly being developed and modified, and prevention interventions require thorough understanding and technical proficiency of the specific program being used. Moreover, each online environment has its own unique microculture and norms that are usually acquired and learned through active participation. For those reasons, it is highly recommended to include a program collaborator who is an expert in the field of online technologies and cultures as early as possible in the planning process (Gold et al. 2012; Young 2012). Second, different populations have different levels of technological skills, knowledge, and health literacy. Thus, it is important to be familiar with these characteristics of the target population and design the program with its intended users in mind. Third, when dealing with online settings, it is somewhat more difficult to measure and monitor which health information and delivery mechanisms users were exposed to. Hence, it is crucial to predetermine desirable outcomes and to define what would be considered as a successful prevention program (Gold et al. 2012). Fourth, it is necessary to address users' privacy and

confidentiality issues by getting familiarized with the common concerns of the targeted population as well as the hosting technology (e.g., social networking website, smartphone app; Young 2012). Fifth, the Internet may introduce ethical, legal, and organizational concerns and, as a result, further delays in implementing and launching the prevention program. In order to avoid unexpected delays and schedule changes, it is important to plan the program ahead of time and consult with the Institutional Review Board during the planning phase (Gold et al. 2012). Sixth, it is helpful to have an established base of prospective users prior to the launching of the program, which can be cultivated by spreading the word about the planned prevention program in both online and offline venues (Gold et al. 2012). Seventh, researchers should investigate what future users would find most appealing and design the program accordingly. In case of using an existing website (e.g., Facebook), researchers can explore the available features to maximize participation. For example, Gold et al. (2012) used an *edutainment* (education and entertainment) approach to maximize appeal to their target audience (MSM and young people aged 16–29 years). Eighth, in order to attain a large enough pool of participants and to recruit high-risk populations, prevention messages should be designed to spread virally on the Internet. Imaginative, emotion-inspiring, fun, and intriguing messages that are accessible and engaging have been found to be most viral (Gold et al. 2012). Ninth, it is important to anticipate the loss of participants over time, which is usually due to loss of interest of participants (Gold et al. 2012). Finally, when the prevention program comes to end, it is imperative to revisit the original aims and outcomes of the program to evaluate the level of success achieved.

Future Directions for Research and Practice

As suggested above, the use of technology and social networking in HIV prevention presents

innovative mechanism for this purpose and is growing rapidly. Yet, increased participation of the public health community is crucial to improve the quality of existing HIV prevention information on the Internet, as well as offer new programs and applications. Providing Internet users worldwide access to comprehensive, reliable, intelligible, and up-to-date HIV prevention information is an important step for decreasing HIV/STI incidence. Furthermore, following and adopting newer technologies for the purpose of HIV prevention will keep this field relevant and accessible to diverse populations, including high-risk populations. In addition, most online HIV/STI prevention interventions have not been thoroughly evaluated, and therefore, more studies are needed to evaluate the quality and outcomes (short- and long-term) of these programs. It would also be informative and of high relevance to have longitudinal (i.e., prospective) data on the effects of different online HIV/STI prevention applications on sexual behavior. It is even more crucial for future studies to focus on evaluation of prevention programs dedicated to high-risk and HIV-positive populations. Lastly, it is necessary to better understand how to functionally translate and disseminate online prevention efforts into real-world settings, more specifically, into high-risk and HIV-positive populations' lives where they can have a positive impact (Noar and Willoughby 2012).

Conclusion

The emergence of new technologies, particularly the Internet, social networking websites, and mobile devices, has had far-reaching consequences for HIV/STI prevention and continues to introduce exciting platforms for the delivery of health-related information. High-speed technological changes may also present new ethical, legal, and organizational concerns and thus require further examination of these issues. These ever-developing technologies may strongly influence the future spread of HIV and other STIs;

adopting and thoroughly investigating the characteristics of such technologies will allow for a better understanding of their influences and consequences on the health of individuals.

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V

Vaccine Efforts Against AIDS

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Definition

A vaccine is an antigenic preparation that confers immunity or protection against a particular pathogen or disease. In the classic approach to vaccination, a microbial immunogen is delivered, there is an immune response to the immunogen, and it is hoped that immune response is protective. Mainly used as preventive, vaccines can also be of benefit in a therapeutic setting. The historical success of vaccines in preventing a variety of viral diseases has raised hopes that the world would soon have an effective vaccine against HIV/AIDS. In this chapter we discuss the global efforts of the scientific community in obtaining a prophylactic vaccine against HIV and highlight the reasons why it has thus far proven difficult.

Background: Viral Vaccines in Human Use

The world we live in is bursting with pathogens: viruses, bacteria, fungi, prions, protists, and helminths. Many individual members of these

microbial classes are relatively innocuous; some can cause mild diseases, while others cause death in a high percentage of those infected. Historically, vaccines have unquestionably been our most powerful weapon against viral diseases of medical importance. The list of viral diseases that have been controlled, or even eliminated, is indeed quite impressive (Table 1). The most remarkable example is of course smallpox. As recently as 1967, it is estimated that two million deaths occurred that year from smallpox (Koplow 2003). As a consequence of the worldwide eradication campaign organized by the World Health Organization and the effectiveness of the live attenuated vaccine that was used, smallpox was declared eradicated from the face of the earth on December 9, 1979 by a commission of eminent scientists. The eradication was subsequently endorsed by the World Health Assembly on May 8, 1980. The last naturally occurring case was recorded in Somalia in 1977. The last actual case was laboratory-acquired in England in 1978. Stocks of live wild-type smallpox virus remain stored in freezers at two locations, one in the United States and one in Russia.

While poliovirus has not been eradicated from the face of the earth, at least as yet, families living in the developed world no longer need to live through the harrowing experience of poliovirus circulating in the neighborhood, and cases of paralytic poliomyelitis in their neighborhood, in the summer months (Roth 2010). Only three countries report polio infections at the current time:

Vaccine Efforts Against AIDS, Table 1 List of human viral diseases for which there are vaccines

Disease	Status	Virus	Vaccine type
Smallpox	Eradicated worldwide	Variola major and Variola minor (<i>Fam. Poxviridae</i>)	Live attenuated
Poliomyelitis (polio)	Eradication attempts underway	Poliovirus, types I, II, and III (<i>Fam. Picornaviridae</i>)	Inactivated (Salk) Live attenuated (Sabin)
Measles	Regional elimination established or underway	Measles virus (<i>Fam. Paramyxoviridae</i>)	Live attenuated
Rubella		Rubella virus (<i>Fam. Togaviridae</i>)	Live attenuated
Rabies		Rabies virus (<i>Fam. Rhabdoviridae</i>)	Inactivated
Mumps	Controlled or at least preventable by vaccination	Mumps virus (<i>Fam. Paramyxoviridae</i>)	Live attenuated
Hepatitis B		Hepatitis B virus (<i>Fam. Hepadnaviridae</i>)	Subunit (particle)
Yellow fever		Yellow fever virus (<i>Fam. Flaviviridae</i>)	Live attenuated
Influenza		Influenza virus, types A, B, and C (<i>Fam. Orthomyxoviridae</i>)	Live attenuated/ inactivated
Hepatitis A		Hepatitis A virus (<i>Fam. Picornaviridae</i>)	Inactivated
Hepatitis E		Hepatitis E virus (<i>Fam. Hepeviridae</i>)	Subunit
Tick-borne encephalitis		Tick-borne encephalitis virus (<i>Fam. Flaviviridae</i>)	Inactivated
Chickenpox and shingles		Varicella-zoster virus (<i>Fam. Herpesviridae</i>)	Live attenuated
Cervical carcinoma		Human papilloma virus (<i>Fam. Papillomaviridae</i>)	Subunit
Rotavirus gastroenteritis		Rotavirus enteritis (<i>Fam. Reoviridae</i>)	Live attenuated
Japanese encephalitis	Japanese encephalitis virus (<i>Fam. Flaviviridae</i>)	Inactivated/DNA	

Afghanistan, Pakistan, and Nigeria. Nigeria, which had not reported a case of wild poliovirus since July 24, 2014 and was taken off the list of polio endemic countries in September 2015, has recently confirmed a few cases during August 2016. International efforts are currently underway to completely eradicate poliovirus, including extensive vaccination campaigns. Additionally, a global switch to inactivated polio vaccines instead of live attenuated shall eliminate prolonged excretion of poliovirus in immunodeficient individuals and vaccine-associated paralytic poliomyelitis,

intending to facilitate complete worldwide eradication.

Viral vaccines currently in human use are of three types: live attenuated, whole killed (inactivated), and subunit. Live attenuated vaccines replicate sufficiently in the host to elicit a protective immune response, but not so much as to cause disease, at least in the vast majority of individuals. Given the historic nature of live attenuated vaccines in human use, they have been attenuated in general by nonspecific means. These include extensive passage in cell culture,

extensive passage in cells of a different species, selection of small plaque variants, or the use of related virus from a different species. Inactivated vaccines use purified whole virus which has been inactivated, usually with heat or chemicals. Since this process can alter the protein structure and antigenicity, inactivation needs to be done in a way that preserves antigenic structures but ensures complete inactivation of the virus. Last but not least, subunit vaccines consist of purified protein components of virions. In some cases (hepatitis B and papilloma), the protein component may assemble into a virion-like structure. Besides the antigenic component, inactivated and subunit vaccines typically contain an adjuvant, used to improve immune responses to the antigens in the preparation. See Table 1 for examples of each type of viral vaccine.

The term *vaccine* likely originated from the Latin *vaccinus* “from cows,” deriving from *vacca*, “cow.” Its origins rest with Edward Jenner (1749–1823), the discoverer of the world’s first vaccine, that for smallpox. Milkmaids who had acquired cowpox infection through their daily work suffered little or no disease from this infection with the cow virus. However, they appeared to be protected against subsequent exposure to the more dangerous and lethal smallpox virus. Jenner soon started *vaccinating* people with cowpox with great success; the rest is history. Jenner’s smallpox vaccine can thus be categorized as a live attenuated vaccine, the most common form of viral vaccine currently in human use (see Table 1). Prior to Jenner’s development of the cowpox virus as the vaccine for smallpox, the risky procedure known as *variolation* was commonly used worldwide as a preventive measure against smallpox. *Variolation* involved scarification of the skin of the subject with material obtained from the lesion of an individual with actual smallpox virus infection. The smallpox disease resulting from this intentional smallpox virus transmission was often, but not always, less severe than naturally occurring smallpox. The origins of the vaccinia virus used as the vaccine for worldwide eradication are a bit murky, but it is generally believed to be derived from passaged cowpox virus.

In this modern era of recombinant DNA, it has become possible to envision other means to approach vaccine development efforts: recombinant viral vectors, recombinant bacterial vectors, and use of DNA expression vectors. DNA vaccines deliver genetic material coding for one or more viral antigens, so that the immunogen can be properly expressed in the body and then recognized by the immune system. The advantages of this approach are that the immunogen would be expressed in its native structure (preserving the antigenicity) and that DNA is cheaper to produce than recombinant protein. A DNA vaccine approved for use in humans has been recently developed against the Japanese encephalitis virus.

Scientific Barriers to an HIV Vaccine: It’s the Virus!

Many consider the search for a safe, effective, affordable vaccine for HIV to be the greatest public health challenge of our time. With more than 36 million people currently living with HIV worldwide, more than 5400 new infections per day, and 3300 AIDS-associated deaths daily, HIV infection is one of the worst pandemics we have ever faced. Due to a broader access to anti-retroviral therapies and other interventions worldwide, AIDS-associated morbidity and mortality have dropped considerably in recent years. But long-term use of antiretroviral therapy is not without complication. Some of the problems appear to relate to long-term side effects associated with the HIV medications themselves. These include lipodystrophy, insulin resistance, decrease in bone density, and lipid abnormalities. Others, particularly chronic immune activation, inflammation, and hypercoagulation, appear to relate to the HIV. While a number of underlying causes have been proposed, microbial translocation across the gut is almost certainly a critical factor. Although it is possible that some low level of HIV replication in the presence of antiviral drugs could contribute to the chronic immune activation, it also seems likely that damage to the gut mucosa during the initial weeks of HIV infection never gets adequately repaired and is an important factor

in the chronic activation of the immune system in the face of effective therapy. In any event, effectively treated HIV-infected adults are increasingly manifesting signs of “accelerated aging,” to include cardiovascular disease, neurocognitive disease, osteoporosis, and kidney disease. These issues, compliance issues, and the difficulties of achieving lifetime supplies of antiviral drugs to developing nations underscore the continuing need for a “one shot and you are good for life” vaccine for HIV/AIDS.

Unfortunately, there are great difficulties associated with creating a successful HIV vaccine. The problem all relate to the properties of the virus itself. First is the huge sequence variability of the virus. It has been estimated that the variability in the HIV sequences found in one single individual is greater than the variability found for the influenza virus worldwide in one whole season (Korber et al. 2001). To put this into perspective, the influenza virus is variable enough so that a new vaccine must be created each year. When an individual becomes infected with HIV, the neutralizing antibody response is specific for the infecting strain but these antibodies have no neutralizing activity against the vast majority of other HIV strains circulating in the population. While this initial anti-HIV antibody response likely serves to limit the extent of viral replication to some extent, it is certainly of limited effectiveness. Viral sequence variants that emerge in the subsequent weeks and months resist neutralization by the antibodies made to the infecting strain (Richman et al. 2003). We currently have no idea what to put in an immunogen to cover the enormous sequence variability among HIV strains.

The very same high mutation rate that is responsible for the extreme diversity of HIV in the population confers upon the virus a unique ability to stubbornly adapt to immune responses within a single infected individual. The virus mutates and by so doing is able to generate escape mutants for both arms of the immune system: the humoral (antibodies) and the cellular (cytotoxic T-lymphocytes or CTLs). HIV is therefore able to persistently replicate in the face of an apparently strong host immune response, staying one step ahead of the host’s ability to respond. Even the

strongest host natural immune response to HIV is indeed completely unable to clear the infection. In fact, infection of an individual with one HIV strain does not routinely protect against superinfection by another strain.

Besides this very unique ability of this member of the lentivirus subfamily of retroviruses to generate and tolerate a wide range of mutations during the course of infection, the virus has also evolved an additional collection of mechanisms to evade the host’s immune system. Among those mechanisms is the poor immunogenicity of the HIV envelope spike. HIV has constructed its envelope glycoprotein in such a way that makes it difficult for antibodies to be elicited and difficult for antibodies to access. Generation of neutralizing antibodies to the viral envelope protein is hampered by its tightly closed configuration in which the variable loops mask or occlude vulnerable epitopes. The trimer also has a huge amount of glycosylation, which further reduces the protein immunogenicity. Gp120 envelope is actually one of the most heavily glycosylated proteins in the mammalian protein database; glycans account for approximately 50% of its molecular mass. This unusual glycosylation found on the envelope spike is an important factor for its low immunogenicity and high resistance to neutralization by antibodies. Indeed it has been suggested that HIV/SIV has an *ever-evolving glycan shield* in which the virus accumulates persistent glycosylation changes surrounding neutralizing epitopes that can block neutralizing antibody recognition of neighboring epitopes (Pikora 2004).

Additionally, several proteins coded by the virus have specific immune evasion activities. These include Nef (Negative factor) which is known to downregulate MHC-I thus preventing the infected cell from being recognized and killed by CTLs, Vif (Viral infectivity factor) which overcomes the host antiviral factors of the APOBEC3 family, and Vpu (Viral protein U) which counteracts the antiviral function of tetherin/BST-2 (also known as CD317).

Besides the CD4 helper cell depletion, which is a hallmark of HIV infection, many other cell types are directly or indirectly negatively affected by the virus (macrophages, B cells, dendritic cells,

monocytes, microglia, etc.). Since HIV primarily targets the very cells in charge of fighting back the infection, it leaves the host in an immunocompromised state characterized by a particular vulnerability to opportunistic infections in which persistent viral replication can occur.

Furthermore, HIV has an ace in the hole: the seeding of a viral reservoir. Soon after infection, the virus can hide in some cells by integrating a copy of its genome into the host cell genetic material. The integrated copy has the potential to remain silent for months or even years, eventually allowing for virus production. The existence of reservoirs is a continuous source of virus *in vivo* and allows viral loads to rebound quickly after antiretroviral therapy interruption.

Because of the reasons outlined above, once HIV enters the body, it is able to replicate continuously and relentlessly, no matter what the host immune system throws at it. Therefore, we do not know what constitutes a protective immune response. Additionally and perhaps more strikingly, HIV infection does not confer immunity: superinfection or reinfection with a second strain of HIV can occur. If the natural immune responses to HIV infection does not routinely protect against the virus, it becomes clear that generating an immunogen able to generate a better immune response than the natural infection is going to be a highly challenging task.

Given these factors, it is perhaps not surprising that development of an effective prophylactic vaccine against HIV is proving to be a difficult uphill struggle. Nonetheless, extensive efforts to find an effective vaccine approach are continuing.

Summary of Efficacy Trials in Humans

Soon after the discovery of HIV in the 1980s, many thought that finding a vaccine would be straightforward. Initial attempts followed the traditional vaccine paradigm and focused on attempting to generate neutralizing antibodies by immunizing with recombinant envelope proteins from various strains. Two phase III trials were conducted between 1999 and 2003, the VaxGen trials (with [ClinicalTrials.gov](https://www.clinicaltrials.gov) identifiers:

NCT00002441 and NCT00006327), in which the use of gp120 protein in adjuvant resulted in no protection against acquisition and no lowering of viral loads in those vaccinated individuals who did become infected. The first study enrolled 5403 healthy volunteers that were randomized in a 2 to 1 vaccine-to-placebo ratio. Volunteers received seven intramuscular vaccinations (at months 0, 1, 6, 12, 18, 24, 30) containing either the AIDSVAX B/B vaccine (MN rgp120/HIV-1 and GNE8 rgp120/HIV-1 in alum adjuvant) or a placebo (alum adjuvant only). They were followed for a total of 16 visits beginning at screening and continuing until month 36. Individuals who became HIV infected were followed every 4 months for at least 24 months. The second study enrolled 2546 participants. Volunteers were randomized (1:1) to receive AIDSVAX B/E (MN rgp120/HIV-1 and A244 rgp120/HIV-1 in alum adjuvant) or placebo (alum adjuvant only) and were followed for a minimum of 2 years. Individuals who became HIV infected were followed every 4 months for up to 36 months. While these studies showed that both binding and neutralizing antibodies could be generated, they also demonstrated that the latter were mostly limited to the very strain used in the vaccine. After these initial attempts, the field focused on eliciting cytotoxic cellular responses, hoping that they would target relatively conserved epitopes and could have breadth to achieve significant levels of protection.

The first T-cell vaccine to achieve efficacy trials was a replication-defective recombinant adenovirus serotype 5 (Ad5) vector with HIV-1 clade B *gag/pol/nef* inserts. This first study was divided in two: the Step trial and the Phambili (Zulu term for forward) trial. Both, Step and Forward (Phambili), were double-blind randomized placebo-controlled phase IIB test-of-concept studies to evaluate the safety and efficacy of a three-dose regimen (intramuscular injection on a 0, 1, 6 month schedule) of the clade B-based Merck trivalent Ad5 vaccine (MRKAd5 HIV-1 *gag/pol/nef*) in HIV-1 uninfected and sexually active adults. Both trials were aimed at assessing the efficacy of a cell-mediated immunity vaccine to protect against HIV-1 infection or change in

early plasma HIV-1 levels. The Step trial (Identifier: NCT00095576) involved 3000 HIV-1-seronegative participants in North America, the Caribbean, South America, and Australia. This study had to be stopped before completion because it unexpectedly met the prespecified futility boundaries in the first interim analysis. It was determined that vaccine recipients with pre-existing antibody titers to Ad5, and/or were uncircumcised, had a statistically significant increased rate of infection when compared with the placebo group.

While still somewhat uncertain, increased risk of HIV acquisition in uncircumcised vaccine recipients with higher preexisting adenovirus titer was initially related to a higher immune activation in those individuals, which could have made them more susceptible to infection. Further follow-up and analysis suggested that the vaccine may have undermined some immune mechanism that normally protected uncircumcised penises through the mucosa of the foreskin. It was also proposed that the Ad5 antibody titers could just be a surrogate for some other factor, like risk behaviors.

In light of the Step trial results, enrolment and vaccination in the Phambili trial (Identifier: NCT00413725) were halted although follow-up continued. This study involved only 801 participants of the 3000 initially scheduled in South Africa. With the same clade B-based vaccine designed to elicit T-cellular immunity and the same aims of safety and protection as the Step trial, the Phambili study was targeting people in an area where the major circulating clade is subtype C: the study was ultimately aimed at investigating cross-clade protection. While the vaccine elicited IFN- γ -secreting T cells that recognized both clade B and C antigens, it was eventually shown that it also increased the rate of HIV acquisition among vaccinated individuals. The outcome of these two trials, with increased rate of infection in vaccine recipients, was a huge setback for the scientific community.

Because the Step data showed that vaccine recipients with preexisting antibody titers to Ad5 and/or that were uncircumcised had an increased risk of infection when compared to the placebo

group, an additional clinical trial known as the HVTN 505 trial (NCT00865566) recruited only Ad5-seronegative circumcised men (and male-to-female transgender persons) that have sex with men. It was also a phase IIB, randomized, placebo-controlled test-of-concept study. The HVTN 505 trial utilized a DNA prime using *gag*, *pol*, and *nef* from clade B and *env* from clades A, B, and C followed by recombinant Ad5 boost using clade B *gag-pol* fusion protein and *env* glycoproteins from clades A, B, and C. This trial, which had enrolled 2504 individuals, used a DNA/Ad5 immunization regimen, which is qualitatively different from the three Ad5 immunizations used in the Step and Phambili trials. Additionally, the HVTN 505 included three different *env* genes to investigate whether the inclusion of Env, missing in the Step and Phambili trials, could result in measureable protective effects. The HVTN 505 trial did not see an increased rate of HIV infection, but also resulted in no protection against acquisition and no lowering of viral loads among vaccinated individuals who did become infected.

Most recently, and perhaps most controversially, is the RV144 Thai trial (NCT00223080), where claims of protection against acquisition have been made. The RV144 recruited 16,402 subjects and was a phase III trial of Sanofi-Aventis Pasteur live recombinant ALVAC-HIV priming (vCP1521, a recombinant canarypox vector vaccine with *env* and *gag* inserts) with two booster inoculations of VaxGen gp120 B/E (AIDSVAX B/E, a recombinant glycoprotein 120 subunit vaccine) in HIV-uninfected Thai adults. In the per-protocol analysis involving 12,542 subjects (those who remained eligible to participate in the study and received the entire series of vaccinations within the defined time period), the vaccine efficacy was 26.2% with a nonsignificant P value of 0.16. In the intention-to-treat analysis involving 16,402 subjects, there was a trend toward the prevention of HIV-1 infection among the vaccine recipients, with a vaccine efficacy of 26.4%, but this was not statistically significant with a P value of 0.08. When excluding from the analysis seven subjects who were found to have had HIV-1 infection at baseline, the efficacy was 31.2% with a

significant P value of 0.04. This has been the first trial of a candidate HIV vaccine regimen to demonstrate some level of apparent protection against HIV acquisition. Vaccination did not affect viral loads or the CD4⁺ T-cell count in subjects that became infected (Rerks-Ngarm et al. 2009, Gilbert et al. 2011).

Due to the surprising lack of effect on viral loads initially seen in RV144, the RV152 trial (NCT00337181) followed up a proportion of participants in RV144 to investigate whether longer-term effects on viral load could be seen. The conclusions drawn from this study confirmed that vaccination did not affect the clinical course of HIV disease after infection. An additional trial that began in 2012 in Thailand, RV305 (NCT01435135), is currently investigating late boost strategies for HIV-uninfected participants in the RV144 study. In fact, a number of additional trials are building on RV144: a study similar to RV305 was initiated in 2013, RV306 (NCT01931358). This trial is using the RV144 vaccine regimen, and it will study boosting strategies after vaccination in new individuals that did not participate in RV144. Similarly, the RV328 trial (NCT01933685), which started in 2014, will evaluate the sequential administration of gp120 B/E (AIDSVAX B/E) with 1-year boosting in HIV-uninfected Thai adults. Another follow-up of RV144 is the HVTN 100 study (NCT02404311). This is a phase I/II randomized, double-blind, placebo-controlled clinical trial of clade C ALVAC-HIV (vCP2438) and bivalent subtype C gp120 with MF59 adjuvant in HIV-uninfected adults at low risk of HIV infection. This trial is currently ongoing and is estimated to be completed at the beginning of 2017. On 2016 HIV Vaccine Awareness Day (May 18), the NIH announced the decision to move forward with HVTN 702, a new phase IIB/III HIV vaccine efficacy clinical trial in South Africa. According to the NIH, this decision was taken based on early data from the HVTN 100 trial showing that the new vaccine regimen is safe and produces a robust immune response. This new trial began in November 2016, after obtaining regulatory approval. It is testing a new HIV vaccine regimen designed to improve upon the efficacy observed in

the RV144 trial and to evaluate the vaccine in a higher risk population. HVTN 702 will enroll 5400 HIV-uninfected men and women who are at risk for HIV infection. Both the DNA and the protein vaccine have been modified from RV144 to be specific to HIV subtype C, the predominant in southern Africa. The adjuvant to be used with the protein vaccine will be MF59, an oil-in-water adjuvant that uses squalene (a natural organic compound originally present in shark liver oil and some plants) and two nonionic surfactants: polysorbate 80 and sorbitan trioleate. This is a different adjuvant than the one used in RV144, thus aiming for a more robust antibody response. In addition, the vaccine regimen will include boosters at the 1-year mark in an attempt to prolong the early protective effect seen in RV144. A summary of the efficacy trials in humans, including the ones undergoing, can be found in Table 2. For more details on RV144 follow-up studies, please see Kim et al. (2015).

Chemoprophylaxis

Preexposure prophylaxis, or PrEP, is one approach where unambiguous protection against acquisition has been achieved. PrEP is when people take a drug before being exposed to the virus thus aiming to diminish the risk of infection. Because of the side effects of these drugs, including the risk of serious adverse events, this strategy is only indicated for HIV-negative people who are at substantial risk of HIV infection.

Indeed PrEP has proven very effective, but for achieving the desired level of efficacy, it is very important that individuals take it as directed. As reported in a phase III clinical trial in 2499 men who have sex with men (the iPrEx study, NCT00458393), taking one pill per day of FTC/TDF (emtricitabine/tenofovir disoproxil fumarate) antiretroviral drugs can reduce an individual's risk of contracting HIV infection by up to 92%. Similarly, the FTC/TDF PrEP has shown strong protection against HIV acquisition in heterosexually active men and women, reducing the risk of HIV infection by 62% (the BOTUSA MB06 trial, NCT00448669). Moreover, up to

Vaccine Efforts Against AIDS, Table 2 Summary of human efficacy trials

Trial	Identifier	Phase	Vaccine	Outcome
VaxGen	NCT00002441	III	gp120 B/B in adjuvant	No protection
VaxGen	NCT00006327	III	gp120 B/E in adjuvant	No protection
Step	NCT00095576	IIB	<i>gag/pol/nef</i> B rAd5	Enhancement
Phambili	NCT00413725	IIB	<i>gag/pol/nef</i> B rAd5	Enhancement
HVTN 505	NCT00865566	IIB	<i>env</i> A,B,C/ <i>gag/pol/nef</i> B DNA + rAd5	No protection
RV144	NCT00223080	III	<i>env/gag/pro</i> B rPox + gp120 B/E	Modest protection?
RV152	NCT00337181	III	Long-term follow-up on RV144	Unaffected viremia
RV305	NCT01435135	II	Late boost on RV144	(Ongoing trial)
RV306	NCT01931358	II	RV144 vaccine + boost	(Ongoing trial)
RV328	NCT01933685	II	gp120 B/E + 1-year boost	(Ongoing trial)
HVTN 100	NCT02404311	I/II	<i>env</i> C, <i>gag/pro</i> B + gp120 C	(Ongoing trial)
HVTN 702	NCT02968849	IIB/III	<i>env</i> C, <i>gag/pro</i> B + gp120 C	(Ongoing trial)

90% protection was seen with the same drugs in discordant couples that adhered to the regimen (the Partners PrEP study, NCT00557245). TDF alone also reduced the risk of HIV infection in injection drug users by almost 50% (the Bangkok Tenofovir Study, NCT00119106). Another interesting study is the VOICE trial or MTN-003 (NCT00705679), aimed at assessing the effectiveness of TDF and FTC/TDF tablets in preventing HIV in women. Besides investigating prophylaxis with oral drugs, this trial also evaluated the effectiveness of tenofovir 1% gel, a vaginal microbicide, with the same purpose. While initial analysis suggested that none of the drug regimens reduced the rates of HIV-1 acquisition, post-trial analysis suggested that the application of the tenofovir gel could confer a good degree of protection but only when consistent adherence was observed. This and other studies evidenced the surprisingly low adherence of the enrolled individuals to the PrEP, despite continuously reporting its proper use (Marrazzo et al. 2015). In the same line of chemoprophylaxis, a separate study (NCT00074581) demonstrated that initiating antiretroviral therapy in therapy-naive, HIV-infected people could prevent the sexual transmission of HIV among HIV-discordant couples. Other drugs such as maraviroc, an entry inhibitor (it is a CCR5 receptor antagonist), have also been explored in clinical trials alone or in combination (see, for instance, clinical trials NCT01505114, NCT01719627, and NCT01749566).

Another form of prevention is the post-exposure prophylaxis (PEP). PEP should be used in emergency cases and consists of taking a strong dose of antiretroviral drugs soon enough after viral exposure (usually less than 72 h) and during approximately a month, in an attempt to inhibit viral infection. A number of different clinical trials have compared different drugs alone or in combination, for instance, the phase IV trial NCT01533272 in Barcelona, Spain, in which no seroconversions were observed during the study.

Use of Antibodies for Prophylactic Immunization

Another strategy to prevent HIV infection is the antibody-based approach. It is reasonable to think that the presence of antibodies with potent neutralizing activity against a broad range of HIV isolates would be able to provide a sterilizing barrier to infection against most HIV strains circulating in the population. Unfortunately, even HIV-infected people rarely make such antibodies. Certainly no one has yet figured out how to elicit them. But over the last several years, a remarkable collection of monoclonal antibodies with such broad, potent activity has been isolated and characterized (Halper-Stromberg and Nussenzweig 2016). Novel high-throughput technologies, including single-cell sorting combined with the use of improved baits and

single-cell antibody cloning, have made possible the isolation of a good number of potent and broadly neutralizing antibodies from HIV-infected people. One creative strategy is to bypass the immune system and deliver already known and well-characterized broadly neutralizing antibodies isolated from selected donors directly to the host. Delivery of these antibodies as an attempt to prevent HIV infection will be endeavored in two trials: the HVTN 703/HPTN 081 trial (NCT02568215) and the HVTN 704/HPTN 085 trial (NCT02716675). The first is a phase IIB study that started on April 2016 and will evaluate the safety and efficacy of the broadly neutralizing antibody VRC01 in reducing acquisition of HIV-1 Infection in 1500 women in Botswana, Kenya, Malawi, Mozambique, South Africa, Tanzania, and Zimbabwe. The latter will similarly evaluate VRC01 efficacy against HIV acquisition but among men (and transgender persons) who have sex with men. The study is currently enrolling 2700 individuals and will take place in several locations in Brazil, Peru, and the United States.

It is worth mentioning that broadly neutralizing antibodies can also be used as a therapeutic approach in already infected individuals. For instance, 3BNC117, 10-1074, and VRC01 are currently being used for that purpose in human clinical trials. While passive administration of neutralizing antibodies can be considered for both the prevention and treatment of HIV infection, the large-scale applicability of passive administration is hampered by cost and compliance considerations. Long-term delivery of such broadly neutralizing monoclonal antibodies using vectors such as adeno-associated virus (AAV) has enormous potential for greatly impacting the worldwide epidemic (Fuchs and Desrosiers 2016).

Summary of Preclinical Vaccine Trials in Monkeys

Two monkey model systems have been used extensively for preclinical vaccine research in the HIV/AIDS arena: SIV in macaque monkeys

and SHIV in macaque monkeys. The SIV strains that have been most extensively used were derived from sooty mangabey monkeys (SIV_{sm}) or from macaque monkeys (SIV_{mac}). SIV_{mac} has its origins from cross-species transmission from sooty mangabey monkeys in captivity, and thus, these SIVs are closely related. While SHIV strains have been less commonly used than SIV for challenge of vaccinated monkeys, recent advances in the consistency of SHIV performance (Li et al. 2016) may portend greater use in the coming years. While rhesus monkeys (*Macaca mulatta*) of Indian origin have far and away been the most frequently used species, other species of macaque have also sometimes been used. These include the pig-tailed macaque (*Macaca nemestrina*) and the cynomolgus macaque (*Macaca fascicularis*) (Ansari and Silvestri 2014). Some investigators have preferred to use cloned virus stocks for challenge, while others have preferred to use passaged stocks of uncloned virus, whether SIV or SHIV. Advantages of using cloned virus stocks include homogeneity of a defined virus sequence, better control of the laboratory research conditions, clarity in the meaning of antibody-mediated neutralization, and ability to exactly match, or mismatch, the sequences in the challenge virus with the sequences in the vaccine (Korioth-Schmitz and Schmitz 2014). Arguments for using uncloned virus include a better reflection of the real-world situation of HIV infection of humans. Additionally, many of the initial cloned SHIV stocks have required passage in monkeys in order to yield reasonably consistent viral loads upon monkey infection. Cloned SIVs used for vaccine studies include the most frequently used SIV_{mac}239, SIV_{mac}766, and SIV_{sm}E543; uncloned SIVs include the most frequently used SIV_{mac}251. Cloned SHIVs include the nicely performing SHIV-AD8eo cloned virus, while examples of uncloned SHIVs include SHIV 89.6PD and SHIV DH12R. Notably, a very interesting version of SHIV with an envelope that shows enhanced CD4 binding and replication in rhesus macaques has been recently described (Li et al. 2016). Modified versions of HIV-1 able to infect monkeys weakly have also been generated.

Besides having the choice of the appropriate virus strain, there are additional advantages of using the macaque model: the dose of virus and the challenge route can also be carefully selected (Del Prete et al. 2016). Studies in monkeys can also be more invasive than in humans and therefore more thorough and detailed. An additional advantage of doing vaccine trials in monkeys is that a variety of approaches and strategies can be compared for far less cost than efficacy trials in humans, and the studies can be completed faster. Different approaches and strategies can be rank-ordered in order to inform and guide selections for efficacy trials in humans (Shedlock et al. 2009).

The greatest degree of protection has unquestionably been achieved using live attenuated strains of SIV, including strains with an inactivating deletion of the *nef* gene (Koff et al. 2006). However, even live attenuated SIV has provided unimpressive protection against SIV strains not matched in sequence to the vaccine. This situation is perhaps analogous to the inability of infection with one HIV-1 strain to routinely protect against superinfection by a different HIV-1 strain following an infectious exposure. In any event, live attenuated strains of HIV are not seriously being considered for use in humans because of concerns for safety.

In terms of viable vaccine approaches, perhaps the most impressive results have been obtained by Dr. Louis Picker's group in Oregon using recombinant cytomegalovirus. The recombinant rhesus monkey CMV strain that has been used contains a deletion of several nonessential genes and results in a highly unusual, nonclassical cellular immune response. Approximately 50% of the challenged monkeys showed aviremic control of infection with the highly pathogenic strain SIVmac239. Replication-competent SIV was not detected in protected vaccine recipients, providing evidence for clearance of a pathogenic lentiviral infection (Hansen et al. 2013). While the results are indeed impressive, this vaccine approach has unfortunately not provided protection against acquisition (or delayed the time to acquisition), and these studies have utilized challenge virus (SIVmac239) exactly matched to the sequences present in the vaccine.

It is worth mentioning that a different viral vector, the Ad5 like the one used in the Step trial, was tested beforehand in macaques. Results were initially very encouraging since good T-cell responses could be measured, and even reduction in viral loads was observed in vaccine recipients when challenged with SHIV. Nevertheless, it was soon realized that these vaccines were only efficient in protecting against the same SHIV strain used for the vaccine and were not offering the same degree of protection against a more pathogenic SIV.

As mentioned above, the human trial RV144 used both DNA inoculation (ALVAC-HIV) with *env* and *gag* inserts and protein boosts (VaxGen gp120) in alum adjuvant. Various follow-up clinical trials to the RV144 study are now using MF59 as a new adjuvant, which is considered more immunogenic. Interestingly, recent monkey studies have shown that the SIV equivalent vaccine ALVAC-SIV + gp120 with MF59 adjuvant was not efficacious in delaying the onset of infection with SIVmac251 in rhesus macaques, while ALVAC-SIV + gp120 with alum did show some efficacy in this regard (Vaccari et al. 2016).

In the antibody arena, successful passive immunization studies in macaques showing that broadly neutralizing human monoclonal antibodies could prevent the acquisition of infection via many different routes have definitely encouraged the use of these antibodies in clinical trials in humans (as seen in previous sections). Thanks to additional monkey studies, we also now know that the seeding of the viral reservoirs after infection happens very quick (at least as soon as in 3 days, during the eclipse phase and before detectable viremia). In line with this, early passive immunotherapy with broadly neutralizing antibodies can prevent the establishment of viral reservoirs when inoculated 24 h after SHIV oral challenge in 1-month-old rhesus macaques as a model of mother-to-child transmission (Hessell et al. 2016).

It is also important to mention that in the last few years, a better understanding of the HIV envelope trimer configuration and structure has been achieved, thus allowing a better design of immunogens, like the SOSIP trimers. Pathways for the generation of broadly neutralizing antibodies

from the germ line have also been investigated in the macaque model and are currently better understood. Attempts at these approaches in humans will probably happen sooner rather than later. It is also worth mentioning that one of the most potent and broad entry inhibitors discovered so far, the eCD4-Ig (a fusion of CD4 and the Fc portion of an IgG with a small CCR5-mimetic sulfopeptide), is planned to reach clinical trials at the end of 2018 after very promising results in monkey studies in which it provided complete sterilizing protection against SHIV challenges (Gardner et al. 2015).

Conclusion

After more than three decades on the quest to find a prophylactic vaccine against HIV, success remains elusive. The difficulties all relate to the properties of the virus itself. These include but are not limited to immune evasion strategies employed by the virus, the ability to replicate continuously in the face of apparently strong host immune responses to the virus, extreme heterogeneity in the sequences of the virus, and the inability of infection by one strain to protect against superinfection by another strain. The predicted difficulties have more or less been borne out by six large efficacy trials in people. Promising strategies emerging from studies in monkeys include live, replication-competent, persisting, recombinant herpesvirus (CMV) and long-term delivery of monoclonal antibodies with potent broadly neutralizing activity. It is likely that studies in monkeys will become increasingly important in order to inform and guide the field regarding most promising strategies.

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Viral Fitness in Hosts

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Definition

The fitness of an organism can be defined as its ability to survive and successfully replicate in a specific environment.

Introduction

Once the host has been effectively invaded, the virus needs to spread and replicate successfully, finding target cells and escaping host defenses at the same time. During this time, virus-host interactions take place, impacting viral fate. Each player has thus to obey certain constraints to survive and resist to selective pressures at the same time, implying some needs to adapt and evolve.

Viral fitness estimates the ability of a virus (or a virus genome sequence) to survive and reproduce in a specific environment (Fig. 1). Although conceptually simple, viral fitness results from a complex interplay between the virus and its host and implies that to adapt and survive, the virus has to evolve in a dynamic environment. Therefore, viral fitness is a relative value rather than an absolute measurement.

Assessing viral fitness is a challenging task, and replication assays are common surrogate assays to investigate virus-host interactions. In the last decade, many efforts have been concentrated in identifying the factors promoting and facilitating HIV replication, as well as the factors from intrinsic cellular defenses that restrict its replication at the cellular level.

Virus Evolution and Constraints

Human immunodeficiency virus (HIV) is an enveloped RNA virus from the *Retroviridae*

family; it is thus characterized by two hallmarks: reverse transcription of the viral RNA genome into a double-stranded DNA and its subsequent integration into the viral host genome. The evolution potential of HIV is determined by three features: (i) a high mutation rate, (ii) a short generation time, and (iii) a large production size (Fig. 1a; Dykes and Demeter 2007; Clementi and Lazzarin 2010; Lauring and Andino 2010; Domingo et al. 2012).

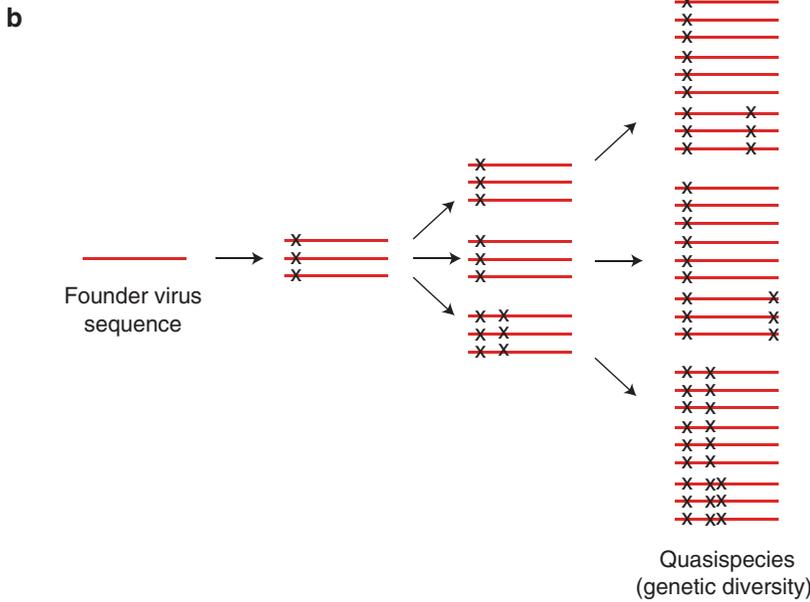
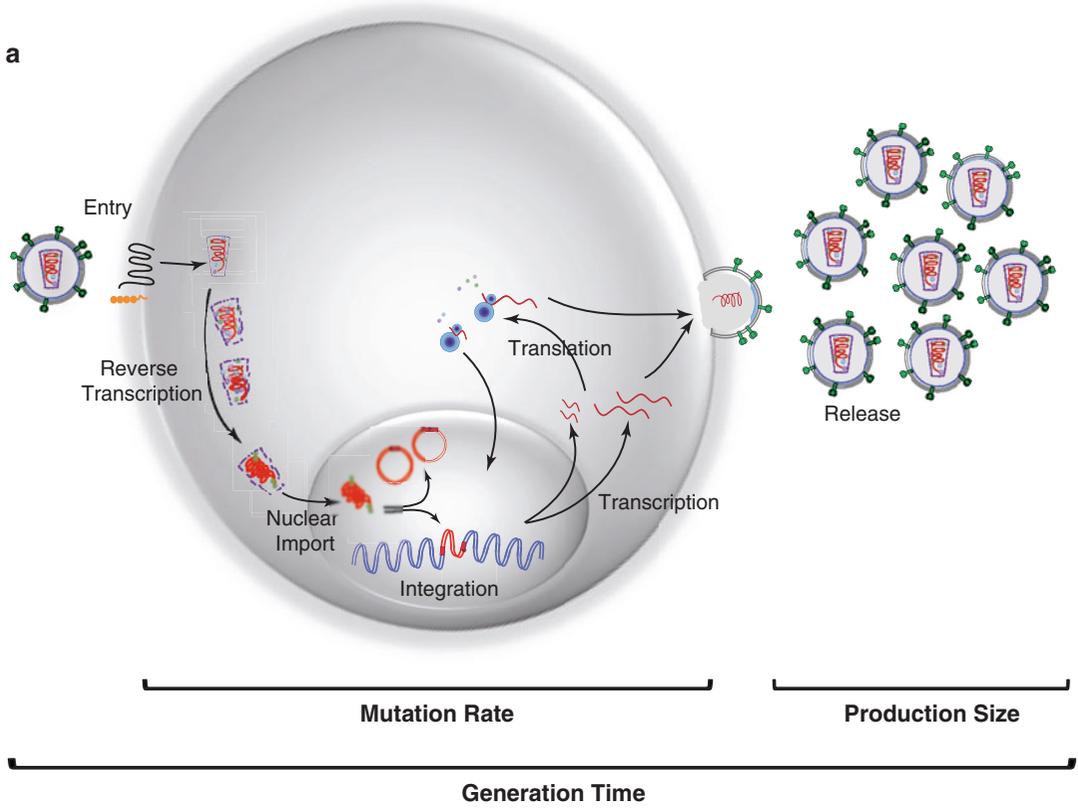
Mutation Rate

A high mutation rate is necessary to generate new variants (or mutants) and thus provides an opportunity to increase genetic diversity.

The mutation rate provides the opportunity to generate a variant sequence. In the case of HIV, mutations are thought to occur principally at the reverse transcription step (Hu and Hughes 2012; Coffin and Swanstrom 2013). The high mutation rate is mainly attributed to the error-prone reverse transcriptase (RT) activity that produces a double-stranded DNA molecule starting from the viral RNA genome. Indeed, this enzyme has no proof-reading activity and thus a poor fidelity. In addition to the RT enzyme activity *per se*, four additional events occurring at the reverse transcription or at the transcription steps can contribute to HIV mutagenesis.

The first one is known as template switch or copy-choice determination: As mentioned earlier, the virion contains two copies of viral RNA genomes, and both can be used as template by the RT to produce the viral DNA copy. During reverse transcription, the RT can thus switch from one copy to the other, thereby providing additional opportunity to add genetic diversity if the two copies are not identical.

The second one is due to the host cellular enzyme APOBEC3G and its cytidine deaminase activity occurring during viral reverse transcription, which results in G-to-A mutations in the reverse-transcribed DNA. Therefore, cellular enzymes can also participate and increase the mutation rate during HIV reverse transcription. Of note, APOBEC3G is a part of the innate immunity, and its hypermutating activity aims at introducing an excessive number of mutations that



Viral Fitness in Hosts, Fig. 1 Evolution rate and genetic diversity. (a) HIV life cycle and processes contributing to genetic diversity. HIV viral particles contain

two copies of viral RNA molecules. Upon binding to CD4 and a CCR5 coreceptor at the surface of the target cell, the viral core is released in the cytoplasm. The viral RNA

would be incompatible with viral functions and thus virus survival (discussed below).

In addition to error incorporations during reverse transcription, HIV genome can also evolve through viral genome recombination (Simon-Loriere and Holmes 2011; Simon-Loriere et al. 2011; Vuilleumier and Bonhoeffer 2015). To occur, several requirements need to be fulfilled: (i) a cell has to be infected by two HIV variants; (ii) during packaging of two copies of viral RNA genomes, one of each viral RNA has to be selected, thereby generating a “heterozygous-like” virion; and (iii) both viral RNA genomes have to be used for reverse transcription (template switch) to generate a recombinant viral DNA genomic sequence. Viral genome recombination represents an additional mechanism for HIV genome evolution.

Finally, another cellular enzyme unrelated to reverse transcription may contribute to the global HIV mutation rate: the host RNA polymerase II. Like for any host gene, this cellular enzyme transcribes the proviral genome into RNA, more particularly into mRNA. Some of these viral RNA molecules contain the whole viral RNA genome that is subsequently packaged into the nascent virions. As the cellular RNA polymerase II lacks a proofreading activity, it can introduce mutations during this transcription process. Although its exact contribution has not been quantified, the cellular enzyme may still add to the global HIV mutation rate (Coffin and Swanstrom 2013).

For HIV, this mutation rate has been estimated to be $\sim 3 \times 10^{-5}$ mutations/nucleotide, corresponding approximately to one mutated nucleotide arising every three ~ 10 kb viral genomes at each viral replication cycle (Fig. 1b; Coffin and Swanstrom 2013).

Generation Time

The generation time is the time required by the virus to complete its replication cycle, i.e., from entry to release. A rapid replication kinetic implies a short progeny generation time, which accelerates the potential to evolve. The duration of a complete HIV life cycle varies depending on the target cell but can be roughly estimated to be around 1–2 days (Coffin and Swanstrom 2013; Mohammadi et al. 2013).

Production Size

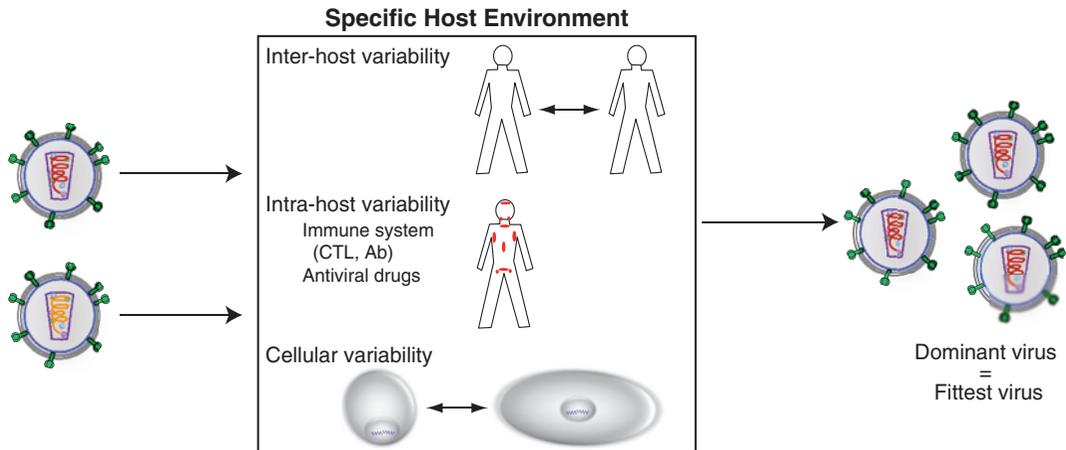
A large population size, i.e., a high virion production rate, increases exponentially the evolution rate as it increases the number of possibilities to evolve. In an infected individual, 10^{10} – 10^{11} particles can be produced per day (Nordkin 2009).

Evolution Rate: Balance Between Adaptation and Survival

All together, these three features ensure that from one transmitted virus genome sequence, HIV rapidly evolves to a swarm of quasispecies that are all genetically distinct but related and that may differ in their viral fitness at a given time and in a specific local environment (Fig. 2). The broad

Viral Fitness in Hosts, Fig. 1 (continued) genome is reverse-transcribed by the viral reverse transcriptase (RT) enzyme in a double-stranded DNA copy, which is in turn translocated to the nucleus and integrated into the host cellular genome (host genome, *blue*; HIV provirus, *red*). The proviral DNA genome is transcribed and translated by the cellular machinery, allowing assembly and release of viral particles to take place. The evolution rate depends on three parameters of the viral life cycle depicted on the bottom. (i) The mutation rate, which provides the opportunity to generate a variant sequence and occurs mostly during the reverse transcription step. (ii) The generation time, which is the time required from virion entry to release of new infectious viral particles. (iii) The production size,

corresponding to the amount of infectious virions produced. All together, these three parameters contribute to the fast evolution rate typical of HIV. (b) Generation of viral genetic diversity. One founder virus sequence (*red line*) can be mutated during reverse transcription (marked by x), estimated to be around one mutation every three genomes (marked by x). The large production size and the short generation time accelerate the evolution rate of HIV, thereby generating a large genetic diversity characterized by a swarm of viral quasispecies (that are genetically related but distinct). Selection of the fittest quasispecies is then dependent on viral constraints and host-specific selective pressures.



Viral Fitness in Hosts, Fig. 2 **Viral fitness.** Two variants of a virus (depicted as virions containing respectively a *red* or an *orange* viral genome sequence on the *left*) may be able to replicate differentially in a specific host environment (*black rectangle*). This environment is variable and can be considered as being the whole organism, a local

environment present in a specific organ or tissue (*red*), or a specific cell type. The fittest virus (here the *red* one) outcompetes the other one (that may be eliminated by the immune system, lethally mutated and thus unable to replicate, or less fit) and is thus overrepresented in the viral population. *CTL* cytotoxic T lymphocytes, *Ab* antibodies

genetic diversity accumulated over time within an HIV-infected individual identifies HIV as being the human virus with the fastest rate of evolution to date (Coffin and Swanstrom 2013).

The evolution potential reflects the ability of a virus to adapt to a specific environment and to its selective pressures, with the mutation rate being one key component (Clementi and Lazzarin 2010; Lauring and Andino 2010). A very low level of mutations leads to low opportunities to adapt and evolve and therefore also lower probabilities to escape to elimination by the adaptive immune system. In contrast, an excessive mutation rate leads to lethal mutagenesis, i.e., to hypermutated sequences likely resulting in nonfunctional proteins and thus incompatible with viable infectious viruses. Indeed, HIV has to obey to specific viral, immunological, and structural constraints in order to successfully replicate. These include (i) the expression of virally encoded proteins able to fulfill specific functions such as catalytic activities, binding to nucleic acids or interacting with other protein partners, (ii) the escape to host recognition and immune-mediated elimination, and (iii) the maintenance of the viral RNA structure necessary for viral function such as tRNA binding, dimerization, binding to viral proteins, and

packaging into virions (Dykes and Demeter 2007; Snoeck et al. 2011; Telenti and Johnson 2012; Bartha et al. 2013).

The equilibrium between mutation rate increasing genetic diversity, adaptation to host-specific environment, and ability to survive and complete replication cycle can also be illustrated at the level of HIV quasispecies that are not equally fit. Viral quasispecies that do not mutate may not survive due to host selective pressures (for instance, adaptive immunity) to which they did not adapt to. In contrast, HIV quasispecies that mutated too much may be incompatible with virus survival. Therefore, adaptation of HIV to a specific environment results from a trade-off between the mutation rate and virus survival.

The number of mutations per genome after which virus viability starts to be compromised is known as the error threshold and is implicated in the error catastrophe scenario (Clementi and Lazzarin 2010; Lauring and Andino 2010). Proteins able to modify the error rate of viruses may thus affect the balance between evolution potential and lethal mutagenesis and start that catastrophe scenario. These proteins may thus represent a cellular defense mechanism. In the case of HIV, this is exemplified by the cytidine deaminase

enzyme APOBEC3G that hypermutates the viral genome during the reverse transcription process, rendering it incompatible with virus survival.

Host-Specific Environment

The environment in which HIV evolves needs to consider three levels of variability: (i) inter-host variability, (ii) intra-host variability, and (iii) cellular variability (Fig. 2). It is important to note that specific environments are also not static but rather dynamic and vary over time, for example, upon aging, immune system changes, or cellular activation states. Finally, all these specific environments coexist at the same time, adding complexity in the selective forces and in the characterization of viral fitness.

Due to the variable nature of the viral environment within the same host, assessing viral fitness is a real challenge. To date, multiple assays have been developed to estimate viral fitness, including replication kinetics, competition assays between viruses, and single-round infectivity assays (van Opijnen and Berkhout 2005; Dykes and Demeter 2007). These assays vary according to the virus and to the cell type used. At the virus level, whole patient-derived virus isolates, viral portions subcloned into a reference vector, or mutated reference vectors are used. Target cells used are either T-cell lines, primary cells (peripheral blood mononuclear cells, monocyte-derived macrophages, CD4⁺ T cells), or indicator cell lines. Although these assays provide valuable information, they fail to recapitulate all the selective pressures existing in the host environment.

Inter-Host Variability

Inter-host transmission represents a bottleneck, as only one virus is successfully transmitted: the founder virus (Gutierrez et al. 2012). Thus, both the donor host and the recipient host may affect viral fitness of the founder virus and thus contribute to shape the genetic viral background. Two different hosts that are genetically different provide two specific environments in which a same HIV variant may evolve differently as they will provide different selective pressures, such as

different adaptive immune responses or different concentrations of antiviral drugs (Telenti and Goldstein 2006; Fellay et al. 2010).

Intra-Host Variability

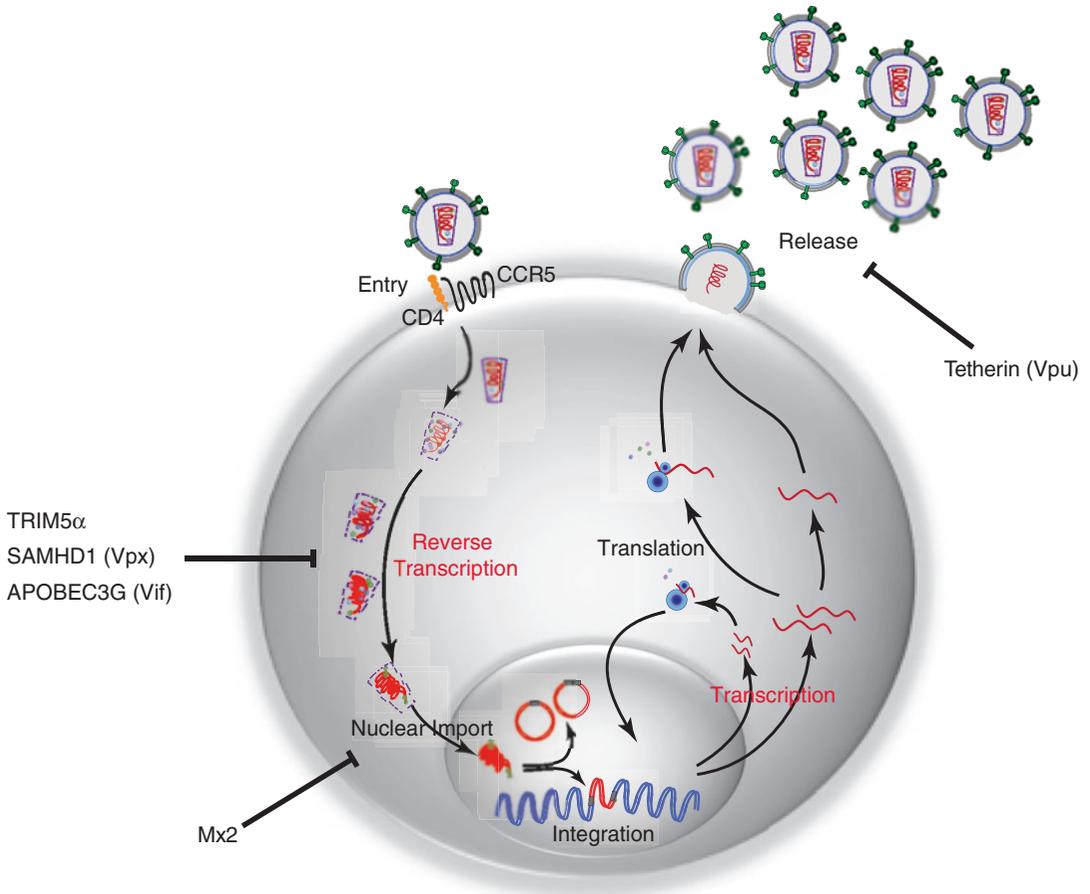
Within the same infected host, different sites of viral replication provide specific local environments. These include blood vessels, lymph nodes, gut-associated lymphoid tissue (GALT), the central nervous system, and the genital tract. The extracellular environment exerts multiple selective pressures, characterized by adaptive immunity (cytotoxic T lymphocytes (CTL), antibodies, cytokines) as well as the presence of antiviral drugs (Fletcher et al. 2014). Of note, these selective pressures change during the clinical course of HIV infection, as illustrated by the absence of specific anti-HIV antibodies during the first weeks of HIV infection or by the increase of CTL upon HIV infection.

Viral replication occurs at multiple sites or organs, thus constituting different local environments that may promote evolution of compartmentalized quasispecies.

Cellular Variability

HIV can infect differently multiple cells, characterized by specific proteomes and transcriptomes, such as memory CD4⁺ T cells, naïve CD4⁺ T cells, activated or resting CD4⁺ cell subsets, and macrophages. The cellular environment plays an essential role in viral replication as HIV interacts with many proteins. Indeed, to survive and successfully reproduce, a virus has to deal with a specific cellular environment, hence profiting from factors promoting and facilitating its replication as well as coping with factors blocking it. Viral replication success will thus result from a balance of HIV interactions with these two types of players, HIV dependency factors (HDF) and restriction factors (Fig. 3).

During its progression through the cell, HIV interacts with a plethora of cellular proteins that are essential to successfully replicate (Bushman et al. 2009; Jager et al. 2012). Among this large list of HIV dependency factors, key cellular proteins include the viral receptor CD4 and the coreceptor CCR5 (or CXCR4) that are essential



Viral Fitness in Hosts, Fig. 3 **Viral replication success in a specific cellular environment.** In a specific cellular environment, viral fitness can be measured as the size of viral progeny particle or in replication kinetic assays. In these systems, success will depend on virus ability to efficiently complete its replication cycle and thus produce progeny virions, by (i) providing viral proteins executing

the required enzymatic activities and exploiting HIV dependency factors (HDF) and (ii) providing viral countermeasures or adaptive mutations to evade restriction factors and cellular antiviral defenses. Restriction factors are indicated at the level where they block HIV replication cycle. Viral countermeasure proteins are indicated in brackets.

for viral entry, TNPO3/TRN-SR2 and LEDGF/p75 that are essential for viral genome nuclear import and integration, multiple cellular transcription factors such as cyclinT1/cdk9 and NF-κB that facilitate viral transcription, proteins involved in nuclear export pathways allowing to translocate viral RNA to the cytoplasm, and ESCRT complex proteins that are required for budding and release.

On the other hand, innate immunity is a natural barrier against infections that aims at recognizing the invading virus, at promoting an antiviral state through the production of interferon and

interferon-stimulated genes, and finally at blocking and eliminating the viral pathogen (Schoggins and Rice 2011; Schoggins et al. 2011, 2014; Sandler et al. 2014; Schoggins 2014). Restriction factors are part of the innate immunity cascade; they are induced by interferon and directly interact with viral proteins in order to impede HIV progression and replication. Examples of restriction factors include TRIM5α, SAMHD1, APOBEC3G, and tetherin (Kirchhoff 2010; Blanco-Melo et al. 2012; Harris et al. 2012; Malim and Bieniasz 2012; Pyndiah et al. 2015).

TRIM5 α binds to the viral core and promotes its premature disassembly, thereby impairing efficient viral reverse transcription. SAMHD1 is an enzyme that hydrolyzes deoxynucleoside triphosphates to deoxynucleosides, thereby reducing the concentration of intracellular nucleotides necessary for viral reverse transcription completion. APOBEC3G is a cytidine deaminase acting at the minus-strand DNA during the reverse transcription process, resulting in G-to-A mutations in the positive-strand DNA and eventually leading to lethal mutagenesis. Tetherin is a membrane-anchored protein that incorporates into virion membranes, thereby retaining viral particles at the cell surface and impeding their full release. Because restriction factors represent selective pressures exerted by intrinsic cellular defenses, escape mutants or counteracting proteins encoded by the virus have been selected over time: a mutation in the viral capsid protein to escape TRIM5 α recognition and mediated degradation; the accessory viral Vif protein that binds and promotes proteasome-mediated APOBEC3G degradation to block its packaging in viral particles and thus its action during reverse transcription in the subsequent infected cells; and the accessory viral Vpu protein that mediates endocytosis and degradation of tetherin. Although no HIV-1 protein counteracts SAMHD1, HIV-2 encodes the Vpx accessory protein that is able to inhibit SAMHD1 restriction activity by promoting its proteasomal degradation. Identifying these restriction factors is essential to understand the role of innate immunity in shaping and selecting for viral sequences within a given cellular environment. The presence of restriction factors and viral countermeasures illustrates the continuous virus-host interplay and evolutionary race toward selective pressures.

Impact of HIV Integration

An additional complexity linked to HIV relies to its ability to integrate. Indeed, integrated HIV implies that viral genome sequences can persist within long-lived cells and can be archived, offering an opportunity for later reemergence. This may provide a second chance to poorly fit viruses under specific environmental conditions at one

time point, to become the fittest viruses under a novel environment at another point in time. The reemerging viruses may thus face a different fitness landscape.

Conclusion

The evolution potential of HIV allows for generation of rapid and unprecedented genetic diversity. Although genetic diversity provides the opportunity to adapt to environmental constraints, it also has a cost at the viral success level. In other terms, adaptation is not free for all possibilities as the virus maintains conserved regions, as compensatory mutations are needed to allow certain mutations to be retained, and as there may be extensive evolutionary dependencies, in particular upon recombination. Because of these multiple viral, cellular, and structural constraints, many quasispecies are dead viruses, unable to replicate and continue spreading.

On another hand, although production of a large amount of viral quasispecies among which only a few are infectious may be considered as a waste of cellular and viral resources, it may also be considered as a way to divert the attention of the host immune system, as the infectious virions could hide and thus possibly escape. All together, viral fitness of quasispecies contributes to replication success, to adaptation to multiple environments, and thus to the epidemiological success of HIV, although the link between viral fitness and clinical outcome has still to be fully established.

Cross-References

- ▶ [APOBEC3F/G and Vif: Action and Counteractions](#)
- ▶ [Attachment/Binding](#)
- ▶ [Budding](#)
- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [Cellular Cofactors for HIV-1 Transcription](#)
- ▶ [Cellular Cofactors of HIV as Drug Targets](#)
- ▶ [Chronic Immune Activation in HIV](#)

- ▶ Counteraction of SAMHD1 by Vpx
- ▶ CXCR4, Coreceptors
- ▶ DDX3, Cofactors, and RNA Export
- ▶ HIV and SIV, CD4 T-Cell Responses to
- ▶ HIV and SIV, CD8 T Cell Responses to
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- ▶ Immunology of Latent HIV Infection
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- ▶ Mucosal Immunity to HIV-1
- ▶ Nef/Env/Vpu/Tetherin
- ▶ Nuclear Import: HIV-1 Goes NUPS
- ▶ Overview of HIV CNS Infection
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- ▶ Pyroptosis in HIV Infection: HIV Offense Meets a Fiery Host Defense
- ▶ Reverse Transcriptase-Catalyzed HIV-1 DNA Synthesis
- ▶ Role of Antibodies in HIV Transmission
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- ▶ Role of Transportin-SR2 (Transportin-3, TRN-SR2, TNPO3) in HIV Replication
- ▶ Transcription (Initiation, Regulation, Elongation)
- ▶ TRIM Protein Family and Viral Restriction
- ▶ TRIM5alpha
- ▶ Uncoating and Nuclear Entry
- ▶ Virus Assembly
- ▶ Virus-Host Evolution and Positive Selection

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Viremic Nonprogressors

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Definition

Viremic nonprogressors are untreated HIV-1-infected individuals who maintain stable CD4+ T cell counts within the normal range for more than 10 years, despite moderate to high levels of HIV-1 replication. However, in most viremic nonprogressors, slow disease progression with gradual CD4+ T cell decline occurs at one point, and these individuals may eventually develop AIDS when left untreated. Viremic nonprogressors are therefore also referred to as viremic slow progressors. Viremic nonprogressors should not be confused with HIV-infected controllers who are able to control viral replication resulting in very low or undetectable viremia and stable CD4+ T cell counts.

Introduction

Prior to the introduction of effective ART, it was observed that a minority of HIV-infected patients did not progress to AIDS or death. These individuals, termed long-term nonprogressors (LTNP), maintained normal CD4+ T cell counts in the absence of treatment for several years – in some cases for more than two decades, as opposed to the majority of HIV-1-infected persons who typically progressed to AIDS and death within a median of approximately 8 years. When PCR assays for measuring HIV RNA were introduced, it was evident that some of these patients with preserved CD4+ T cell counts had low or even undetectable viral replication. This phenomenon leads to the classification of HIV-infected controllers defined by their ability to control viral replication in the

absence of ART. Controllers can be further divided into elite controllers (EC) and viremic controllers (VC), most commonly with HIV RNA <50 copies/mL and 50–2,000 copies/mL, respectively, although variations with higher levels are found as well (Okulicz et al. 2009; Hunt 2009; Saag and Deeks 2010). In contrast, true viremic nonprogressors typically display HIV-1 viral loads >10,000 copies/mL (Poropatich and Sullivan 2011), while maintaining similar or only slightly lower levels of CD4+ T cells compared to HIV-1 controllers. Today, the term “long-term nonprogressors” is widely used as a collective name for controllers and viremic nonprogressors.

It is well established that viremic nonprogressors and controllers are different subpopulations (Okulicz et al. 2009; Grabar et al. 2009; Petrucci et al. 1997; Lambotte et al. 2005), suggesting that different immunological mechanisms are responsible for the preserved CD4+ T cell counts. Viremic nonprogressors and controllers are described as rare populations, with little overlap between them (Okulicz et al. 2009; Hunt 2009; Grabar et al. 2009; Petrucci et al. 1997; Lambotte et al. 2005; Boufassa et al. 2011). Viremic nonprogressors are, along with elite controllers, reported from different cohorts to comprise <1% of HIV-infected individuals, whereas viremic controllers are more frequent depending on the level of viremia (Okulicz et al. 2009; Hunt 2009; Grabar et al. 2009; Petrucci et al. 1997; Lambotte et al. 2005; Boufassa et al. 2011). However, the definition of the populations suffers from lack of consensus in terminology and inclusion criteria, impeding the comparison of findings. In addition, a central problem in defining the nonprogressor phenotype is a complete lack of inclusion of the viral load in some cohorts, thereby confusing viremic nonprogressors with controllers and vice versa.

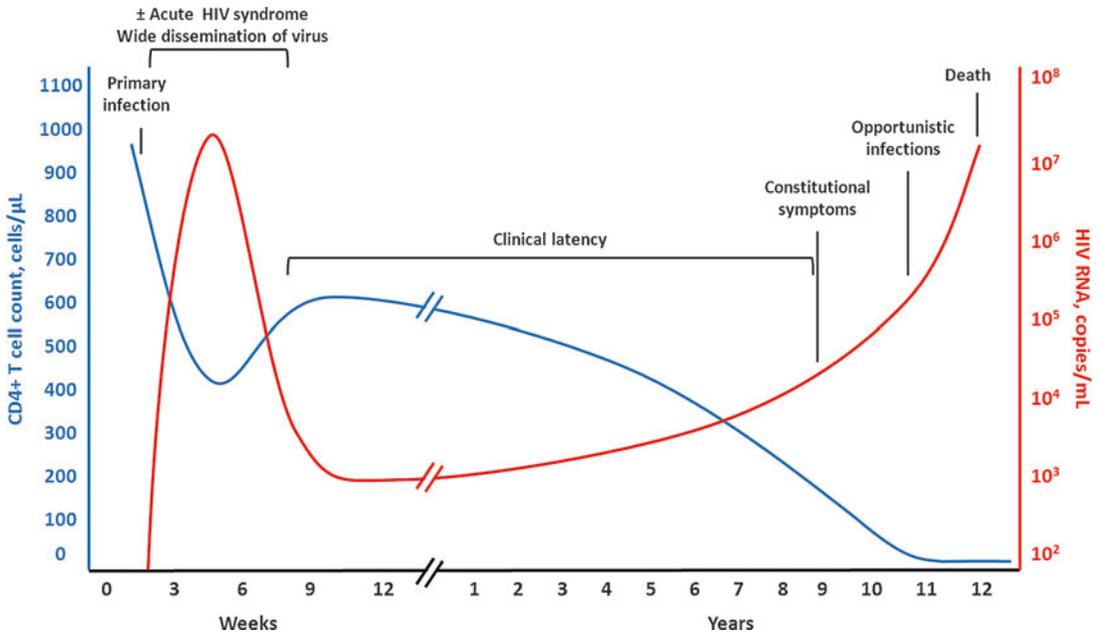
Patients need not necessarily be infected for a long period of time in order to be categorized as controllers. Thus, a single measurement of spontaneous control may be used as sufficient for defining a controller, whereas others demand several years of infection. In contrast, viremic nonprogressors due to the nature of the case definition

have a long duration of infection, most often 7–10 years. Viremic nonprogressors present with more or less stable CD4+ T cell count within the normal range (390–1,600 cells/ μ L) (Okulicz et al. 2009; Grabar et al. 2009; Petrucci et al. 1997; Rodes et al. 2004). Due to the low prevalence of viremic nonprogressors, it is tempting to relax the inclusion criteria. However, the stringency of inclusion criteria seems to predict the clinical outcome. Thus, a better survival among viremic nonprogressors defined by 10 years of stable CD4 T cell counts versus 7 years is reported (Okulicz et al. 2009), suggesting that even 7 years of stable infection does not distinguish adequately between true viremic nonprogressors and progressors.

This entry focuses on viremic nonprogressors, while controllers are discussed in a separate entry of this encyclopedia. However, as data on viremic nonprogressors are very limited, some data referring to LTNP without further classification based on viral load or plasma viremia are also included.

The Normal Course of HIV Infection

Three to 6 weeks following primary infection, 50–70% of infected individuals experience an acute clinical syndrome characterized by fever, “flu-like” symptoms, and other neurological, dermatological, or hematological manifestations. This acute infection is characterized by severe depletion of CD4+ T cells in the gut-associated lymphoid tissue (GALT), where most CD4+ T cells reside. During acute infection, high-level viral replication takes place in almost all body compartments, particularly in the GALT and other lymphoid organs, leading to increased immune activation. As activated cells are more susceptible to HIV infection, this massive viral replication is the beginning of a vicious cycle where viral replication drives the infection of newly activated CD4+ T target cells (Fig. 1). Along with the establishment of a partly efficient adaptive immune response leading to suppression of replication and elimination of virus-producing CD4+ T cells, the balance between viral replication and target cells eventually reaches equilibrium. This leads to a decrease in viral replication



Viremic Nonprogressors, Fig. 1 CD4+ T cell count (*blue*) and viral load (*red*) during untreated HIV infection (Adapted from Pantaleo et al. 1993)

and a subsequent stabilization of the levels of HIV RNA, *the viral set point*, which is specific for a given individual. This is accompanied by an increase in CD4+ T cells to normal or more often subnormal levels. The viral set point predicts the rate of disease progression, including the rate at which the CD4+ T cells are lost. When the CD4+ T cell count decreases below 200 cells/ μ L, HIV infection is often associated with symptoms of immune deficiency. The infection has now progressed to late stage disease, and the patient may acquire AIDS and may die within a short time if treatment is not initiated (Harrison's).

SIV Infection

Given that the case definition of nonprogressors requires multiple years of follow-up, patients are typically not identified as nonprogressors until late in the disease process. This fact represents a considerable challenge for investigations of viremic nonprogressors, as data and biological samples from acute infection of patients who eventually develop a viremic nonprogressor phenotype are

typically not readily available. Therefore, animal models of non-pathogenic SIV infection in natural hosts, such as sooty mangabeys and African green monkeys, are helpful to delineate the pathogenesis of HIV/SIV infection not leading to CD4+ T cell losses and clinical immune deficiency. In fact, despite clinical nonprogression, natural hosts of SIV infection experience high levels of viral replication and can therefore be considered as functional analogues to viremic nonprogressors (Brenchley and Paiardini 2011). This assumption is supported by gene expression similarities between non-pathogenic SIV infection in sooty mangabeys and human viremic nonprogressors, while HIV-infected rapid progressors exhibited a gene expression profile more closely related to rhesus macaques with progressive SIV infection (Rotger et al. 2011).

Reasons for Nonprogression in Viremic Nonprogressors: Virology

No specific viral variation has so far been identified in viremic nonprogressors that distinguish

these individuals from other HIV-1-infected persons. Also, viral Nef proteins that modulate T cell activation and play a key role in the pathogenesis of AIDS have not been found to be abnormal in viremic nonprogressors (Heigele et al. 2014). Thus, host genetics or host immune responses appear to be the main reason responsible for viremic nonprogression of HIV-1 infection (Gaardbo et al. 2012).

Reasons for Nonprogression in Viremic Nonprogressors: Host Genetics

Prior genome-wide association studies have demonstrated that a number of factors are involved in viral control and a delayed disease progression. In contrast, little is known about genetic determinants for viremic nonprogression. Recently, a single nucleotide polymorphism (SNP) 35 kb upstream of the HLA-C gene locus (−35C/T) that correlates with increased HLA-C expression and improved control of HIV-1 was shown to be possibly enriched in viremic nonprogressors (Ballana et al. 2012). However, these findings will have to be verified in future studies. To what extent other genetic variations, such as specific HLA class I alleles, KIR alleles, or chemokine receptor polymorphisms, are associated with a viremic nonprogressor phenotype is not known. Given that the frequency of viremic progressors is extremely low, it is difficult to appropriately power immunogenetic association studies in this specific patient population.

Immunology in Viremic Nonprogressors: Production of Cells

T cells originate from hematopoietic progenitor cells and mature in the thymus. HIV influences both bone marrow and hematopoietic progenitor cells, and impaired hematopoiesis in HIV infection was demonstrated years ago (Marandin et al. 1996; Moses et al. 1998). Furthermore, progenitor cells themselves may be susceptible to HIV infection as they express CD4, CXCR4, and CCR5. Preservation of cellular immunity in

viremic nonprogressors may therefore be possibly related to a selective sparing of hematopoietic and lymphoid precursor cells from cytopathic effects of HIV infection. Indeed, in a study comparing HIV controllers with normal or reduced CD4+ T cell counts, controllers with progressive CD4+ T cell losses displayed signs of exhausted lymphopoiesis compared to controllers with normal CD4+ T cell counts (Sauce et al. 2011). This supports the idea of a sufficient hematopoiesis as a contributing factor to nonprogressive disease, implicates that viral replication in itself is not the only driver of disease progression, and may suggest that a preserved hematopoietic resource is involved in maintaining CD4+ T cell counts in viremic nonprogressors. Also, it was recently shown that viremic nonprogressors display limited HIV infection of central memory and stem cell memory CD4+ T cells compared to patients with progressive infection who had similar total CD4+ T cell counts (Klatt et al. 2014). This suggests that more immature and long-lived CD4+ T cell populations from viremic nonprogressors may be partially resistant against HIV-1 infection. Notably, a reduced ability to support SIV infection was also noted in central memory and T memory stem cells from sooty mangabeys (Cartwright et al. 2014).

The CD4+ T cell count is maintained by de novo production in the thymus and lymphoid tissue or by homeostatic proliferation of naive CD4+ T cells in the periphery. It is now evident that the thymus is active not only in childhood but also in adulthood, particularly during lymphopenic conditions such as HIV infection (Douek et al. 1998). Thymic function can be assessed as T cell receptor excision circles (TRECs) or as the naive CD4+ T cell count. HIV infection leads to reduced numbers and impaired function of naive CD4+ T cells in blood as well as in lymphoid tissue (Douek et al. 1998). As illustrated by the sink model (Ho et al. 1995), a well-functioning thymopoiesis (the tap) is central in preserving the CD4+ T cell count and is therefore likely to be involved in nonprogression in viremic nonprogressors. In nonprogressors, similar and lower numbers of naive CD4+ T cells have been found compared to progressors (Gaardbo

et al. 2012), and TRECs may be higher in viremic nonprogressors compared to progressors (Gaardbo et al. 2013). The contribution of a well-functioning thymopoiesis to nonprogression is further supported by the findings of strong correlations between TRECs and nonprogression in SIV-infected rhesus macaques (Ho Tsong et al. 2005). Also, normal levels of memory cells and preserved IL-2 secretion capacities have been shown in nonprogressing SIV-infected rhesus macaques compared to progressors (He et al. 2011). For this reason, it seems plausible that viremic nonprogressors possess an extraordinary ability to produce CD4⁺ T cells possibly contributing to the preserved CD4⁺ T cell counts in viremic nonprogressors. Furthermore, it is worth noticing that with increasing age, thymic output is reduced. Thus, the reported loss of the non-progressor status in some viremic nonprogressors may be due to age-related decrease in thymic output. This age-related output could potentially be enhanced by a premature aging which have been suggested to take place in HIV-infected individuals (Sauce et al. 2011).

Secondary Lymphatic Tissue

One of the central challengers in understanding how HIV interacts with the immune system is why lost CD4⁺ T cells are only partly being replaced. One reason may be an HIV-induced damage of the structures in lymphoid tissue where the functional space becomes replaced with collagen (Estes et al. 2008). Lymphoid tissue is important for production, survival, and proliferation of cells. The amount of the collagen-deposition has been associated with the CD4⁺ T cell count and number of naive CD4⁺ T cells (Schacker et al. 2002). At present, it is unknown if viremic nonprogressors are less likely to generate fibrosis in their lymphatic tissue, or if the lymphatic tissue is more effective in producing new cells. In contrast, the amount of lymphoid tissue in viremic non-progressors was found to be reduced in a single study, suggesting that a larger amount of lymphoid tissue is not the main reason for viremic nonprogression (Gaardbo et al. 2013).

Destruction of Cells

Activation of the immune system is a main feature in HIV infection and a recognized predictor of disease outcome. Immune activation has a stronger prognostic value than CD4⁺ T cell count or viral load alone (Grossman et al. 2002). It is therefore reasonable to assume that limited immune activation may be involved in lack of progression in viremic nonprogressors. In non-progressors, immune activation is one of the better examined features, and lower immune activation in comparative studies of controllers and progressors is well documented (Gaardbo et al. 2012). In SIV infection immune activation distinguishes pathogenic infection from the benign outcome seen in the natural hosts, as these animals do not show signs of increased immune activation or T cell turnover during the chronic phase despite significant viral replication (Brenchley and Paiardini 2011; Broussard et al. 2001; Kaur et al. 1998). However, this does not seem to be the case in the human immune system as viremic nonprogressors have been demonstrated to display elevated levels of immune activation compared to HIV-negative individuals (Gaardbo et al. 2013).

Destruction of cells in HIV infection – the drain in the sink model – is partly a consequence of increased immune activation as activated cells are both more likely to become infected with HIV and to undergo apoptosis. However, much of the uncontrolled immune activation is also believed to be a consequence of non-HIV-specific mechanisms including microbial translocation.

Immune Regulatory Cells and Microbial Translocation

Regulatory T cells (Tregs) are anti-inflammatory T cells with an important function in preventing immune responses from becoming overactivated. Tregs are more prevalent in HIV-infected, untreated, progressing patients compared to healthy controls (Kanwar et al. 2010; Chevalier and Weiss 2013), suggesting high levels of Tregs to be harmful, possibly because they

downregulate beneficial HIV-specific immune responses. Indeed, controllers exhibit lower levels of Tregs than progressors according to some studies, while the prevalence of Tregs in viremic nonprogressors may be elevated like in progressors (Gaardbo et al. 2014). Interestingly, it may be of importance if the Tregs are activated or not, based on expression levels of CD45RA+ and the level of FoxP3. Thus, both elite controllers and viremic nonprogressors displayed elevated percentages of activated Tregs in one study (Gaardbo et al. 2014). Finally, Tregs may increase with age possibly contributing to the slow progression or loss of nonprogression in viremic nonprogressors. Thus, the role of Tregs in regard to HIV infection is likely to be a two-edged sword being beneficial in some settings and harmful in others.

Tregs are closely related to IL-17-producing Th17 cells which are pro-inflammatory cells and involved in autoimmune responses. Tregs and Th17 cells function together in opposing ways to control the inflammatory response upon infection, and while Tregs are likely to become more prevalent during HIV infection, Th17 cells are depleted. Th17 cells are believed to play a pivotal role for the mucosal integrity in the gut by stimulating epithelial proliferation and inducing a pro-inflammatory environment by recruiting neutrophils. GALT is the main defense against infectious microorganisms in the gastrointestinal tract and host of the majority of Th17 cells. Thus, the depletion of CD4+ T cells in the GALT during acute HIV infection includes depletion of Th17 cells. The consequence is damage to the mucosal barrier resulting in a continuing leak of microbial remnants to the systemic circulation, a phenomenon termed microbial translocation. These microbial products may activate the immune system and may therefore contribute to HIV progression (Brenchley et al. 2006). In non-pathogenic SIV-infection CD4+ T cells including Th17 cells in the gastrointestinal tract are relatively preserved. One study of HIV nonprogressors, most of whom met the viremic nonprogressor criteria, demonstrated elevated levels of Th17 cells compared to progressors (Gaardbo et al. 2012). Another study of 4 viremic nonprogressors

suggested lower microbial translocation in these patients compared to rapid progressors (Rotger et al. 2011). However, more studies are needed to more definitively analyze the possible contribution of Th17 cells to a viremic nonprogressor phenotype.

HIV-Specific Immune Responses and Neutralizing Antibodies

HIV-specific CD8+ T cells and neutralizing antibodies are believed to be an important but in many cases insufficient element in suppressing viral replication. While the importance of HIV-specific T cell responses on viral control is well established (Gaardbo et al. 2012; Migueles et al. 2004; Porichis and Kaufmann 2011), little is known about HIV-specific T cell responses in viremic nonprogressors. A small study of three viremic nonprogressors found a preserved HIV-specific T cell response, while responses in progressors were found to be reduced (Choudhary et al. 2007). The contribution of neutralizing antibodies on viremic nonprogression is even less clear, but represents an important area for future investigations.

Innate Immune Activation

The innate immune response is the earliest response to HIV infection. Effectors of the innate immune system include plasmacytoid dendritic cell, Toll-like receptors (TLRs), and NK cells. One study evaluated the phenotypic and functional properties of CD56+ CD16+ natural killer (NK) cells in controllers and viremic nonprogressors. Cytolytic activity against autologous CD4+ T cells was found to be abrogated after treatment with an antibody to NKp44L, the cellular ligand of the natural cytotoxicity receptor NKp44 which is specifically induced on CD4+ T cells during HIV-1 infection in LTNP and HIV progressors. In contrast, in HIV controllers and uninfected persons, NKp44L expression on CD4+ cells and autologous NK lysis were both poorly detected (Vieillard et al. 2010).

Conclusion

The reasons for lack of HIV-associated immune deficiency in viremic nonprogressors are multifactorial and appear to involve a broad range of components. However, data on viremic nonprogressors are very limited, studies are small, and case definitions differ among studies. Nevertheless, it is clear that these rare individuals are infected with replication-competent virus, and therefore, host immune factors or specific host-virus interactions seem to represent the main determinants. An extraordinary ability to produce and regenerate T cells and a lower rate of CD4⁺ T cell death are currently assumed as main driving forces that lead to the viremic nonprogressor phenotype. However, only few empiric studies investigating CD4⁺ T cell production or destruction in these patients are available at present to support this hypothesis. Likewise, solid evidence of reduced immune activation in the innate and adaptive compartment in viremic nonprogressors is currently lacking. Thus, understanding the underlying mechanisms underlying viremic nonprogression remains one of the most difficult but also one of the most important fields of future investigation.

Cross-References

- ▶ [Chronic Immune Activation in HIV](#)
- ▶ [HIV and SIV, CD4 T-Cell Responses to](#)
- ▶ [HIV and SIV, CD8 T Cell Responses to](#)
- ▶ [Host Genetics and Genomics](#)
- ▶ [Long-Term Nonprogressors and Elite Controllers](#)
- ▶ [Role of Regulatory T Cells During HIV Infection](#)
- ▶ [Th17 Cells](#)

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Virus Assembly

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Definition

Assembly is the process whereby the components necessary for transmission of HIV to a new cell come together to form a viral particle or virion. These principal components are the proteins Gag and GagPol, the envelope glycoproteins, the viral genome, and some of the HIV accessory proteins. Assembly occurs on cellular membranes; immature virions bud through the cell membrane and are released by the action of host-cell cofactors recruited by Gag. Maturation, which occurs during or immediately following ► [budding](#), involves the cleavage of Gag and GagPol by HIV protease and subsequent modification of the internal structure of the virion necessary for infectivity. Assembly can be considered complete once an infectious virion has been generated.

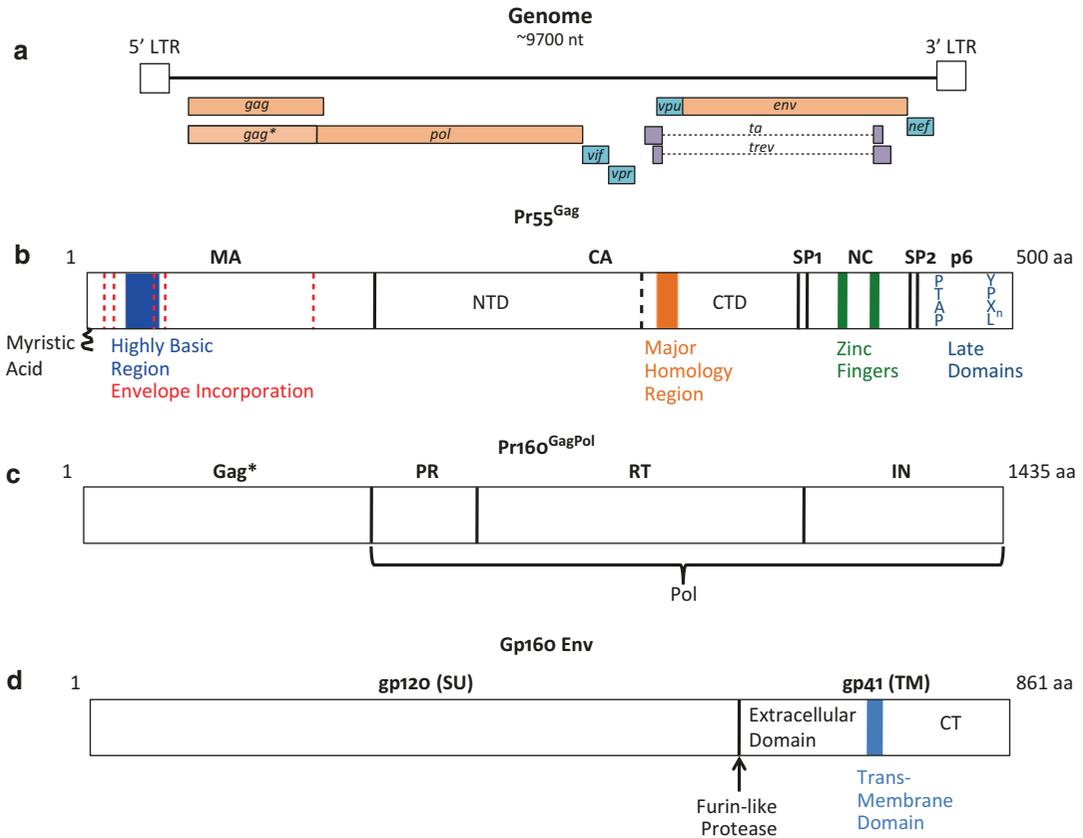
Although AIDS is caused by both HIV-1 and HIV-2, the majority of human infections and the majority of research involve HIV-1. This review will therefore deal almost exclusively with HIV-1; however, it is probable that HIV-2 behaves similarly if not identically for the processes discussed in this review.

HIV-1 Replication

HIV-1 infection begins when the viral envelope (Env) glycoproteins on the virion bind to the viral receptor (CD4) and coreceptor (CCR5 or CXCR4) on the surface of the target cell. Conformational changes in Env, induced by receptor and coreceptor binding, trigger the fusion of viral and target cell membranes, leading to the entry of the viral core into the cytoplasm. After target cell entry, the viral RNA genome is converted to double-stranded DNA by the enzyme reverse transcriptase (RT). The newly synthesized viral DNA translocates across the nuclear envelope as part of the preintegration complex. The process of nuclear import remains poorly defined, though recent evidence suggests that interactions between the Gag-capsid (CA) protein and components of the nuclear pore may play a positive role. Having gained entry to the nucleus, the HIV genome is integrated into the host genome by the viral integrase (IN) protein. The integrated provirus is transcribed by host RNA polymerase II to produce the viral mRNAs encoding the viral proteins and the full-length viral RNA that is packaged into new particles. The integrated provirus is maintained in infected cells until they die; cell death may occur rapidly in some cell types, such as activated T cells, or cells may survive for many years, as in memory T cells. The viral structural proteins assemble, together with two single-stranded copies of the viral RNA, into a new generation of viral particles (Freed and Martin 2012). The assembly process is the focus of this chapter.

The HIV-1 Proteins

HIV-1 is a complex retrovirus that encodes 15 proteins (Fig. 1). These can be categorized into five groups: Gag, Pol, Env, regulatory, and accessory proteins. The principal driver of virus assembly is the Gag polyprotein, a 55-kDa precursor (Pr55^{Gag}) to the mature Gag proteins matrix (MA), CA, nucleocapsid (NC), and p6. Each of these proteins is derived from functional domains in the Gag precursor, playing key roles in virion



Virus Assembly, Fig. 1 Schematic of the HIV-1 genome and polyproteins. (a) The HIV-1 genome with long terminal repeats (LTRs) and protein products indicated. The polyproteins (Gag, GagPol, and Env), regulatory proteins (Rev and Tat), and accessory proteins (Vif, Vpr, Vpu, and Nef) are indicated. HIV-2 has similar genomic organization, but lacks Vpu and encodes an extra protein, Vpx. (b) Pr55^{Gag}. The domains comprising the mature protein products are separated by *solid lines* (matrix, MA; capsid, CA; spacer peptide 1, SP1; nucleocapsid, NC; spacer peptide 2, SP2; p6). Major domains involved in virus assembly and release are indicated. The N-terminal and C-terminal domains of CA are separated by a *dashed line*. (c) Pr160^{GagPol}. GagPol is generated through a mechanism of ribosome slippage during translation of Gag, resulting in a frameshift. The resultant Gag* lacks the p6 domain and its stop codon; translation therefore

continues through Pol generating the GagPol protein that assembles with Gag in the viral particle and during maturation releases the enzymes (protease PR, reverse transcriptase RT, integrase IN). (d) Gp160 Env. The gp160 Env glycoprotein precursor is translated in the endoplasmic reticulum and traffics to the cell membrane via the Golgi apparatus. In the Golgi, it is cleaved by a host furin-like protease to generate the mature products, gp120 and gp41. The mature proteins remain non-covalently associated and form a trimer. Gp120 is responsible for the recognition of the receptors and coreceptors for HIV entry, while gp41 contains an extracellular domain necessary for membrane fusion and a cytoplasmic tail (CT). The function of the cytoplasmic tail is not entirely clear, but it appears to affect cell signaling, immune functions, and incorporation of Env into viral particles

assembly and ► **budding**. Via a process known as ribosomal frameshifting, Gag protein synthesis occasionally (5–10% of the time) leads to the synthesis of a longer GagPol product. Pol, like Gag, is a precursor polyprotein; it encodes the viral enzymes protease (PR), RT, and IN. While RT and IN function early in the infection process,

PR is required for the final step of the replication cycle – virion maturation. The third viral precursor protein is the Env glycoprotein. Synthesized as a 160-kDa polyprotein, gp160, it is cleaved by a cellular protease during trafficking through the Golgi apparatus to form the surface Env glycoprotein gp120 and the transmembrane Env



glycoprotein gp41. The Env glycoproteins are exposed on the surface of the virion; gp120 is responsible for recognizing the viral receptors on target cells, and gp41 catalyzes a membrane fusion reaction between viral and cellular membranes to release the contents of the virion into the target cell.

The regulatory proteins, Tat and Rev, control viral protein expression. The accessory proteins, Vif, Vpr, Vpu, and Nef, play roles in regulating the cellular environment to make it compatible with HIV-1 replication. While in many cases they are dispensable for virus replication in cultured cells, they are critically important during infection of the human host (Freed and Martin 2012).

Following successful infection of a cell, the HIV-1 genetic material resides in the host genome in the form of an integrated provirus. The host-cell transcriptional and translational machinery generates HIV-1 proteins much as it would human proteins, and many of these go on to form the virions responsible for initiating subsequent rounds of infection.

HIV-1 Gag

The Pr55^{Gag} precursor is able to drive the formation and budding of virus-like particles (VLPs) in the absence of any other viral component; for this reason it is sometimes referred to as the assembly machine (Sundquist and Kräusslich 2012). Like any machine, the Gag precursor possesses multiple components each of which plays a specific role in virus assembly (Fig. 1).

The MA domain directs Pr55^{Gag} to bind membranes. Gag–membrane binding is achieved primarily through two features: a myristic acid group covalently attached to the N-terminus of MA and a highly basic region. The MA domain also promotes the incorporation of the Env glycoprotein complex into virions.

The CA domain is comprised of two structural and functional units – the amino-terminal domain (NTD) and carboxy-terminal domain (CTD) –

which are connected by a flexible linker. CA-CA interactions mediated by both the NTD and the CTD allow CA to form a continuous hexameric lattice. The CTD also includes the major homology region (MHR), an essential domain conserved throughout many genera of retroviruses that plays an important role in Gag multimerization and virus assembly.

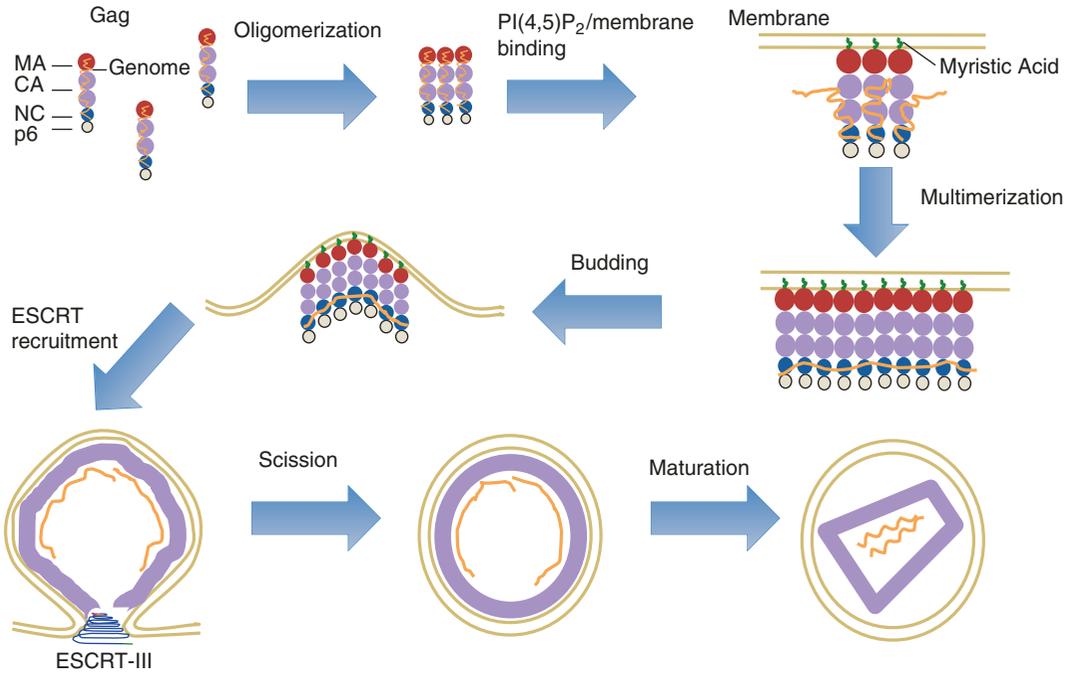
NC forms the third domain of Gag. It is small, highly basic, and possesses as its most distinctive feature two zinc-finger motifs. These motifs are well conserved among retroviruses and are connected by a flexible linker. NC can bind RNA nonspecifically because of its overall positive charge, but exhibits selectivity for HIV-1 genomic RNA. This selectivity is conferred to a large extent by the zinc fingers. In addition to its role in assembly, NC has important post-entry functions as a nucleic acid chaperone.

P6, the most C-terminal domain in Gag, possesses the “late domains,” so-called because they function late in the virus budding process. Gag mutants that lack either the late domains or the entirety of p6 are able to assemble particles but are unable to bud from the cell and instead remain tethered to the cell membrane.

In addition to the four major Gag domains discussed above, Pr55^{Gag} contains two small spacer peptides located between CA and NC (SP1) and between NC and p6 (SP2). These spacer peptides help regulate the kinetics of Gag processing, and SP1 contributes to Gag multimerization.

Gag Trafficking

The newly translated Gag molecules begin the process of assembly in the cytoplasm (Fig. 2) (Balasubramaniam and Freed 2011). Early in the assembly process, Gag forms low-order oligomers, comprising just a few Gag molecules that bind to the membrane. Gag oligomerization and membrane binding are thought to be primed by Gag–RNA interactions, mediated by NC. Phosphatidylinositol-4,5-bisphosphate [PI(4,5)P₂], a phospholipid concentrated on the inner



Virus Assembly, Fig. 2 A schematic representation of the processes that take place in particle assembly and release. The Gag polyprotein (MA, maroon; CA, pink; NC, blue; p6, beige) is synthesized in the cytoplasm where it oligomerizes. MA binds PI(4,5)P₂ at the cell membrane, promoting exposure of the myristic acid (green), anchoring Gag to the membrane. Binding of the

genome (orange) by NC leads to large-scale multimerization of Gag, driven by CA-CA interactions. The nascent Gag particle buds from the cell and recruits ESCRT machinery via p6 to achieve membrane scission. Maturation to the infectious particle occurs during or immediately following release

leaflet of the plasma membrane, is a major cellular determinant in directing Gag to the plasma membrane. PI(4,5)P₂ binding by the MA domain of Gag triggers exposure of the myristic acid group. This so-called myristyl switch permits the hydrophobic acyl chain of the myristic acid moiety to insert into the hydrophobic interior of the lipid bilayer, anchoring Gag to the membrane. HIV-1 assembly takes place in membrane microdomains (often referred to as lipid rafts) that are enriched in cholesterol and highly saturated sphingolipids; the HIV-1 lipid bilayer is therefore enriched in PI(4,5)P₂, cholesterol, and sphingolipids relative to bulk plasma membrane. In addition to promoting virus assembly, this specific lipid composition may also function in the infection process, as virions depleted of cholesterol and raft lipids demonstrate reduced infectivity.

Assembly of the Gag Lattice

After initial membrane binding, low-order Gag oligomers assemble into a more extensive Gag lattice (Mateu 2009). Interactions between Gag and RNA play a crucial regulatory role in the assembly process. Mutating the regions of NC necessary for RNA binding inhibits virion assembly. The current model is that RNA provides a scaffold for assembly, bringing together Gag molecules, thereby promoting their multimerization. The CA-SP1 region of Gag is sensitive to Gag concentration, and the concentration-dependent modification of the structure in this region contributes to the multimerization of thousands of Gag molecules (2,000–5,000/particle) into an ordered lattice. Support for the hypothesis that NC–RNA interactions help to initiate the assembly process derives from the observation that Gag

will efficiently assemble into a multimeric lattice if NC is replaced with a leucine zipper motif. Leucine zippers oligomerize, increasing the clustering of Gag independently of RNA. Thus, the local concentration of Gag is increased by activities encoded at both ends of the Gag precursor; the N-terminal MA domain binds to specific lipid microdomains, and the more C-terminal NC domain interacts with RNA. Once Gag has been concentrated, Gag multimerization domains in CA and SP1 drive assembly into an organized protein lattice. In the immature lattice, the hexameric sheet formed by the CA domain adopts a closed, roughly spherical structure via inclusion of gaps in the lattice. In contrast, the hexameric CA lattice in the mature core closes off by inclusion of pentamers (see below).

Virion Budding

Although p6 plays no role in the formation of the Gag lattice, it is required to promote ► budding and release of the virion after the immature particle has been assembled. The membrane scission event that allows the virus particle to pinch off from the plasma membrane requires the late domains. The common role of all retroviral late domains is to recruit components of the endosomal sorting complex required for transport (ESCRT) machinery. ESCRT functions in processes requiring the formation of a bud protruding out of the cytoplasm, such as during cell division and the formation of intracellular multivesicular bodies. Such processes topologically resemble virus budding, and a number of enveloped virus families take advantage of this cellular machinery to effect particle release. The ESCRT machinery is comprised of four multisubunit complexes known as ESCRT-0, -I, -II, and -III. A variety of accessory molecules also function in ESCRT-mediated membrane budding and scission events (Martin-Serrano and Neil 2011).

Retroviruses interact with ESCRT or accessory endosomal sorting machinery via three types of late domain. Specifically, a Pro-[Thr or Ser]-Ala-Pro [P(T/S)AP] motif interacts with Tsg101; a Tyr-Pro-Asp-Leu (YPDL) motif, or

related sequence, binds the ESCRT accessory factor Alix; and a Pro-Pro-Pro-Tyr (PPPY) motif interacts with Nedd4 family ubiquitin ligases. HIV-1 has both a PTAP and a YPDL-like motif in its p6 domain. Tsg101 is a component of ESCRT-I, whereas Alix bears interaction sites for both ESCRT-I and ESCRT-III. The PTAP motif plays the primary role in HIV-1 budding, at least in cell culture; however, the high degree of conservation of both sequences suggests that both are important to virus replication in a natural setting.

While the various retroviral late domains interact with different components of the ESCRT machinery, the ultimate outcome of these interactions is to recruit ESCRT-III to the site of budding (Fig. 2). ESCRT-III is composed of the charged multivesicular body proteins (CHMPs 1–7). Typically, ESCRT-III function is nucleated by CHMP6, which in turn recruits multiple CHMP4 monomers, in an ever-tightening helix, to pinch off the budding vesicle. Addition of CHMP4 monomers ends with capping by CHMP3 and CHMP2 and the final recruitment of the ATPase Vps4, which disassembles the ESCRT-III complex. Surprisingly, HIV-1 requires only a subset of the CHMP proteins (CHMP2 and CHMP4) to achieve budding and does not require ESCRT-II, while most analogous cellular processes seem to require the full complement of ESCRT components (Morita 2012).

Restriction of Virus Release by Tetherin

After membrane scission has been effected by ESCRT-III and PR has cleaved the Gag polyprotein into the mature Gag proteins, mature, cell-free virions can initiate new rounds of infection. However, under some circumstances, release of budded virions is blocked by the cellular innate immune system. A principal signal in the induction of an “antiviral state” is interferon. A well-studied interferon-stimulated gene (ISG) with respect to HIV-1 replication is tetherin, originally known as CD317, BST2, or HM1.24 (Martin-Serrano and Neil 2011). Tetherin is a raft-localized protein that possesses two

membrane-anchored regions and a surface-exposed coiled-coil domain that mediates dimerization. Dimerization between tetherin molecules on the cell surface and within the viral membrane leads to the accumulation of virions on the cell surface, where they remain “tethered.” HIV-1 encodes viral protein U (Vpu) that counteracts tetherin by removing it from sites of virus ► [budding](#) and inducing its degradation. Other lentiviruses use their Env glycoproteins or the accessory protein Nef to counteract tetherin. The precise role of tetherin in the natural course of HIV-1 replication remains uncertain. Although tetherin clearly inhibits the release of cell-free virus *in vitro*, there is some evidence that, under certain circumstances, tetherin may actually promote virus transmission between cells. Nevertheless, the fact that lentiviruses have evolved diverse strategies to counteract tetherin argues that it serves a predominantly restricting role in lentiviral replication.

The Site of HIV-1 Assembly and Budding

HIV-1 predominantly infects T cells, leading to their eventual depletion and the induction of AIDS. However, lentiviruses including HIV-1 also infect cells of the myeloid lineage such as macrophages. Although currently a matter of debate, macrophage infection may contribute to the virus’ ability to persistently infect its host. The site of virus assembly and budding differs in certain respects between T cells and macrophages. In T cells, the overwhelming majority of Gag traffics to the plasma membrane where virus assembly occurs and virions are released to the extracellular medium or are transferred across synapses between T cells. In macrophages, Gag localizes to apparently intracellular compartments (Benaroch et al. 2010). Initially, these sites were thought to be multivesicular bodies, but more recent studies using a range of histological and imaging techniques have revealed that the internal virus-containing compartments in macrophages are in fact deep invaginations of the plasma membrane that are contiguous with the extracellular environment. These invaginations appear to function as sites of particle assembly and storage from

which mature virions can be released into the synapse formed between infected macrophages and uninfected T cells. Such a strategy allows virus particles to remain relatively hidden from the host immune system until the opportunity for cell–cell spread arises.

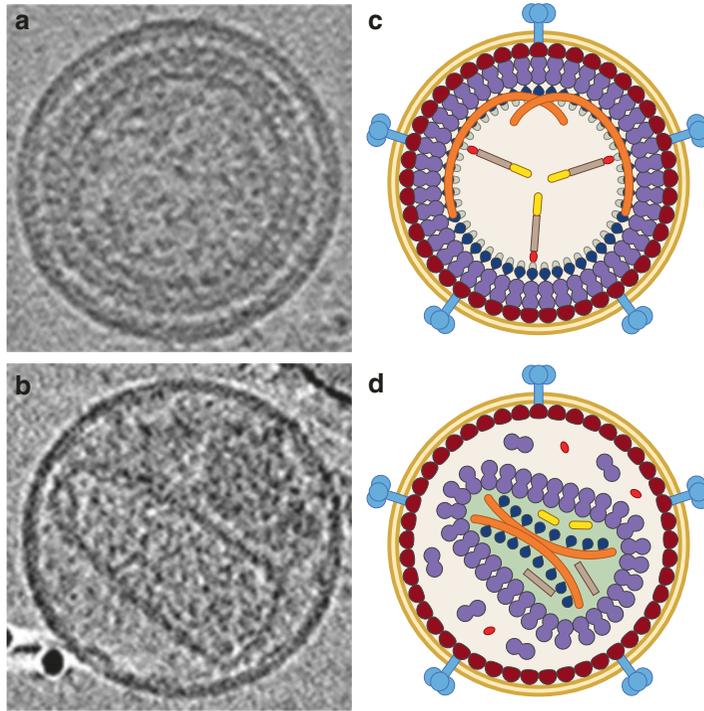
HIV-1 Particle Maturation

Pr55^{Gag} contains five major cleavage sites for the viral PR, located between functional domains of the polyprotein (Fig. 1). The final step in the generation of the infectious HIV-1 particle is maturation (► [HIV-1 Maturation](#)), which is triggered by PR-mediated processing at each of these cleavage sites (Sundquist and Kräusslich 2012). Gag cleavage by PR occurs concomitant with virion release from the host membrane, leading to the liberation of CA from the immature Gag lattice and its subsequent reassembly to form the outer shell of the conical capsid core that is characteristic of the mature virion (Fig. 3). PR-mediated Gag processing is a highly ordered, sequential process, with some cleavage events taking place early in the cascade and others occurring later and less efficiently. In the core, the CA protein is arranged in a hexameric lattice that is closed off at both ends by the asymmetric inclusion of twelve CA pentamers within the lattice (five at the narrow end, seven at the broad end) to produce a core with the characteristic conical shape. Virion maturation is critical for productive infection in the next cycle of replication; immature virions are both fusion-defective – likely owing to suppression of Env fusion activity resulting from gp41 interactions with the immature Gag lattice – and are incompetent to initiate reverse transcription in the target cell.

Additional Virion Components

The Env Glycoproteins

The Env glycoproteins are not required for particle assembly and budding, but they are necessary for the formation of an infectious virion (Fig. 1). The precise mechanism of Env glycoprotein



Virus Assembly, Fig. 3 Cryoelectron micrographs and schematic representations of immature and mature HIV-1 particles. Central slices through cryo-EM tomograms of immature (a) and mature (b) virus particles that are ~130 nm in diameter. Schematic representation of immature (c) and mature (d) HIV-1 particles. Color scheme is consistent with Fig. 2: MA, maroon; CA, pink; NC, blue;

p6, beige; genome, orange. Additionally, GagPol products are shown: protease, red; reverse transcriptase, gray; integrase, yellow. Env glycoprotein trimers are shown in light blue on the particle surface (Figure originally appeared in Balasubramaniam and Freed (2011)). Parts of figure were adapted from Ganser-Pornillos et al. (2008) and used with permission from Elsevier)

incorporation remains under investigation, particularly the question of whether or not a direct interaction between Gag and Env is required for Env recruitment into virions. It is nevertheless clear that two viral components are critical for Env incorporation. In cells that are physiologically relevant to HIV-1 infection *in vivo* (e.g., primary lymphocytes and macrophages), the cytoplasmic tail of gp41, which is unusually long for a viral glycoprotein, is required for Env incorporation (Postler and Desrosiers 2012). The long tail is not a universal requirement; however, in some laboratory cell lines (e.g., HeLa and 293T), a tail-deleted gp41 is incorporated into virions and is functional. The gp41 cytoplasmic tail plays a role in Env glycoprotein trafficking and localization to specific plasma membrane microdomains

(e.g., lipid rafts) and may interact, directly or indirectly, with Gag (see below).

The second viral determinant that is required for Env glycoprotein incorporation is the MA domain of Gag. Various single-amino-acid mutations in MA prevent the incorporation of Env without affecting any other aspect of virus assembly or release. Indeed, when such MA mutants are expressed in the presence of gp41 cytoplasmic tail-deleted Env in permissive cells (e.g., HeLa or 293T), the resulting particles are fully infectious. Evidence for an association between MA and the gp41 cytoplasmic tail is also provided by the observation that a mutation in gp41 that blocks Env incorporation can be rescued by a mutation in MA. Together, these findings are consistent with the existence of an interaction between Env and

MA. It remains unclear, however, whether the inability to incorporate Env in the presence of MA or gp41 cytoplasmic tail mutations is due to a loss of a positive Env recruiting signal or the introduction of a steric block to Env incorporation. In other words, is the long gp41 cytoplasmic tail actively recruited, or simply accommodated, by MA? This continues to be an active area of investigation.

RNA Packaging

The viral RNA genome plays two roles in the assembly of infectious HIV-1 particles. The role it plays in regulating the assembly process itself is detailed above. No less important is that the virus particle exists as a vehicle to transfer the viral genome – full length, capped, and polyadenylated – to new cells (Lu et al. 2011). To this end, a dimer of genomes is recognized and packaged by Gag, primarily via the NC domain. Although NC is highly basic and binds non-specifically to negatively charged molecules, such as RNA, it achieves selectivity for the HIV-1 genome by recognizing a packaging site in the 5' end of the genome. The packaging site (often referred to as ψ) is composed of four stem-loop structures that encompass the dimerization initiation signal (DIS), the splice donor, and the Gag start codon. The positioning of a large portion of ψ after the splice donor ensures that full-length genomes, rather than spliced mRNAs, are preferentially packaged into the particle. During reverse transcription, the RT enzyme jumps frequently from one molecule of RNA to the other, increasing the frequency with which reverse transcription is successfully completed, i.e., a break in the RNA genome would be lethal if only one molecule of RNA were present. This “template switching” also drives recombination between the two packaged RNAs, thereby increasing viral genetic diversity.

The viral genome is not the only RNA in the particle; transfer RNA for lysine (tRNA^{Lys3}) is also incorporated, in part by binding to a specific site at the 5' end of the viral genome. The tRNA is required for the initiation of reverse transcription after the particle infects a cell. Other cellular RNAs are also found in the particle, although it

is not clear whether they play a specific role or are simply incorporated nonspecifically owing to their intracellular abundance and binding to basic residues in NC.

Viral Enzymes

The viral enzymes PR, RT, and IN are not required for particle formation per se, but are critical components of the mature, infectious virion. As discussed above, these enzymes are initially synthesized as domains within the 160-kDa GagPol precursor (Pr160^{GagPol}) which is incorporated into virions primarily by Gag–GagPol interactions; as Pr160^{GagPol} possesses the MA, CA, and NC domains, it is able to bind to the membrane in the same manner as Pr55^{Gag} and oligomerize with Gag into nascent particles. Reflecting the ~5% rate of ribosomal frameshifting during Gag translation, the abundance of Pr160^{GagPol} in virions is ~20-fold lower than that of Gag. If Pr160^{GagPol} is artificially overexpressed relative to Gag, assembly is disrupted, primarily as a result of excess of PR activity cleaving Gag before assembly has been completed. The first event in maturation is PR autoproteolysis, whereby PR cleaves itself out of Pr160^{GagPol}, allowing formation of the fully active, dimeric PR enzyme and subsequent proteolytic processing of the remaining sites in Gag and GagPol (Hill et al. 2005).

Accessory Proteins Packaged into Virions

Vpx (which is unique to HIV-2 and closely related simian lentiviruses) and Vpr are recruited into particles via binding to sequences in p6. A number of functions have been ascribed to Vpr, including the induction of cell-cycle arrest and increasing particle infectivity in nondividing cells. Vpx has recently been shown to induce the degradation of the myeloid-specific host restriction factor SAMHD1 early postinfection. In the absence of Vpx, SAMHD1 suppresses the cellular levels of deoxynucleotide triphosphates, which are required for reverse transcription. The absence of Vpx in HIV-1, and its presence in HIV-2/SIV, likely accounts for the relative inefficiency with which HIV-1 infects myeloid cells (macrophages and dendritic cells) relative to HIV-2/SIV (Fujita et al. 2012).

Vif is also found at low levels in virions, probably due to interactions with RNA. The primary function of Vif is to induce the degradation of the host restriction factor apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3). Nef is also found in HIV-1 particles, where it is cleaved by PR. There is limited evidence for direct (active) incorporation of Nef; its presence in virions is likely due to its membrane association. Vpu, discussed above as the viral protein that counteracts tetherin, is not packaged into virions to an appreciable extent.

Host-Cell Proteins in HIV-1 Particles

A large number of cellular proteins are incorporated into HIV-1 virions. Mass spectrometry analysis of highly purified HIV-1 virions revealed the presence of ~250 host proteins; these include membrane proteins, cytoskeletal elements, soluble cytosolic factors, and nuclear components (Ott 2008). In most cases, these proteins are probably incorporated nonspecifically and are unlikely to serve any particular function in the virus replication cycle. However, some host proteins are actively incorporated into virions through direct interactions with viral structural proteins. An example is the peptidyl prolyl *cis-trans* isomerase cyclophilin A, which binds to a Pro-rich loop in the NTD of CA. Despite this active incorporation, there is no definitive evidence for a functional role of cyclophilin A in HIV-1 assembly and budding. Instead, it seems likely that CA–cyclophilin A interaction plays a role early postentry in the next round of infection.

APOBEC3G and a subset of other APOBEC3-family proteins (most notably APOBEC3F) are host-cell restriction factors that are incorporated into virions. These cytidine deaminases possess the ability to introduce mutations into the HIV-1 genome during reverse transcription, blocking viral infectivity. APOBEC proteins are packaged into virions most likely by binding to RNA or RNA in complex with NC. The viral accessory protein Vif targets APOBEC3G and APOBEC3F proteins for degradation in the virus-producer cell, preventing their incorporation into particles and preserving particle infectivity.

Particle Assembly and Maturation as Drug Targets

Steps in assembly and maturation mediated by the Gag proteins are crucial to the virus replication cycle; however, there are currently no approved drugs targeting these steps. In contrast, drugs that target the PR enzyme have been in widespread clinical use for more than 15 years. By blocking PR-mediated cleavage of Gag, PR inhibitors prevent the generation of mature infectious virions from immature particles. PR inhibitors such as ritonavir, darunavir, and atazanavir are among the most potent components of highly active antiretroviral therapy (HAART).

Because resistance to PR inhibitors and other antiretroviral drugs can emerge in treated patients, efforts are underway to develop inhibitors that block maturation by an alternative strategy, i.e., by disrupting processing at specific sites in Gag rather than by targeting PR itself (Waheed and Freed 2012). Thus far, two so-called maturation inhibitors with this mode of action have been reported. The first-in-class compound is bevirimat, a betulinic acid derivative. Bevirimat is thought to bind to the assembled Gag complex and, by a still poorly defined mechanism, prevent PR cleavage at the CA-SP1 junction. The accumulation of the CA-SP1 processing intermediate interferes with particle infectivity by disrupting formation of the conical core. Although in clinical trials bevirimat was able to significantly reduce viral loads in many HIV-1-infected patients, naturally occurring sequence polymorphisms in the SP1 region of Gag limited the efficacy of the compound in a significant percentage of patients. More recently, Pfizer developed another maturation inhibitor, PF-46396, again targeting CA-SP1 processing but possessing a chemical structure distinct from that of bevirimat. It is hoped that development of such structurally diverse inhibitors, and second- and third-generation derivatives, will ultimately make the maturation inhibitor class of compounds a valuable addition to the current portfolio of therapeutic options.

Attempts to target Gag oligomerization and particle assembly are at a much earlier stage of development. A variety of CA assembly inhibitors

have been reported (e.g., CAP-1, CAI, NYAD-1, PF74). These compounds are peptide-based or small molecules that target Gag at several distinct steps in the virus replication cycle, affecting immature Gag–Gag interactions (CAI, NYAD-1), mature core formation (CAP-1, PF74), and potentially post-entry functions (NYAD-1). At present all of these compounds are at the proof-of-concept stage; a great deal of work remains before these compounds or their derivatives can be brought to clinical trials.

Conclusions

Studying the process of HIV-1 assembly has provided fresh insight into basic cellular processes, such as the regulation of protein trafficking by phosphoinositides and ESCRT-mediated membrane budding and scission. Nevertheless, there remain many unanswered questions. For example, the route taken by Gag to reach the plasma membrane and the role played by host-cell factors in Gag trafficking remain incompletely understood. Elucidating the structures of viral proteins has had a transformative impact on the development of antiretroviral compounds targeting the viral enzymes RT, PR, and IN. Other structures, such as the Gag lattice, are now being actively pursued as potential novel drug targets. While much can be gleaned from structural studies of the mature CA lattice, it is the uncleaved Gag precursor that drives virus assembly. Thus, an atomic-resolution structure of Gag in the immature virion would be of great value from both a basic science and a drug development perspective. Recent developments in structural and cellular biology are likely to have a profound impact on the rapidly moving field of retroviral assembly.

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Virus-Host Evolution and Positive Selection

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Definition

Viruses, including HIV-like viruses, have been infecting primates and other hosts for millions of years. During this long-term common history, viruses and hosts have constantly put pressure

on each other for survival. Over time, hosts have evolved first lines of defense against viruses, the cellular restriction factors, while pathogens have developed mechanisms of evasion and antagonism. These antagonistic relationships have set up genetic conflicts between viruses and hosts that drive their evolution and interactions. Therefore, over evolutionary time and as a result of virus-host arms races, host restriction factors have been rapidly evolving and display signatures of positive selection, in particular at the virus-host interface.

Introduction

Lentiviruses are retroviruses that have been infecting various mammals and in particular primates for millions of years (Gifford 2012). The virus responsible for the AIDS (acquired immune deficiency syndrome) pandemic in humans, HIV (human immunodeficiency virus), has originated from cross-species transmissions of simian immunodeficiency viruses, SIVs, from non-human primates (Sharp and Hahn 2011). Recent studies using various temporal markers and strategies have estimated that ancestors of the modern-day lentiviruses existed in primates more than 10 million years ago (Compton et al. 2013). The long-term antagonistic coevolution of lentiviruses with their primate hosts has set up an evolutionary arms race between the two adversarial entities. These ancient genetic conflicts can notably be witnessed today by signatures of positive selection in the host genome as a result of constant innovations at the virus-host interface. Understanding these long-term virus-host evolutions help in understanding current virus-host interactions and host susceptibility to modern-day pathogenic viruses.

Hosts have Evolved Specialized Genes, the Cellular Restriction Factors, to Block Viral Replication

Cellular restriction factors are host proteins from the innate immune system that potentially block viral replication. These antiviral proteins target

many stages of the viral life cycle, they may have different mechanisms of viral inhibition that can be direct or indirect, and some are broadly acting while others are very specific to a virus or a viral protein. Despite this diversity of antiviral function, restriction factors share a common evolutionary feature: restriction factors are immune genes that are rapidly evolving and display signatures of positive selection as a result of genetic conflicts with pathogens (Duggal and Emerman 2012).

In lentiviral infection, cellular restriction factors play a key role in host susceptibility and pathogenesis. Although there is currently no strong evidence that restriction factors control viral replication of HIV in humans, they may be a first line of defense and protect a host species against the emergence of new lentiviruses. Examples of restriction factors with anti-lentiviral activity include: APOBEC3G (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G), a cytidine deaminase that targets the reverse transcription step of HIV, TRIM5 (tripartite motif protein) that impairs viral uncoating, SAMHD1 (SAM domain and HD domain-containing protein 1) that blocks reverse transcription, and Tetherin/BST-2 (bone marrow stromal antigen 2) that restricts viral budding.

Viruses have Evolved Means to Counteract Restriction Factors by Evasion or Antagonism

Because viruses are obligate intracellular pathogens, they have evolved means to evade or counteract host restriction factors to complete their life cycle.

Certain restriction factors directly target a viral protein to restrict viral replication. In these cases, viruses can escape from this restriction by avoiding to be recognized by the host protein, a mechanism of *evasion or escape*. Viruses will therefore evolve very rapidly at the virus-host interface to evade host recognition by single amino acid changes or more complex evolutionary strategies such as recombination. Evasion is for example used by the capsid of lentiviruses to

escape recognition from the restriction factor TRIM5 (see details below).

Viruses have also evolved *antagonists* to directly target the cellular restriction factors and counteract their action. This mechanism is largely used by lentiviruses. It is particularly efficient to antagonize cellular factors that target another viral protein constrained in its evolution or to antagonize restriction factors that do not directly target the virus (i.e., indirect restriction). Indeed, several cellular restriction factors broadly block viruses by restricting steps of viral replication that are common to several virus families. For example, host factors can restrict several viruses by decreasing the level of nucleic acids in cells necessary for viral replication, inhibiting viral budding, recognizing viral RNA in the cytoplasm, or inhibiting mRNA translation. Viral antagonists are therefore specialized viral proteins that can interact with cellular restriction factors and inhibit their action, often by inducing their proteasomal degradation, relocalizing them to a different compartment, producing competitive inhibitors by mimicry, or using other functional inhibition mechanisms. The lentiviral antagonists are mainly encoded by viral accessory genes that are not strictly necessary for replication and therefore bear more plasticity. For example, the accessory proteins Vpr and Vpx antagonize SAMHD1, the accessory protein Vif counteracts APOBEC3G and other APOBEC3 proteins, and the viral proteins Vpu, Nef, or Env antagonize Tetherin/BST-2.

Adversarial Virus-Host Interactions Set Up a Genetic Conflict

These antagonistic relationships between the host and the virus proteins set up an evolutionary genetic conflict between the two entities (Daugherty and Malik 2012). This genetic battle is often referred as a “virus-host arms race,” which follows the Red Queen hypothesis where organisms constantly evolve and adapt to survive in an ever-changing environment (Van Valen 1973). Indeed, when a host protein restricts a virus, it puts pressure by natural selection on the virus to evolve and evade or counteract the host protein in

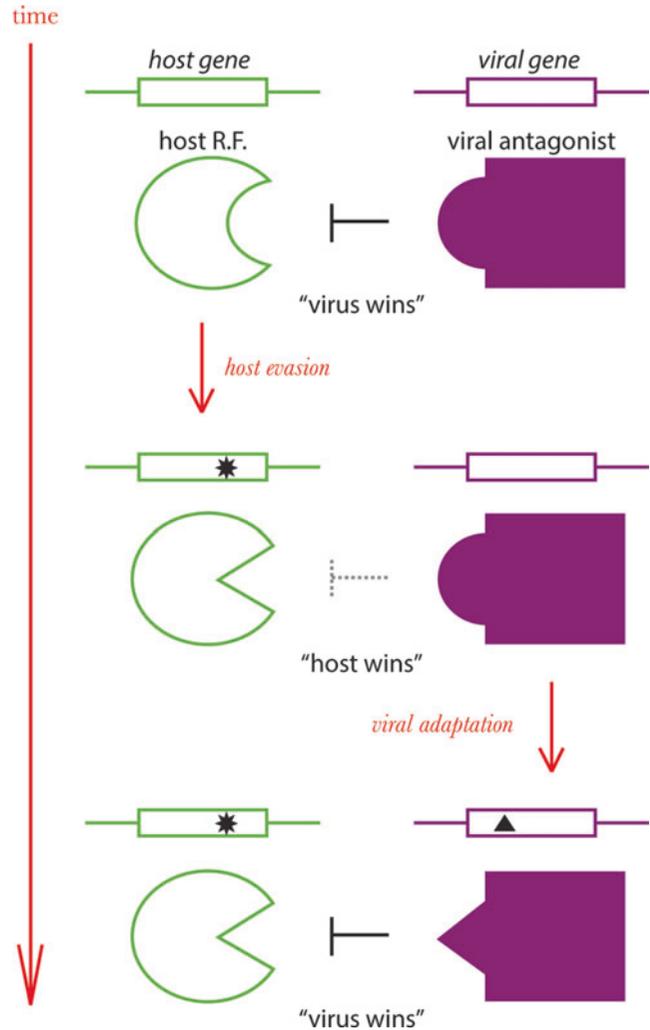
order to complete its viral cycle. As a result, the “successful virus” is the one with changes that allow the pathogen to win the virus-host battle. In response to the new selection exerted by the virus, the host will in turn evolve and adapt to re-gain antiviral capacity. Over evolutionary times, these recurrent cycles of rapid virus-host evolution leave signatures of positive selection in both the virus and the host genome, in particular at sites of interactions (Fig. 1). The primate restriction factors capable of blocking lentiviruses, such as APOBEC3G, SAMHD1, TRIM5, or Tetherin/BST-2, all bear signatures of positive selection. Furthermore, the identification of the specific sites that are under strong positive selection within the gene has powerfully aided the discovery of virus-host interacting domains and the characterization of host protein functions (Daugherty and Malik 2012).

Host Evolutionary Analyses of Long-Term Virus-Host Genetic Conflicts: Methods to Analyze Positive Selection

Evolutionary analyses of the host genome may be performed to discover which host genes have been under genetic conflict and identify potential virus-host interfaces. During a viral-host arms race, changes that result in a fitness gain are frequently due to mutations that result in amino acid changes. Therefore, at sites of virus-host interaction, a greater likelihood of non-synonymous substitutions (changes of amino acids; dN) are observed over synonymous changes (changes in nucleotides that conserve amino acids; dS), which is a signature of positive selection. The estimation of the dN/dS ratio, ω , can be used to identify such sites of adaptive evolution. Positive selection is characterized by $dN/dS > 1$, while purifying and neutral selection (characteristics of most host genes) are characterized by $dN/dS < 1$ and $dN/dS = 1$, respectively. Because the antagonistic virus-host evolution has been ongoing for million years, one can use orthologous gene sequences from host species that have diverged millions of years ago to reconstruct ancestral gene sequences and infer the dN/dS ratio. Studies of

Virus-Host Evolution and Positive Selection,

Fig. 1 Long-term viral-host genetic conflict. Virus and host proteins are engaged into a “Red Queen” conflict inducing rapid adaptive evolution of the host antiviral gene over evolutionary time. This leaves signatures of positive selection in the host genome, in particular at the virus-host interface. Steps: The viral protein is able to antagonize the restriction factor (i.e., the “virus wins”). This puts a selective pressure on the host that will evolve and evade the antagonist, leading to a situation where the “host is winning.” Such selective pressure will, in turn, induce a rapid viral evolution and only viruses able to block the new host protein will be selected. These virus-host interactions therefore set up unceasing evolutionary arms races. *R.F.* restriction factor. *Black stars* and *triangles* within genes represent amino acid changes. *Dashed lines* represent loss of block



primate phylogeny are therefore ideal to understand the viral-host arms races that have been ongoing in the primate lineage, including in the ancestors of humans. The analyses of where and when the virus-host genetic conflicts have occurred improve our understanding of the current viral epidemic, pathogenesis, and evolution.

Several statistical methods exist to detect such adaptive evolution (Pond et al. 2005; Yang 2007; Delpont et al. 2010); a summary of the most commonly used tools is given here. First, to identify overall signature of positive selection, one can estimate the dN/dS ratio across the gene using PARRIS from HYPHY/Datamonkey or Codeml from the PAML package. Second, to identify specific sites evolving under strong positive selection,

one can estimate the dN/dS ratio at individual sites along a gene (i.e., site-by-site selection analyses) using MEME from HYPHY/Datamonkey or BEB from PAML. Third, to identify lineage-specific selection (i.e., episodic selection), one can estimate the dN/dS ratio for each lineage, using BranchREL from HYPHY/Datamonkey or the “free-ratio model” from PAML.

Examples of Major Virus-Host Genetic Conflicts in Lentiviral Infection

Several genetic conflicts between cellular restriction factors and lentiviral proteins have been characterized in recent years. The main ones are

Virus-Host Evolution and Positive Selection, Table 1 Examples of major genetic conflicts between primate cellular restriction factors and lentiviral proteins

Cellular restriction factor	Viral evasion or antagonism		Evidence (in the host genome) of	
	Mechanism	Viral protein	Long-term genetic conflict	Ongoing or recent genetic conflict
APOBEC3G	Antagonism	Vif	Positive selection in primates	Polymorphism* in AGMs and rhesus macaques
APOBEC3D, APOBEC3H, APOBEC3F	Antagonism	Vif	Positive selection in primates. Gene family (duplication)	Polymorphism* in humans
SAMHD1	Antagonism	Vpr or Vpx	Positive selection in primates	Polymorphism* in AGMs
TRIM5 α and TRIMcyp	Evasion	Capsid	Positive selection in primates. Gene fusion that led to the creation of TRIMcyp	Polymorphism* in rhesus macaques
Tetherin/BST-2	Antagonism	Vpu, Nef, or Env	Positive selection in primates	Specific 5-aa deletion in the human lineage

AGMs African green monkeys, *aa* amino acids, * polymorphism that impacts virus-host interaction or the host antiviral gene function

summarized in Table 1 and some are further described here as examples.

APOBEC3G (and Other APOBEC3 Members) and the Viral Protein Vif

In early 2000s, the HIV-1 accessory protein Vif was found to be necessary for efficient HIV infection in primary human cells and certain cell lines (Sheehy et al. 2002). Several studies then characterized that Vif was allowing viral replication by inhibiting a cellular factor called APOBEC3G (Malim and Bieniasz 2012). APOBEC3G is a member of the *APOBEC3* gene family that is constitutively expressed in many cell types. APOBEC3G primarily restricts lentiviral replication by being incorporated in nascent virions and deaminating cytidine to uracil in single-stranded viral DNA during reverse transcription, thereby inducing lethal G-to-A hypermutation in the viral genome. In turn, the viral antagonist Vif has the capacity to bind host APOBEC3G and the Cul5-EloBC ligase complex, targeting the cellular factor for proteasomal degradation. This antagonistic function of Vif is essential for lentiviral infection as it has been retained in all extant primate lentiviruses and is therefore one of the most studied viral-host arms races. Because no crystal structure

of the complex has been solved yet, the interaction between Vif and APOBEC3G has only been studied through mutational screens and evolutionary analyses. The analyses of positive selection as well as of the inter-species variations in the host gene have therefore aided our understanding of this ancient and ongoing genetic conflict (reviewed in: (Duggal and Emerman 2012)). The viral protein Vif is further the antagonist of other APOBEC3 protein members (i.e., APOBEC3D, APOBEC3H, and APOBEC3F) that also have anti-lentiviral activities. Vif is thereby involved in multiple viral-host arms races against several cellular proteins (Desimmié et al. 2014).

Tetherin/BST-2 and the Viral Proteins Vpu, Nef, and Env

Tetherin/BST-2 inhibits viral infection by primarily blocking the release of enveloped viruses. This host transmembrane protein, stimulated by interferon, tethers budding virions to the plasma membrane, thereby blocking their release and inhibiting infection of a new target cell (Sauter et al. 2010). Recent studies have shown that Tetherin is involved more broadly in viral immune sensing and activation of the host innate immune response (Hotter et al. 2013). Lentiviruses have

therefore evolved antagonistic mechanisms to counteract Tetherin activity and allow an efficient spread of viral particles between cells. Primate lentiviruses have evolved different antagonists to inhibit Tetherin. HIV-1 encodes the Vpu accessory protein that targets the transmembrane domain of Tetherin, downregulates it from the cell surface, and allows efficient viral release. On the other hand, most primate lentiviruses antagonize Tetherin by encoding a Nef protein that targets the cytoplasmic tail of Tetherin and impairs its trafficking to the plasma membrane. In contrast, HIV-2 seems to use another viral protein, its envelope protein, to sequester Tetherin away from the plasma membrane (Sauter et al. 2010). Overall, Tetherin is targeted by lentiviral antagonists in several domains of the protein. Consistent with this, Tetherin is evolving under positive selection across primates, and specific sites in both the transmembrane domain and the cytoplasmic domain demonstrate signatures of positive selection (Lim et al. 2010). In addition, a five-amino acid deletion in the transmembrane domain of Tetherin, a genetic innovation not detected by standard tests for positive selection, has further played a role in the virus-host arms race in humans (more below).

SAMHD1 and the Viral Accessory Proteins Vpr and Vpx

SAMHD1 is a deoxynucleotide triphosphohydrolase that notably lowers the dNTP pool in the cytoplasm and blocks the reverse transcription step of lentiviruses in certain cell types (Laguette et al. 2011). As a countermeasure, most lentiviruses encode for an antagonist, Vpr or Vpx, which binds to SAMHD1, recruits it to the Cul4/DCAF1/DDB1 ubiquitin ligase complex, and targets it for degradation. As expected, SAMHD1 has evolved under strong positive selection throughout primate evolution (Laguette et al. 2012; Lim et al. 2012; Spragg and Emerman 2013). The identification of two hot spots of positively selected sites aided in the identification of two distinct virus-host interaction sites, demonstrating the power of an evolutionary approach to understand virus-host antagonism (Fregoso et al. 2013). Of note, HIV-1 does not encode any

direct antagonist to SAMHD1. It is likely that HIV-1 and its ancestors SIVcpz and SIVgor have lost this antagonistic function as a result of another evolutionary selective constraint (Etienne et al. 2013) and have evolved other means, which remain to be discovered, to efficiently infect their hosts.

Host Evolutionary Analyses of Ongoing and/or Recent Virus-Host Genetic Conflicts

On recent time scales, the analysis of the dN/dS ratio to look for evidence of positive selection in host genome is not appropriate as it relies on accumulation of significant numbers of mutations over time. Recent genetic conflicts can be witnessed by the presence of polymorphism within a host species where the amino acid changes functionally impact the virus-host interaction (Table 1). For example, APOBEC3G is polymorphic within African green monkey species and several amino acid changes were found to impact Vif antagonism (Compton et al. 2012). Spragg and Emerman further identified genetic variants of SAMHD1 in the African green monkey populations that resist viral antagonism (Spragg and Emerman 2013). Together, these studies show evidence of ongoing and/or recent genetic conflicts between African green monkeys and lentiviruses, in particular between the restriction factors APOBEC3G and SAMHD1 and the lentiviral antagonists Vif and Vpr, respectively.

To test whether adaptive selection has occurred in recent years within a species, analyses based on allele frequency differences and variation of allele frequencies between populations can be performed. The main statistical methods to detect the type of selection acting on recent genetic conflicts have been reviewed by Quintana-Murci and Clark (2013) and are briefly listed here: F_{ST} statistics to identify allele differences between populations; Tajima's D , Fay and Wu's H test, and others to identify unusual allele frequencies; HKA test to estimate reduction or excess in diversity.

Virus-Host Evolution: Beyond Positive Selection

On both ancient and recent time scales, host proteins can undergo different types of evolution as a result of evolutionary arms races with pathogens.

First, selection to maintain polymorphism within a species, also known as *balancing selection*, can be the result of frequency-dependent selection or heterozygote advantage. When a single antiviral protein is facing multiple selective pressures (e.g., from different viruses), different haplotypes can be maintained in the population to “keep up” in the virus-host arms race. Some of the best-known examples of genes evolving under balancing selection are the *MHC* (major histocompatibility complex) genes (Hughes and Yeager 1998), which by maintaining multiple alleles can recognize a wide variety of pathogens. Various haplotypes may also provide a heterozygous advantage to the host where the virus is forced to adapt to multiple alleles with various interfaces to replicate efficiently. Heterozygous African green monkeys that have two alleles of *APOBEC3G* with different virus-host interfaces apply a strong adaptive constrain on the viral antagonist Vif that is “facing two battlegrounds” (Compton et al. 2012). This may ultimately be advantageous to the host during lentiviral challenges (Fig. 2, Box 1).

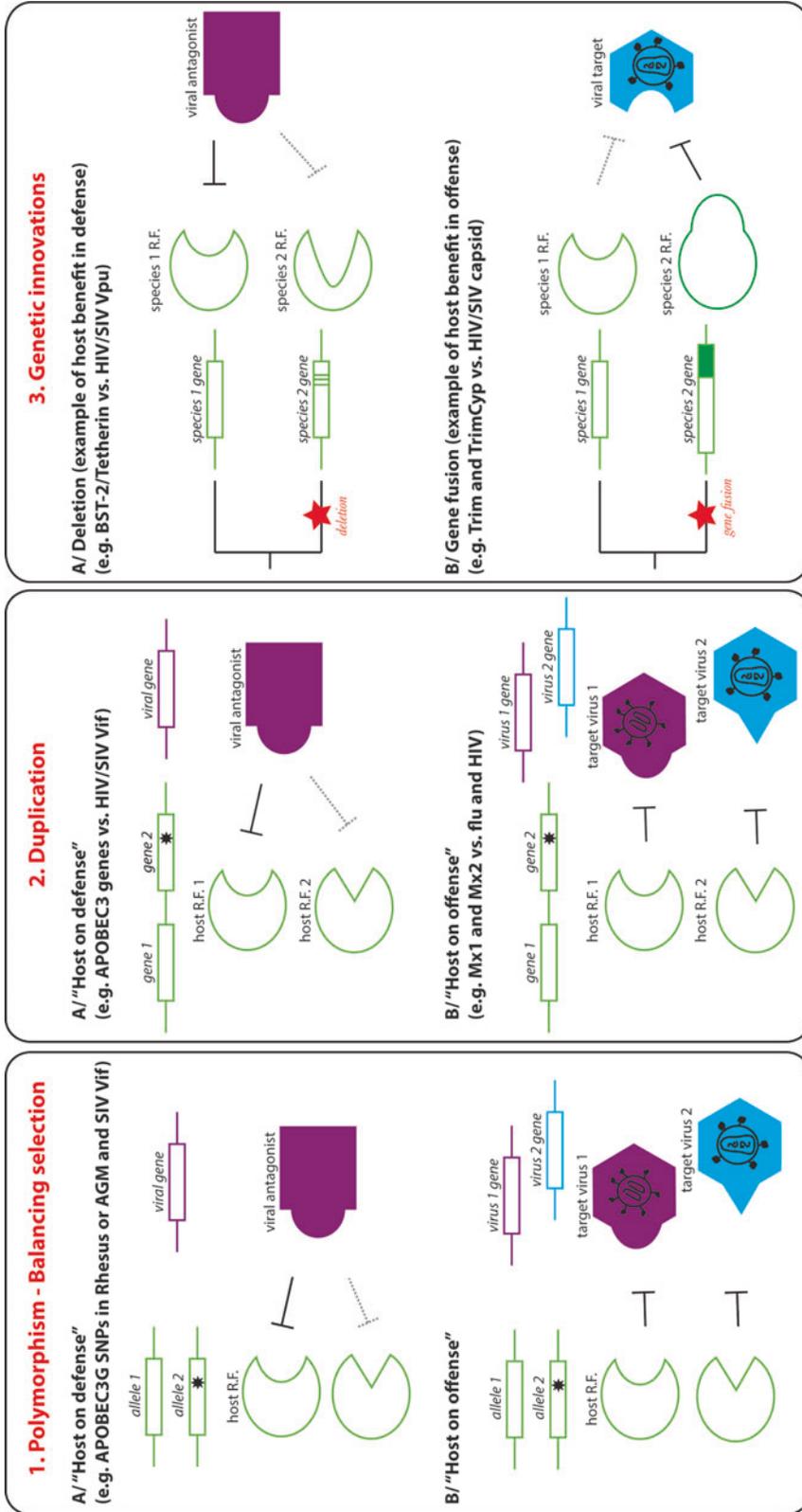
Second, the *duplication* of antiviral genes may be another major evolutionary strategy for the host to remain competitive in the virus-host arms race. Indeed, multiple restriction factors are part of a gene family that results from gene expansion by duplication. *APOBEC3G* is for example one gene of the *APOBEC3* family that comprises seven members in primates. Other examples of antiviral gene duplications come from the *TRIM* family, the *IFITM* genes, or the *Mx* genes. The advantage to maintain several genes for the host may be seen as an extension of the heterozygous advantage (a) to target different type of viruses (i.e., diversification of the offense) and/or (b) to provide multiple virus-host interfaces (i.e., diversification of the defense). (a) For example, the Mx2 restriction factor, thanks to its N-terminal tail, can restrict HIV as opposed to Mx1, which

cannot block HIV but can restrict influenza and other viruses (Busnadiego et al. 2014; Goujon et al. 2014). This gene duplication has = allowed the host to restrict very different viral families (Fig. 2, Box 2B). (b) On the other hand, gene duplication can also benefit the host evasion from a viral antagonist. For example, the different *APOBEC3* proteins with anti-lentiviral activities are targeted by the viral antagonist Vif at different protein interfaces, constraining strongly the viral protein evolution (Desimmie et al. 2014) (Fig. 2, Box 2A).

Third, *genetic innovations*, such as insertions (from insertion of few amino acids to entire gene fusion) and deletions, are other evolutionary strategies observed during viral-host arms race (Fig. 2, Box 3). Because these events include dramatic changes to the host genome, they are often deleterious and are less frequent than single amino acid changes. However, there are few instances where such events were selected during evolution, as they may have provided a strong advantage to the host under a major selective pressure from a pathogen. An example of deletion can be found in the evolution of BST-2/Tetherin, which lost five amino acids in its N-terminal domain in the human lineage specifically, possibly as a result of a viral-host arms race with a lentiviral antagonist like Nef (Sauter et al. 2009; Lim et al. 2010) (Fig. 2, Box 3A). Examples of gene fusion in an antiviral gene can be found in several primate species (e.g., owl monkeys, Asian macaques), where convergent evolution has led to the generation of TRIMcyp fusion proteins. The retrotransposition of *CypA* (cyclophilin A) downstream or within the *TRIM5* gene in several primate lineages has led to various forms of TRIMcyp proteins. These fused restriction factors have gained new antiviral specificities thanks to the capsid-binding properties of CypA (Malfavon-Borja et al. 2013) (Fig. 2, Box 3B).

Beyond Restriction Factors: Arms Races Between Viruses and Other Host Genes

Amongst host genes, few bear signatures of rapid evolution as restriction factors do. However,



Virus-Host Evolution and Positive Selection, Fig. 2 Beyond positive selection: other mechanisms of host evolution in viral-host genetic conflict. Boxes 1, Balancing selection (A, the host is “winning” on defense; B, the host is “winning” on offense). Box 2, Duplication (A, the host is “winning” on defense; B, the host is “winning” on offense). Box 3, Genetic innovations (A, deletion; B, gene fusion). Examples of known genetic conflicts involving lentiviral proteins and host restriction factors are given. R.F. restriction factor. *Black stars* and *triangles* in genes represent amino acid changes. *Dashed lines* represent loss of block

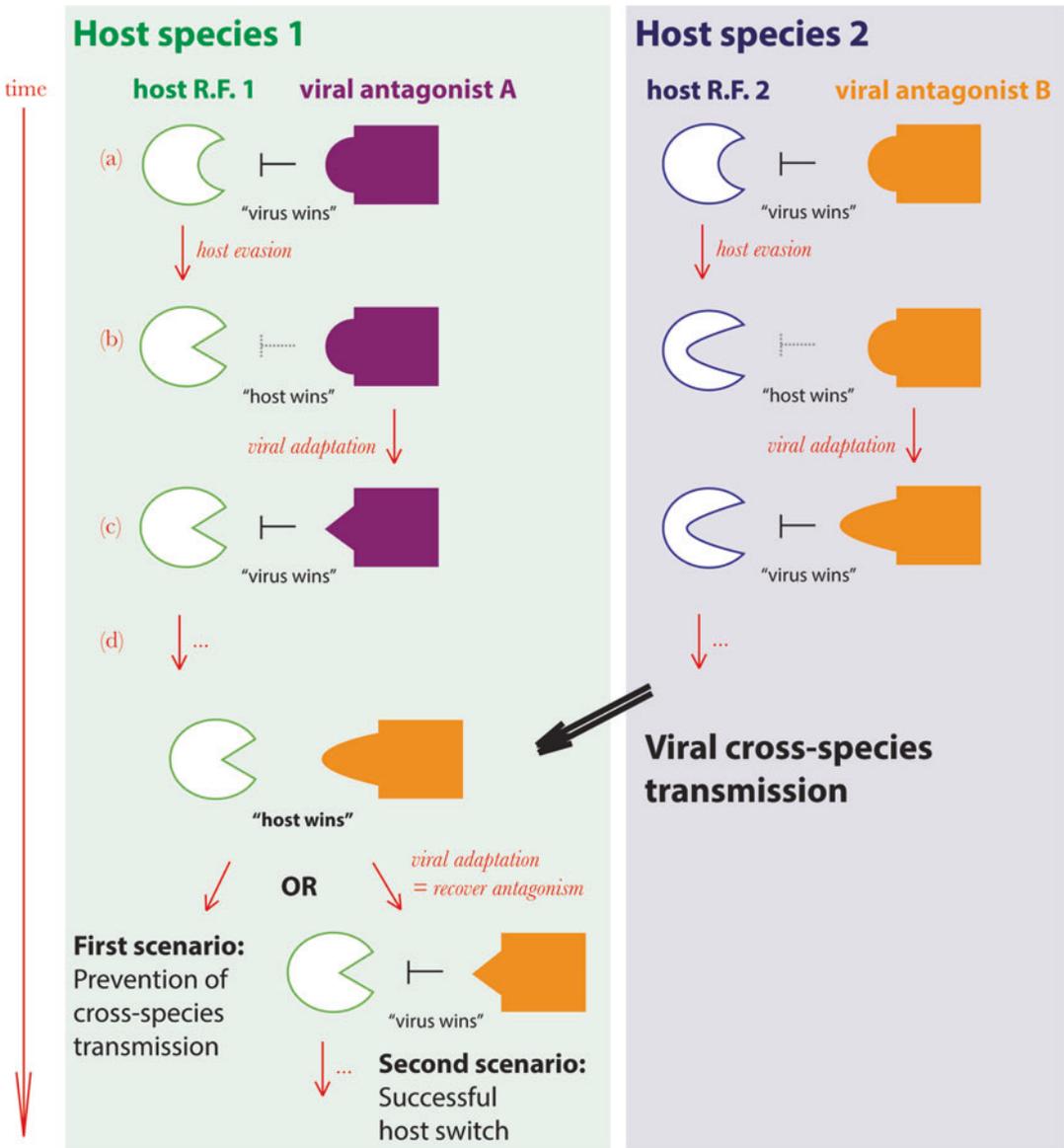
because the virus interacts with numerous other host proteins, it has been recently proposed that host cofactors may also be evolving under positive selection as a result of viral pressure. Unlike restriction factors that are “specialized intrinsic immune genes,” host factors that are usurped by the virus for replication also perform essential cellular functions. Therefore, like most host proteins that are important for cellular physiology processes, they are expected to be mostly conserved throughout evolution (i.e., evolve under purifying selection). However, recent studies have highlighted that, despite this evolutionary constrain, host factors that are necessary for virus replication may also be evolving under recurrent positive selection as a result of “host evasion” mechanisms. This was first shown in an elegant study for Tfr1, which is a receptor for multiple viruses (Demogines et al. 2013), and it was recently shown that some HIV cofactors may also be under positive selection (Meyerson et al. 2014). For example, the HIV receptor CD4 is found under strong positive selection in primates and a site that is bearing a strong signature of positive selection is implicated in the specificity of HIV entry (Meyerson et al. 2014). Therefore, even though most host cofactors of HIV seem to be generally conserved, a subset of them may evolve under positive selection driven by virus-host interactions that have played out over evolutionary time. More evolutionary studies of HIV cofactors associated with functional analyses will help in the characterization of these “unusual” viral-host arms-races and increase our understanding of the biology and evolution of these cellular cofactors that could be potential anti-HIV drug targets.

Consequences of Ancient and Ongoing Virus-Host Arms Races on the Cross-Species Transmission Potential of Modern-Day Lentiviruses

Ancient and ongoing virus-host arms races have therefore shaped modern-day host species and in particular have driven the specificities of interactions between the host restriction factors and

viruses. As viruses can adapt very rapidly to their host, modern-day viruses efficiently counteract the antiviral genes of the host species they infect. However, differences in restriction factors between species may be sufficient to create a barrier to viruses and may shape the host species spectrum of viruses (i.e., species-specificities). These differences between orthologous antiviral genes have mainly been shaped by ancient virus-host genetic conflicts (Daugherty and Malik 2012; Duggal and Emerman 2012) and current selective pressures continue to shape our antiviral repertoire. Therefore, the evolutionary history of virus-host interactions may in part explain the susceptibility and resistance of modern-day species to viral cross-species transmissions (Fig. 3). In particular, the differential susceptibility of host species to lentiviral cross-species transmissions may lie in the capacity of restriction factors to block SIV emergence (Fig. 3, first scenario). Furthermore, the evolutionary potential of a virus to adapt to the new restrictive cellular environment may determine the ease at which cross-species transmissions can occur (Fig. 3, second scenario).

Lentiviruses have infected primates over millions of years. However, the global picture today is that each primate species is infected by its own lentiviral lineage and few cross-species transmissions have been identified. This lentiviral species-specificity may be driven by the inability of lentiviruses to antagonize or escape from the restriction factors encountered in a new species (Fig. 3, first scenario). Although the influence of host restriction factors on viral cross-species transmission has not been well studied, in particular in natural settings, some *in vitro* and *in vivo* experimental studies have tackled this question. Both APOBEC3G and TRIM5 may represent natural selective barriers to lentiviral cross-species transmissions and only viruses that have the capacity to adapt rapidly to counteract or escape from the antiviral proteins will successfully emerge (Fig. 3, second scenario). Indeed, although the role of APOBEC3G as a species barrier has been mainly investigated in experimental cross-species transmissions, it has been shown that its evolution and its variability between and within primate



Virus-Host Evolution and Positive Selection, Fig. 3 Model for the consequences of long-term viral-host arms race on the potential of viral cross-species transmission. Steps: (a) The viral protein A is able to antagonize the restriction factor 1. This puts a selective pressure on the host that will evolve to evade the antagonist, leading to (b) where the “host is winning.” Such selective pressure will induce a rapid viral evolution. (c) Only viruses able to block the new host protein will

be selected. (d) This virus-host interaction sets an evolutionary “arms-race” in the host species 1. Similarly, another evolutionary “arms-race” is in place in the host species 2 infected by a virus B. So that, when a virus crosses from species 2 to species 1, the host will most likely “win” and this will, in most cases, prevent the cross-species transmission (first scenario). However, if the virus is able to rapidly adapt to the new species, it will successfully jump the species barriers (second scenario)

species have been implicated in the species-specificity of lentiviruses (Johnson 2013). Studies on the origin of the HIV-1/SIVcpz lineage in hominoids also suggest that APOBEC3G has

been a selective barrier for SIVs, and only lentiviruses with some capacity to antagonize the new host APOBEC3G may have the potential to jump efficiently in the new species (Etienne et al. 2013)

(Etienne et al. unpub). Genetic variations in TRIM5 may also influence in some primates the outcome of cross-species transmissions. Indeed, TRIM5 has played a role of selective barrier at the origin of SIVmac during the species-jump of SIVsmm from sooty mangabeys to macaques (Johnson 2013). Future studies on the role of other restriction factors as selective barriers and their potential hierarchy are likely to uncover more examples of host barriers to transmission of lentiviruses among primates.

Conclusion

Over evolutionary time, primate species have been under the selective pressure of many pathogens, including lentiviruses. The adversarial interactions between the cellular restriction factors, principal component of the host intrinsic immune system, and the viral antagonists have set the two entities in a “Red Queen” competition where the host gene imposes a selective pressure on viral replication and the virus exerts pressure on the host for survival. These antagonistic virus-host interactions and evolutions have therefore mainly evolved under positive selection, although other evolutionary strategies have been identified. In conclusion, both ancient and recent virus-host genetic conflicts have shaped our modern-day intrinsic immune genes, largely determining our current sensitivity and resistance to pathogenic and emerging viruses.

Cross-References

- ▶ [APOBEC3F/G and Vif: Action and Counteractions](#)
- ▶ [Cellular Cofactors of HIV as Drug Targets](#)
- ▶ [Cell-Intrinsic Immunity](#)
- ▶ [Counteraction of SAMHD1 by Vpx](#)
- ▶ [MHC Locus Variation](#)
- ▶ [Nef/Env/Vpu/Tetherin](#)
- ▶ [TRIM5alpha](#)

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Women, Epidemiology of HIV/AIDS

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Definition

Women worldwide bear a substantial burden of human immunodeficiency virus (HIV) infection, the majority attributed to heterosexual transmission. A woman's risk of HIV acquisition results from a complex intersection of biological, behavioral, social, and structural factors. Timely diagnosis and linkage to care and treatment can reduce morbidity and mortality as well as perinatal transmission of HIV. Effective HIV prevention efforts among women will require a synthesis of biomedical, behavioral, and structural approaches which address the underlying social structures (e.g., gender inequality) which promote vulnerability to HIV infection among women.

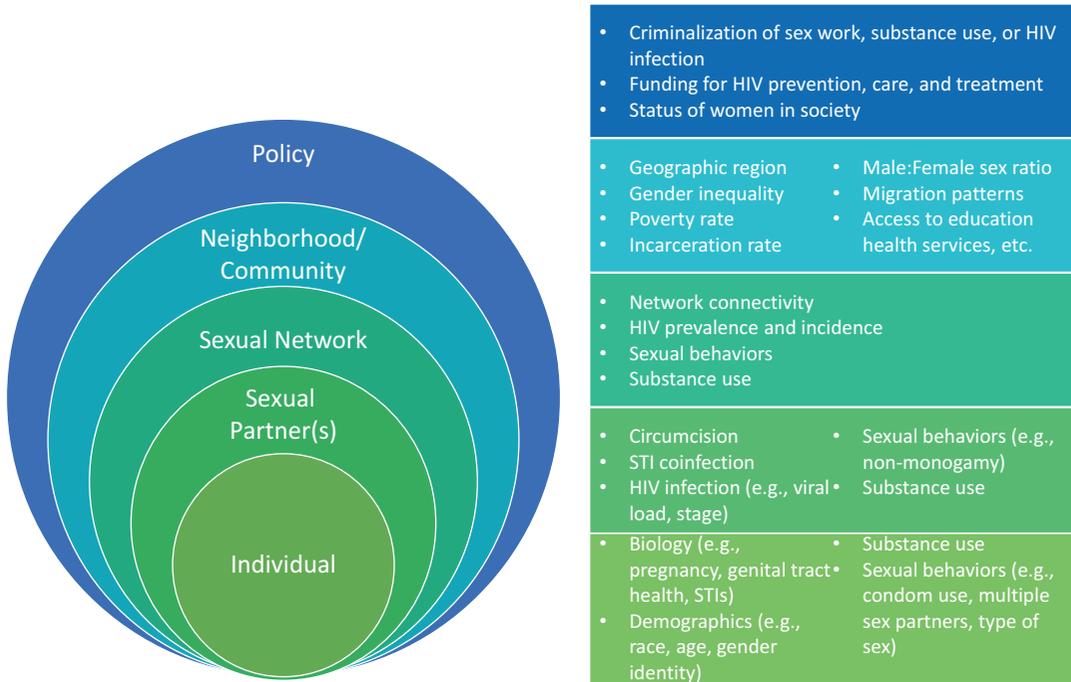
HIV/AIDS in Women

Globally, over 50% of all adults aged 15 years and older living with HIV are women, an estimated

total of 17.8 million women in 2015 (UNAIDS 2017). The burden of HIV among women varies by geographic region of the world. The majority of HIV-infected women live in sub-Saharan Africa, which constitutes roughly 80% of the epidemic among women (UNAIDS 2017).

Risk Factors for HIV Acquisition Among Women

The vast majority of women (>80%) acquire HIV through sex with an HIV-infected male partner. A number of biological, behavioral, social, and structural factors shape women's risk of HIV acquisition (Fig. 1). The probability of HIV transmission per sex act depends on the susceptibility of the female partner (e.g., coinfection with other sexually transmitted infections [STIs], pregnancy status, integrity of the genital tract), the infectiousness of the male partner (e.g., circumcision status, stage of HIV disease, HIV viral load), the mode of sex (i.e., oral, vaginal, or anal), and frequency of exposure. For example, having an STI increases the probability of HIV infection more than 17-fold per vaginal sex act, and the probability of HIV acquisition per anal sex act is more than five-fold the probability of HIV acquisition per vaginal sex act (Boily et al. 2009). The presence of multiple factors may further increase the probability of transmission. Individual behaviors, such as vaginal or anal sex without a condom, substance use, having multiple male sex partners, and



Women, Epidemiology of HIV/AIDS, Fig. 1 Multilevel factors shaping women’s risk of HIV acquisition

exchanging sex for money, drugs, or other commodities, are associated with increased risk of HIV acquisition among women. In addition, characteristics of sexual partners (e.g., non-monogamy) or corresponding sexual networks (e.g., network connectivity and HIV prevalence) may create susceptibility to HIV infection by connecting otherwise low-risk women to higher-risk sexual networks. There is a growing appreciation of how social phenomena, such as gender inequality and poverty, create vulnerability to disease among women. A disproportionate number of women live in poverty globally. Limited economic and educational opportunities may increase women’s reliance on male partners, promote sex exchange, and undermine a woman’s ability to negotiate safer sex practices. Notably, 50% of women living with HIV also report experiencing gender-based violence during their lifetime (UNAIDS 2013). Similarly, social and built characteristics of the places where women live may engender higher-risk environments. For example, high incarceration rates or out-migration of men

for labor may result in a shortage of men relative to the number of women in a community. Imbalanced sex ratios may support multiple sexual partnerships and reduce the ability of women to negotiate monogamy or safer sex practices. Policies may further promote risk. Notably, laws which criminalize behaviors associated with HIV acquisition (e.g., drug use, commercial sex work) may discourage access to healthcare and effective HIV prevention, care, and treatment.

Within the epidemic, certain subgroups of women who represent a substantial or disproportionate share of the HIV epidemic are considered priority populations. These groups include:

Commercial Sex Workers

The majority of commercial sex workers (CSW) are women. Female sex workers are 13.5 times as likely to be HIV infected as their female counterparts (Baral et al. 2012). HIV risk factors associated with commercial sex work include behavioral characteristics, such as high number of sexual partners, illicit drug use, and concurrent

infection with sexually transmitted infection (STIs) as well as social factors such as the power differential inherent in a transactional relationship that may result in high rates of sexual violence, lower condom use, and risky sexual practices. Importantly, structural factors such as policies that criminalize commercial sex work or carrying condoms, stigma, and inadequate access to condoms and healthcare contribute to increased HIV risk among CSW (Baral et al. 2012; UNAIDS 2013).

Women Who Inject Drugs

Central and Eastern Europe and Central Asia, in particular, have well-documented HIV epidemics within populations that inject drugs. The HIV prevalence among people who inject drugs is estimated to be as high as 20% (Jolley et al. 2012). Although the majority of people who inject drugs are men, women who inject drugs are also vulnerable to HIV infection. This risk is in part attributable to the efficiency of HIV infection through the use of intravenous needles contaminated with HIV virus, challenges negotiating safer sex and injection practices, violence experienced by women who inject drugs, commercial sex work, and the criminalization and stigma associated with injecting drug use (Jolley et al. 2012).

Young Women in Sub-Saharan Africa

Although 15% of all women living with HIV aged 15 years and older are young women 15–24 years old, of these, 80% live in sub-Saharan Africa (UNAIDS 2014). The HIV prevalence among young women in sub-Saharan Africa is twice as high as the prevalence in young men, and women acquire HIV infection at least 5–7 years earlier than men (UNAIDS 2014). This imbalance reflects several factors contributing to these women's vulnerability to HIV, including gender inequality, older male sexual partners, high population level HIV prevalence (e.g., male partners are more likely to be HIV infected), fertility desire and decreased use of condoms, low rates of male circumcision, migratory labor and resulting concurrency, and, in some regions, “dry sex” practices which result in increased genital trauma.

African American Women in the United States

Significant racial disparities exist for newly reported cases among women in the United States. HIV incidence is more than 15 times higher among African American (AA) women than among White women (Prejean et al. 2011). Sexual behaviors alone do not explain the dramatic differences in HIV infection by race and ethnicity in the United States. A number of studies have found comparable sexual risk behavior among AA as compared to other racial and ethnic groups, and some demonstrated that Whites engaged in higher-risk behaviors than their White counterparts. Characteristics of sexual networks (e.g., indirect concurrency, prevalence of HIV within network) and of the places where people live (e.g., neighborhood poverty, ratio of men to women) are thought to be powerful drivers of the epidemic among AA women in the United States (Cooper et al. 2014).

Transgender Women

Transgender women, defined as persons born biologically male who identify themselves as female, shoulder a disproportionate burden of the HIV epidemic. A 2013 review of available literature from Europe, Central and South America, Asia-Pacific, and the United States found that the odds of HIV infection among transgender women were consistently 20–90 times the odds of HIV infection among adults living in the same country (Baral et al. 2013). Transgender women are at enhanced risk of HIV infection in part due to the high prevalence of receptive anal sex and high prevalence of HIV within sexual networks but also frequently face social exclusion, economic marginalization, and unmet healthcare needs (Baral et al. 2013).

Incarcerated Women

More men are incarcerated than women worldwide. However, the incarceration rate of women has grown substantially in the United States, a country that incarcerates a larger proportion of the population than other countries in the world. The prevalence of HIV is higher among incarcerated females than among incarcerated males in the

United States. Data on HIV prevalence among incarcerated women in low- to middle-income countries is limited but has been estimated to be as high as 20% (Dolan et al. 2007). Commercial sex workers, substance users, transgender women, and, in the United States, AA women are more likely to be incarcerated than women in the general population; incarcerated women represent an intersection of several high-risk groups and the complex behavioral, social, and structural factors which enhance HIV acquisition.

HIV Testing, Antiretroviral Treatment, and Linkage to Care

Globally, HIV/AIDS is the leading cause of death among women of reproductive age (UNAIDS 2014). Antiretroviral therapy (ART) reduces mortality attributable to HIV, improves quality of life of HIV-infected individuals, and reduces HIV transmission. Timely initiation of ART depends on HIV testing, linkage to HIV care, and access to treatment. Globally, only 48% of persons living with HIV are aware of their HIV status (UNAIDS 2014). Women are more likely to have been tested than men in most regions, which is most likely a result of the integration of HIV testing in many antenatal and reproductive health settings. Treatment guidelines and access vary by region (UNAIDS 2013; World Health Organization 2013; UNAIDS 2017). Women living in high-income countries, such as the United States, in theory, have better access to ART but may not be successfully connected to care; the Centers for Disease Control (CDC) estimates that among HIV-infected women, 88% are aware of their HIV infection, 45% are linked to care, 41% are prescribed ART, and 32% that are virally suppressed are linked to HIV care and taking ART (CDC 2011).

Timely HIV diagnosis, prevention of unwanted pregnancy, and access to ART are especially critical for HIV-infected women, who may transmit HIV to infants during pregnancy, in childbirth, or through breast milk (mother-to-child transmission, MTCT). MTCT has been virtually eliminated in high-income countries such as the United States and Europe due to ART. Although low- and middle-

income countries experienced a 52% decline in new HIV infections among children between 2001 and 2013, significant gaps remain (UNAIDS 2013). In many low- and middle-income countries, pregnant women living with HIV are less likely to receive antiretroviral therapy than other HIV-infected treatment-eligible adults (UNAIDS 2013). In 2012, 62% of pregnant women in low- and middle-income countries living with HIV received antiretrovirals. In contrast, 49% received ART during the breastfeeding period. Estimates suggest that nearly half of all cases of HIV transmission period occur during the breastfeeding period (UNAIDS 2013). As a result, breastfeeding recommendations for HIV-infected women vary by country, replacement feeding alternatives, and access to clean water, sanitation, and health services (World Health Organization 2013).

HIV Prevention in Women

The substantial burden of HIV among women globally and the complex behavioral, social, and structural factors which contribute to HIV acquisition necessitate strategies which combine behavioral, biomedical, and structural approaches to reducing HIV acquisition and increasing diagnosis and treatment among HIV-infected women (UNAIDS 2013).

Some successful biomedical approaches, including treatment as prevention and male circumcision, have emerged as effective HIV prevention tools in recent years and have contributed to a decline in new HIV infections among women in some parts of the world. The treatment of an HIV-infected person with ART may reduce the risk of HIV transmission to his/her HIV-uninfected partner by up to 96% (Cohen et al. 2011). Voluntary adult medical male circumcision reduces HIV infection in men by approximately 60% and may result in population-level reductions in the rate of male-to-female HIV transmission by up to 46% (Hallett et al. 2011). Condoms can also be considered effective biomedical devices for HIV prevention. When used correctly and consistently, condoms are 80% effective in reducing HIV transmission (Weller and Davis 2002). Increases in condom use, which occurred

in parallel with an increase in condom distribution, are credited with declines in new HIV infections in South Africa between 2000 and 2008. However, access to condoms may be limited in a variety of settings. In 2013, only eight male condoms were available per year for each sexually active individual in sub-Saharan Africa (UNAIDS 2014). In addition, there a number of reasons why women may elect not to use condoms, including desire for children and perceived HIV-negative status of sexual partner(s) and partner monogamy.

Condom use, voluntary medical male circumcision, and sexual partner HIV testing and treatment require buy-in by male partners, which may not be feasible in many settings. There is a need for highly effective, female controlled biomedical prevention. Preexposure prophylaxis (PrEP), which involves the topical or oral use of an antiretroviral drug before, during, or after sex, may also be a promising prevention tool for women. However, the evidence supporting PrEP use in women is inconclusive (Baeten et al. 2013). One clinical trial of tenofovir vaginal gel showed a 39% reduction in HIV acquisition and 51% reduction in herpes simplex 2 virus acquisition when used by study participants before and after sex. However, subsequent trials testing tenofovir gel or oral PrEP were halted because HIV acquisition in the participants using the study product was not significantly different than the participants using the placebo product (a gel or pill which looks like the study product but is made with inactive ingredients, also known as “sugar pill”). However, further investigation of these clinical trials suggested that study participants did not use products as prescribed and further research is needed to understand PrEP acceptability and use among women (Baeten et al. 2013).

There are a number of existing evidence-based interventions (EBIs) for individuals, couples, and groups that are designed to reduce risk behaviors associated with HIV acquisition (e.g., unprotected sex) and to promote healthy sexual norms (e.g., partner communication). These interventions have been tested in clinical trial settings and have demonstrated efficacy in changing the target

behaviors relative to the control group (the group that did not get the intervention). Although behavior change is a critical component of HIV prevention, behavior change alone may not reduce the risk of HIV acquisition in women.

Structural approaches, which seek to address the underlying social and structural factors which create vulnerability in women (e.g., gender norms, poverty), may also reduce new HIV infections among women. Approaches currently being explored include cash transfers (e.g., providing financial support to families who keep their young girls enrolled in school), vouchers, and food and nutrition support. Implementing successful structural interventions on a community-wide level is complex, costly, and requires political support. Notably, needle exchange programs, which distribute clean needles and injecting equipment to people who use drugs, have been shown to be effective in reducing HIV transmission, but the implementation of these programs is often stymied by policies that criminalize the distribution of drug-related paraphernalia. Policy change may be an essential component of interventions seeking to change the social and structural determinants of HIV acquisition among women.

Conclusion

In summary, women represent a substantial portion of the HIV/AIDS epidemic globally. Although biomedical approaches offer exciting opportunities to reduce HIV transmission among women, the failure of condoms to control the HIV epidemic, nonadherence to PrEP in recent clinic trials, and incomplete HIV testing and access to treatment highlight the complex behavioral, social, and structural factors which shape women’s HIV risk and necessitate strategies which harmonize behavioral, biomedical, and structural approaches designed to increase access to medical care and reduce HIV acquisition in women and their male partners (UNAIDS 2013). Such approaches will not only improve the health of women but benefit their families and communities.

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